Contents

1 Calibration of Spectrophotometers

UV/Vis spectrophotometry is one of the most frequently applied methods in chemical analysis. It is used for example in clinical chemistry, pharmaceutical industry, scientific research and very often in quality assurance.

In recent years quality requirements, as outlined by EN ISO 9000, Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP) or the recommendations of pharmacopeias (EP, DAB, USP) have become more influential. Formal performance verification of UV/Vis spectrophotometers is now essential. The performance verification tests required by major Pharmacopeias for UV/Vis spectrophotometers are checking the spectral resolution, the wavelength accuracy, the photometric accuracy, and checking for stray light.

The set of liquid Hellma calibration standards enables the checking of spectrophotometers with respect to

- wavelength accuracy
- photometric accuracy
- stray light and
- spectral resolution

at wavelengths from 198 nm to 650 nm.

The set of solid Hellma calibration standards enables the checking of spectrophotometers with respect to

- the photometric accuracy in the visible range of the spectrum and the
- wavelength accuracy in the ultraviolet and visible range

1.1 Calibration Standards for Checking the Wavelength Accuracy

The ideal standard to measure wavelength accuracy has very narrow, well defined peaks at a series of wavelengths throughout the UV and visible range. There are several standards suitable for wavelength calibration, which are described in different regulations. The following table shows an overview of the calibration standards offered by Hellma and their reference in the most important regulations:

Checking for	Calibration Standard	Pharmacopeia			ASTM
		EP	DAB	USP	
Wavelength Accuracy	Holmium Oxide Liquid Filter	✓	✓	✓	✓
	Holmium Oxide Solid Filter			✓	✓
	Didymium Glass Solid Filter			✓	✓

According to European Pharmacopeia checking of the wavelength accuracy is described as follows:

“Verify the wavelength scale using the absorption maxima of holmium perchlorate solution R [...]. The permitted tolerance is ±1 nm for the ultraviolet range and ±3 nm for the visible range”.

European Pharmacopeia specifies four wavelengths for holmium perchlorate solution filters: 241.15 nm, 287.15 nm, 361.5 nm and 536.3 nm. The spectrum contains some additional peaks which can also be used for checking the wavelength in principle.

Compared with the holmium oxide liquid filter, the holmium oxide glass filter possesses a slightly weaker pronounced spectrum. Particularly in the deep UV range the holmium peaks are affected by the absorbance of the carrier glass. A similar effect can be observed with the didymium glass filter.

For common wavelength accuracy standards, because the peaks are not ideally symmetrical, changes in the slit width of the instrument will slightly affect the measured position of the peaks. Because of the narrow bands of the peaks the heights of the measured maxima will vary with a change of the slit width. Therefore standards for checking the wavelength accuracy are in general not suited for checking the photometric accuracy.

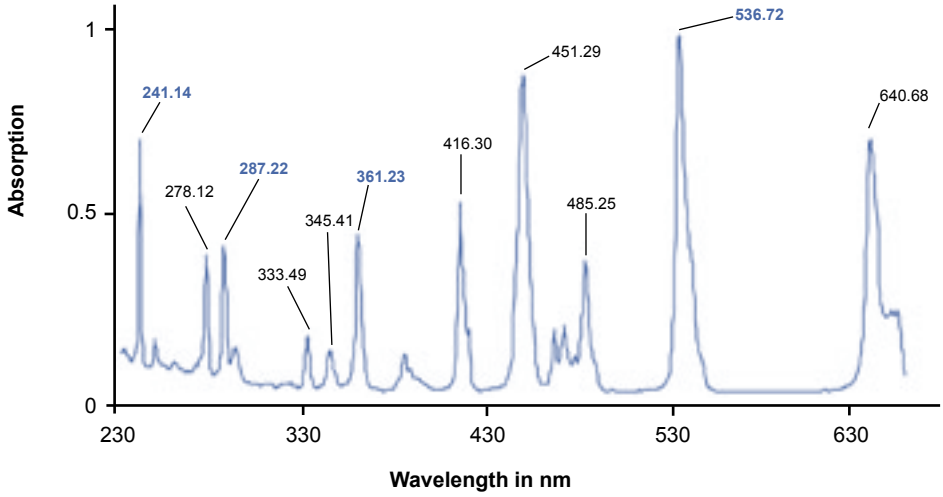
For an overview of all Hellma calibration

1.1.1 Holmium Oxide Liquid Filter

Description	Holmium oxide filter, liquid filter, certified
Use	Assessment of wavelength accuracy in both UV and visible range from 240 nm to 650 nm, at a spectral bandwidth between 0.1 nm and 3 nm
Material	Holmium oxide dissolved in perchloric acid and sealed in Hellma precision cells made from quartz SUPRASIL®

667-UV5 Holmium oxide dissolved in perchloric acid

Holmium oxide dissolved in perchloric acid is highly suitable for checking the wavelength accuracy of spectrophotometers both in the UV and the visible range. It shows a spectrum with a multitude of highly defined peaks.
The heights and positions of these peaks are dependent on and will vary with the chosen slit width of the measuring instrument.



Typical absorption spectrum of holmium oxide dissolved in perchloric acid, recorded with a slit width of 1 nm

The filter with the holmium perchlorate solution is placed into the beam path of the measuring instrument. The smallest slit width has to be used for this measuring (e.g. 1 nm). The smoothing (damping) has to be adjusted to a relatively high level. Alternatively the average of several measurements can be calculated to avoid evaluation errors. The precise peak positions at 241.15 nm, 287.15 nm, 361.5 nm and 536.3 nm are measured and compared with the values given in the certificate. Deviation should not be more than ±1 nm in the ultraviolet range or more than ±3 nm in the visible range. If available the reference filter 667-UV14 filled with perchloric acid can be placed in the reference beam path. This suppresses the background of the spectrum without influencing the peak position.

Ideally the measurement is performed with the slit width specified in the certificate. If this is not possible, choose the smallest possible slit width.

Catalogue-No.	Substance	Theoretical Wavelength (nm) According to Pharm. Eur.
667-UV5	Holmium Perchlorate in Perchloric Acid	241.15, 287.15, 361.5, 536.3

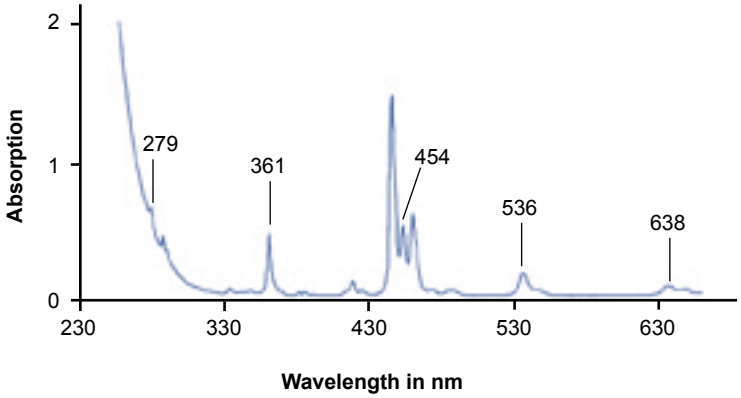
standards please see pages 18 and 19.

1.1.2 Holmium Oxide Glass Filter

Description	Holmium oxide glass filter, solid filter, certified
Use	Assessment of wavelength accuracy in both the UV and visible range (279 nm to 638 nm) with a spectral bandwidth up to 2 nm
Material	Glass doped with holmium oxide

666-F1 Holmium oxide glass filter

The slightly yellow filter marked F1 is made of silica glass doped with holmium oxide (Ho_2O_3). The element holmium exhibits a number of sharp absorption peaks throughout the UV and visible spectral ranges.



Typical spectrum of a holmium oxide glass filter

Because the exact positions of the holmium peaks vary slightly for each production batch (movement due to the glass matrix), the filters are calibrated individually.

Catalogue-No.	Material	Thickness	Absorbance at nm
666-F1	Holmium Oxide Glass	2.1 mm	279, 361, 454, 536, 638

For an overview of all Hellma calibration

1.1.3 Didymium Glass Filter

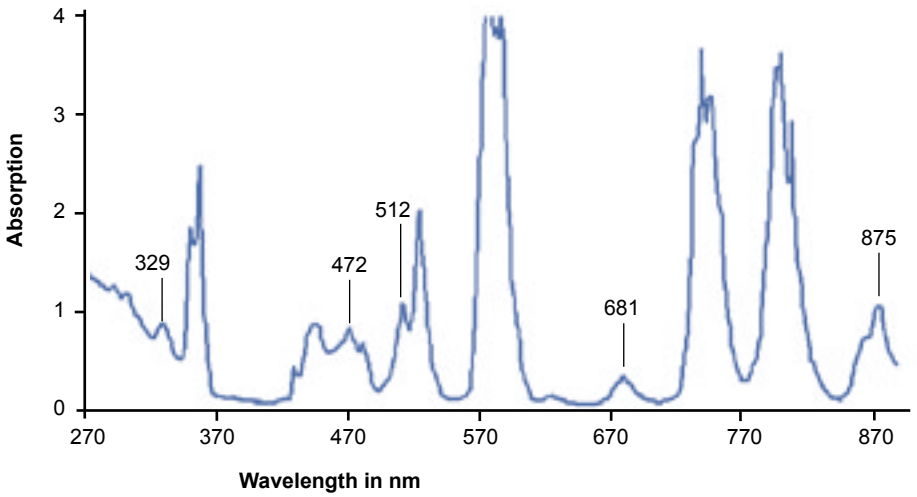
Description	Didymium glass filter, solid filter, certified
Use	Assessment of wavelength accuracy in both the UV and visible range (329 nm to 875 nm) with a spectral bandwidth up to 2 nm
Material	Glass BG 36 of SCHOTT Glaswerke

666-F7 Didymium glass filter

The didymium glass filter 666-F7 made of SCHOTT glass BG 36 can be supplied to check the wavelength accuracy in both the UV and visible range.

This filter can additionally be used to check the photometric accuracy at wavelengths of 270 nm, 280 nm, 300 nm, 320 nm and 340 nm (see page 8).

Didymium glass shows a spectrum with a multitude of highly defined peaks. The heights and positions of these peaks are dependent on and will vary with the chosen slit width of the measuring instrument.



Typical spectrum of a didymium glass filter

Catalogue-No.	Material	Thickness	Absorbance at nm
666-F7	Didymium Glass BG 36	ca. 1.2 mm	329, 472, 512, 681, 875

standards please see pages 18 and 19.

1.2 Calibration Standards to Check the Photometric Accuracy

To check the photometric accuracy Hellma offers different kinds of standards: neutral density glass filters are very well suited for testing in the visible range, checking the photometric accuracy with potassium dichromate dissolved in sulphuric acid is the approved method for the UV region. Typically used standards have broad peaks and valleys. The following table shows the standards offered by Hellma and their compliance references:

Checking for	Calibration Standard	Pharmacopeia			ASTM
		EP	DAB	USP	
Photometric Accuracy	Potassium Dichromate Liquid Filter	✓	✓	✓	✓
	Neutral Density Solid Filter			✓	✓
	Didymium Glass Solid Filter				

1.2.1 Potassium Dichromate in Sulphuric Acid

Description	Potassium dichromate filter, liquid filter, certified
Use	Assessment of photometric accuracy (absorbance) in the UV range (235 nm up to 350 nm) with a spectral bandwidth of 2 nm or less
Material	0.006% Potassium dichromate dissolved in sulphuric acid, sealed in Hellma precision cells made from quartz SUPRASIL®

667-UV7 Potassium dichromate dissolved in sulphuric acid is a highly suitable method to check for photometric accuracy (absorbance) in the UV region. Potassium dichromate gives a spectral scan containing characteristic peak maxima at 257 nm and 350 nm and minima at 235 nm and 313 nm. The potassium dichromate solution is sealed in an airtight sealed quartz precision cell with 10 mm light path. The absorbance values of the filter are determined at the wavelengths 235 nm, 257 nm, 313 nm, and 350 nm and are specified in the certificate. It should be noted that the measurements are carried out with the filter 667-UV8 (0.01 N sulphuric acid) in the reference beam path. Measured values of the reference filter are separately displayed in the certificate.

The individually measured absorbance values are free from any systematic errors in the preparation of the solution and in the light path. Since the maxima and minima in the absorbance spectrum are relatively wide, you should measure them with a correspondingly large band width, e.g. 2 nm. In this way the influence of the noise on the measured values is minimised.

Catalogue-No.	Substance	Wavelength nm	
667.300-UV	667-UV7 Potassium Dichromate in Sulphuric Acid 667-UV17 Potassium Dichromate in Sulphuric Acid (430 nm) 667-UV8 Sulphuric Acid (Blank)	235, 257, 313, 350	also available individually

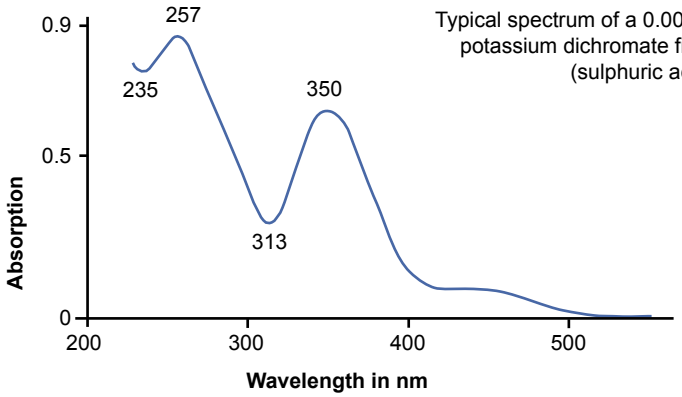
Also available:
Calibration set 667.301-UV, consisting of filter 667-UV13 potassium dichromate in perchloric acid and filter 667-UV14 perchloric acid blank.

For an overview of all Hellma calibration

According to European Pharmacopeia checking of the photometric accuracy (absorbance) is described as follows:

"Check the absorbance using suitable filters or a solution of potassium dichromate R at the wavelength indicated in the table which gives for each wavelength the exact values and the permitted limits of the specific absorbance. The tolerance for the absorbance is ±0.01. For the control of absorbance, use solutions of potassium dichromate R [...] in sulphuric acid [...]."

Wavelength (nm)	Specific Absorption $A^{1\%}_{1\,cm}$	Maximum Tolerance $A^{1\%}_{1\,cm}$
235	124.5	122.9 to 126.2
257	144.5	142.8 to 146.2
313	48.6	47.0 to 50.3
350	107.3	105.6 to 109.0



Typical spectrum of a 0.006% potassium dichromate filter (sulphuric acid)

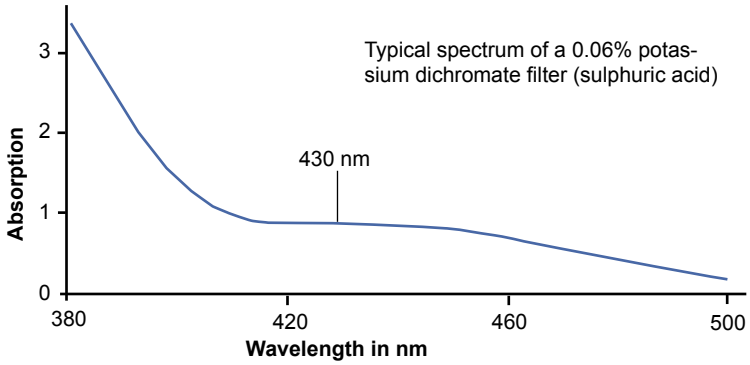
1.2.2 Potassium Dichromate in Sulphuric Acid (430 nm)

Description	Potassium dichromate filter, liquid filter, certified
Use	Assessment of photometric accuracy (absorbance) in the Vis range (measuring wavelength 430 nm) with a spectral bandwidth of 2 nm or less
Material	0.06% potassium dichromate dissolved in sulphuric acid and sealed in Hellma precision cells made from quartz SUPRASIL®

667-UV17 0.06% Potassium dichromate dissolved in sulphuric acid

Fifth revision of European Pharmacopeia stipulates also checking the photometrical accuracy of the spectrophotometer at a wavelength in the visible region (430 nm) besides the checking in the UV region. The testing has to be carried out by means of a solution of 0.06% potassium dichromate dissolved in sulphuric acid. The absorbance value of the filter is determined at a wavelength of 430 nm and is specified in the certificate. It should be noted that the measurement is carried out with the filter 667-UV8 (0.01 N sulphuric acid) in the reference beam path. Measured values of the reference filter are separately displayed.

Wavelength (nm)	Specific Absorption $A^{1\%}_{1\,cm}$	Maximum Tolerance $A^{1\%}_{1\,cm}$
430	15.9	15.7 to 16.1



Typical spectrum of a 0.06% potassium dichromate filter (sulphuric acid)

standards please see pages 18 and 19.

1.2.3 Neutral Density Glass Filters

Description	Neutral density glass filters, solid filters, certified
Use	Checking the photometric accuracy (absorbance) in the visible range (440 nm to 635 nm)
Material	Filter material NG 11, NG 5, NG 4 supplied by SCHOTT Glaswerke

666-F2, 666-F3 and 666-F4 Neutral density glass filters

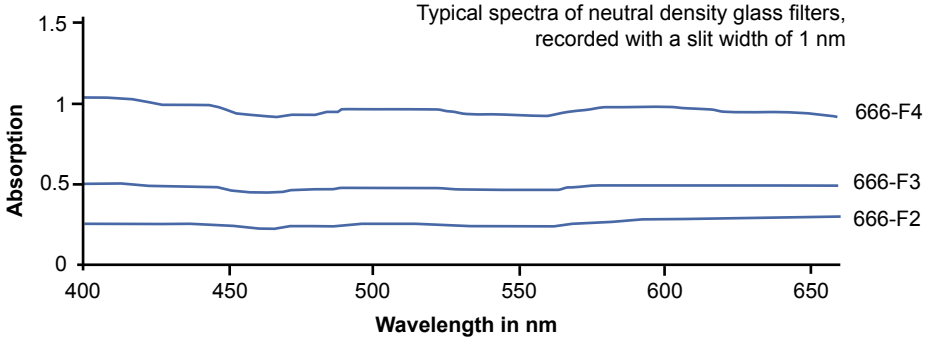
SCHOTT neutral density glasses have been used for decades for checking the photometric accuracy and linearity of spectrophotometers in the visible range.

They show a relatively constant transmission throughout the visible wavelengths range and are calibrated at wavelengths as follows:

440.0 nm, 465.0 nm, 546.1 nm, 590.0 nm, 635.0 nm

The thickness of the glass filters has been chosen to provide nominal absorbance values of 0.25 A, 0.5 A and 1 A.

Typical spectra of neutral density glass filters, recorded with a slit width of 1 nm



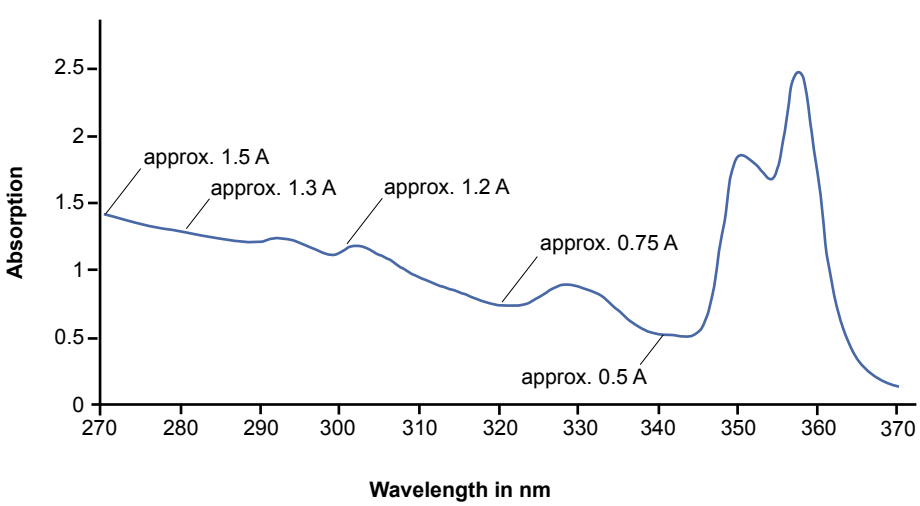
1.2.4 Didymium Glass Filter

Description	Didymium glass filter, solid filter, certified
Use	Assessment of the photometric accuracy in the UV region (270 nm to 340 nm)
Material	Glass BG 36 of SCHOTT Glaswerke

666-F7 Didymium glass filter

The F7 marked didymium glass filter is actually a standard to check for wavelength accuracy in the UV region (see page 5), but it can also be used for checking the photometric accuracy.

Checking the photometric accuracy in the UV range is possible at the following wavelengths: 270 nm, 280 nm, 300 nm, 320 nm and 340 nm. As a standard, the thickness of the filter is manufactured in a way that it will have an absorbance of approx. 0.5 A at a wavelength of 340 nm. Absorbance values will increase with decreasing wavelengths up to ca. 1.5 A at 270 nm.



Typical spectrum of a didymium glass filter in the range of 270 nm up to 370 nm

Catalogue-No.	Description	Material	Thickness mm	Nominal Absorbance	Absorbance value at nm
666-F7	Didymium Glass Filter, Certified	BG 36	approx. 1.2	depending on wavelength	270, 280, 300, 320, 340

1.3 Solid Filter Set

The calibration set 666.000 consists of three neutral density glass filters for checking the absorbance accuracy, and a holmium oxide glass filter for checking the wavelength accuracy. The glass filters are mounted in precision aluminium frames. They are designed for use with the standard 10 mm cell holder provided with spectrophotometers. The set, along with an empty mount, is supplied in a sturdy wooden case.
For identification purposes the set number is engraved on each filter mount.
The absorbance values and/or peak position wavelengths of every filter are quoted in the accompanying calibration certificate. A copy of the values, for laboratory use, can be found on the inside face of the case lid.

Catalogue-No.	Solid Filter Set	Consisting of	Absorbance Values, Certified at nm	
666.000	Set for Checking the Wavelength Accuracy and the Photometric Accuracy	666-F1 Holmium Oxide Glass Filter 666-F2 Neutral Density Glass NG 11 666-F3 Neutral Density Glass NG 5 666-F4 Neutral Density Glass NG 4 666-F0 Empty Filter Mount	279, 361, 454, 536, 638 440; 465; 546.1; 590; 635 440; 465; 546.1; 590; 635 440; 465; 546.1; 590; 635	also available individually

standards please see pages 18 and 19.

1.4 Calibration Standards for Checking for Stray Light

Stray light (false light) is light being detected but does not belong to the bandwidth of the chosen measuring wavelength. The effect of stray light is caused by light scattering, diffraction or malfunction of the instrument. Stray light causes a decrease in the measurable absorbance range and reduces the linear relationship between concentration and absorbance of the instrument.

Stray light can be a problem at any wavelength, but if energy throughput of an instrument decreases, as when moving into the UV range, the influence of increasing stray light will more and more affect the measured values.

To measure stray light, filters are needed which would ideally absorb all light of the wavelength at which the measurement is to be performed and transmit higher wavelengths (so called cut-off filters). Hellma stray light filters do not allow light with wavelengths lower than a certain wavelength to pass (cut-off wavelength). Therefore any indication of light transmission below the cut-off wavelength must be stray light.

According to European Pharmacopeia (5th revision) checking for stray light is described as follows:

„Stray light may be detected at a given wavelength with suitable filters or solutions: for example the absorbance of a 12 g/l solution of potassium chloride R in a 1 cm cell increases steeply between 220 nm and 200 nm and is greater than 2.0 at 198 nm when compared with water as compensation liquid. Suitable certified reference materials may also be used.“

Checking for	Calibration Standard	Pharmacopeia			ASTM
		EP	DAB	USP	
Stray Light	Potassium Chloride Liquid Filter	✓	✓		✓
	Sodium Iodide Liquid Filter				✓
	Sodium Nitrite Liquid Filter				✓

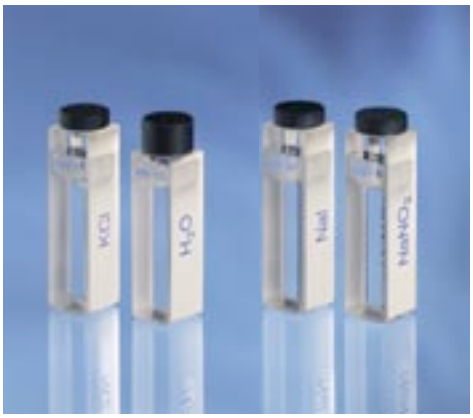
To measure the amount of stray light realistically, a filter with a cut-off wavelength as near as possible to the measuring wavelength should be chosen. The following table shows the stray light filters offered by Hellma. They are suitable for stray light measurements at cut-off wavelengths of 200 nm, 259 nm and 385 nm or lower.

Catalogue-No.	Substance	Cut-Off Wavelength nm	Certified Wavelengths nm
667.100-UV	667-UV1 Aqueous Potassium Chloride Solution 667-UV12 Purified Water	200	198, 200
667.101-UV	667-UV10 Aqueous Sodium Iodide Solution 667-UV12 Purified Water	259	220
667.102-UV	667-UV11 Aqueous Sodium Nitrite Solution 667-UV12 Purified Water	385	340, 370
667-UV12	667-UV12 Purified Water		198, 200, 300, 400

For an overview of all Hellma calibration

1.4.1 Potassium Chloride Filter, Sodium Iodide Filter and Sodium Nitrite Filter

Description	Liquid filters with sharp cut offs in transmission at specified wavelengths, certified
Use	Detection of stray light in the UV range (at wavelengths of 198 nm to 385 nm, depending on the chosen filter)
Material	Aqueous solutions sealed in Hellma precision cells, made from quartz SUPRASIL®

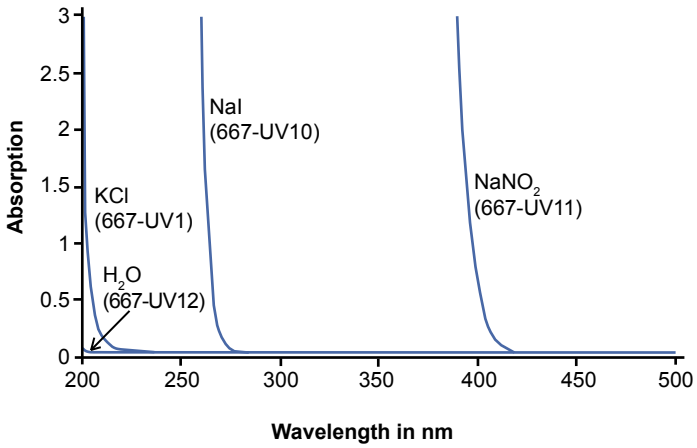


667-UV1 Potassium chloride filter
667-UV10 Sodium iodide filter
667-UV11 Sodium nitrite filter

These calibration standards show a remarkably high absorbance below a particular wavelength (cut-off wavelength) in the UV range. Using this phenomenon, the spectrophotometer can be checked for stray radiation. The measurement of stray light is carried out against a reference filter containing ultra purified water, which is supplied with every set of stray light filters. The procedure is the same for any kind of cut-off filter.

Reference filter: 667-UV12 Ultra purified water

Handling:
The spectrophotometer is set to a wavelength which is about 30 nm higher than the cut-off wavelength of the stray light filter being used. Using for example potassium chloride, it is suggested to start at 230 nm. Then scan down into the UV region and observe the absorbance values below the cut-off wavelength. The stray light value for the measuring device results from the difference between the maximum measurable absorbance and the displayed absorbance.



Typical spectra of the stray light filters offered by Hellma

Checking the maximum indication of the instrument

The filter 667-UV12 is filled with ultra purified water. Its absorbance gradient above 198 nm up to the NIR range is virtually only defined by reflection losses at the glass/air transitions. The maximum indication of a spectrophotometer can be checked with the certified values at 198 nm, 200 nm, 300 nm, and 400 nm at very low absorbance values.

Please contact the manufacturer of your instrument if the measured values differ significantly from the values given on the certificate, especially if the measured values are less than 0.02 A.

standards please see pages 18 and 19.

1.5 Calibration Standard to Check the Spectral Resolution

Spectral resolution is a measure of the ability of an instrument to differentiate between two adjacent wavelengths. Two peaks usually are considered resolved if the minimum of the absorption between the two peaks is lower than 80% of the peak maxima. Resolution of a modern spectrophotometer is closely related to its slit width. The smaller the slit width and associated instrumental spectral bandwidth, the higher is the spectral resolution.

For wavelength and absorbance measurements the dispersive power of the monochromator has to be considered, too. At the same time a small spectral bandwidth leads to a reduction of energy throughput and so the signal-to-noise ratio will fall.

When measuring in a high-resolution spectrophotometer, it is recommended that the spectral bandwidth should not exceed 10% of the natural bandwidth of the peak that is to be analysed.

Regularly checks of the resolution power of an instrument assure that, for example adjacent peaks will be fully resolved and not blended into a combined peak with the associated wavelength. Additionally absorbance errors will be avoided.

Checking for	Calibration Standard	Pharmacopeia			ASTM
		EP	DAB	USP	
Spectral Resolution	Toluene Liquid Filter	✓	✓		

1.5.1 Toluene Liquid Filter

Description	Toluene in hexane, liquid filter, certified
Use	Resolution determination of spectral bandwidth
Material	Solution of toluene in hexane, sealed in Hellma precision cells made from quartz SUPRASIL®

667-UV6 Toluene in hexane

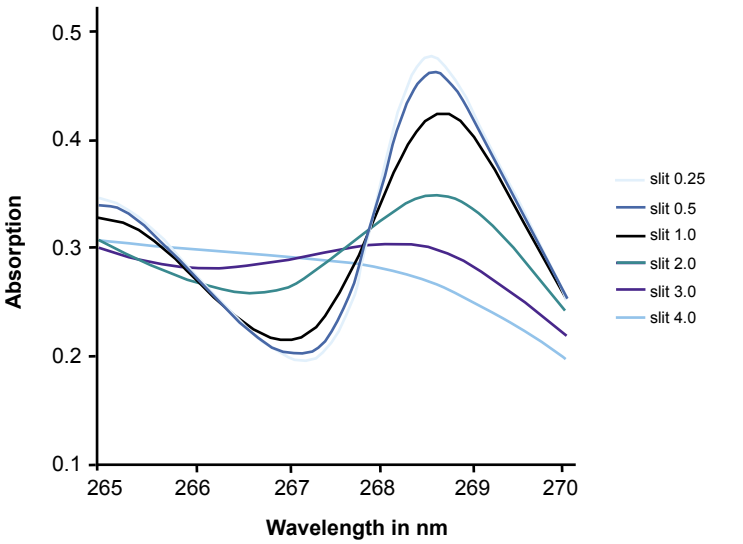
To check the resolution power of a spectrophotometer, the ratio of the peak maximum at λ_{max} (269 nm) to the absorbance of the minimum at λ_{min} (266 nm) is calculated. This gives a measure of the spectral bandwidth of the instrument.

Determination of the resolution is carried out against the 667-UV9 hexane blank reference filter, which is supplied with every filter set. Measurement is the same for all bandwidths. The wavelength range between 265 nm and 270 nm is scanned and the peak maxima and minima are determined. Then the peak/trough ratio is calculated and compared to the reference values.

The ratio of the absorbance value at 269 nm to the absorbance value at 266 nm should not be less than the one calculated from the values given in the certificate. Contact the manufacturer of your instrument if the ratio is considerably lower (e.g. 15%). Please note, however, that the result also depends on your specific measuring conditions; consequently when using particularly small slit widths the integration time should be sufficiently long.

Catalogue-No.	Substance	Wavelength nm	
667.200-UV	667-UV6 Toluene in Hexane 667-UV9 Hexane Blank	266, 269	also available individually

For an overview of all Hellma calibration



Typical spectra of a toluene filter at different slit widths

Slit Width	0.25	0.5	1.0	2.0	3.0	4.0
Ratio (Recommended Values)	2.3	2.2	2.0	1.4	1.1	1.0

1.6 Liquid Filter Set

The calibration set 667.000-UV consists of eight liquid filters which enable the complete checking of the spectrophotometer according to European Pharmacopeia. They are designed for use with the standard 10 mm cell holder provided with spectrophotometers. The set is supplied in a sturdy wooden case. For identification purposes a number is engraved on each filter.
The absorbance values and/or peak position wavelengths of every filter are quoted in the accompanying calibration certificate.

Catalogue-No.	Liquid filter set	consisting of	Wavelength nm	
667.000-UV	Complete Set for Checking the Spectrophotometer According to Pharm. Eur.	667.100-UV Potassium Chloride + Blank 667.200-UV Toluene in Hexane + Blank 667.300-UV Potassium Dichromate in H_2SO_4 + Blank 667-UV5 Holmium oxide in perchloric acid + Blank	198, 200 (certified) 266, 269 235, 257, 313, 350, 430 241.15, 287.15, 361.5, 536.3	also available individually

standards please see pages 18 and 19.

1.7 Calibration Standard for Microplate Readers

666.013 Microplate reader calibration standard

The photometric accuracy of microplate readers can be checked using the calibration standard 666.013. The microplate reader standard has the same dimensions as a microplate and has windows with a 6.6 mm diameter at the same positions as the 96 wells. Neutral density glass strips with varying absorption values are located behind groups of 16 windows. All calibration standards come with a certificate of calibration.

Catalogue-No.	Description	Nominal Absorbance	Absorbance at nm
666.013	Microplate Reader Calibration Standard, NG 11, NG 5, NG 4, NG 3 Certified	0.25, 0.5, 1.0, 1.5, 2.5	405, 450, 490, 650

For checking the wavelength accuracy of microplate readers microplate reader standards are available, where a neutral density glass stripe is substituted by a homium oxid glass stripe (on customer request).

2 Traceability of the Calibration

All Hellma calibration standards are prepared in accordance with NIST (National Institute of Standards and Technology, USA), ASTM (American Society for Testing and Materials) or European Pharmacopeia. Liquid filters are filled under controlled conditions, then the cells are permanently sealed. Solid filters are carefully cleaned and mounted stress free into their holders. The manufacturing process of the filters is completed by certifying them individually.

The manufacturing of the calibration standards is accomplished through the use of a high performance UV/Vis/NIR spectrophotometer. This instrument is used exclusively for calibration purposes and is checked for its accuracy at fixed intervals of time.

For calibration of the spectrophotometer which is used to calibrate the Hellma filters, among others, the following sets of primary standards from the “National Institute of Standards and Technology” (NIST, USA) are used:

Photometrical Accuracy
- SRM 930e Neutral Density Glass Filter

Wavelength Accuracy:
- SRM 2034 Holmium Oxide Liquide Filter

Primary instrumental wavelength calibration is established using the emission lines from mercury and deuterium sources. Linearity is established using double aperture method.

For an overview of all Hellma calibration

3 FAQ - Frequently Asked Questions

What does ”traceability“ mean?

Measurement results have to be internationally comparable. This demand is assured by the relation of the measurements results to standard references, normally maintained by a national standard body. Accuracy of those standards is assured by an unbroken chain of international comparison measurements. Our calibration standards and calibration standard measurement equipment is traceable to international accepted standards of NIST, USA (National Institute of Standards and Technology).

”Traceability“ describes the procedure of comparing the display of a measuring device with the original reference source for the measurement in one or more steps. Each step has to be calibrated against a standard whose metrological quality was also certified against a standard of higher order, creating a calibration hierarchy from the national working standards through a laboratory.

Traceability is characterised by some basic essentials:

- An unbroken chain of comparisons which can be traced back to stated national or international references, approved by the parties involved.
- Measurement uncertainty has to be calculated for each intermediate reference standard using approved methods. It has to be stated in a way that allows calculating the over-all uncertainty for the measuring chain.
- All calibration steps have to be accomplished following generally accepted and described methods, results have to be documented.
- Laboratories or others who accomplish one or more steps in the measuring chain have to prove their technical competence.
- Calibrations should be repeated after reasonable periods of times. The length of this period depends on variables such as the required certainty, the frequency of use, the way of using it or the constancy of the device.

The peak positions found with the calibration standard is beyond tolerance - what is the problem?

Does the setting of the slit match with the specifications on the certificate? Other slit settings can cause errors in the peak maxima.

Do you supply neutral density glass filters with absorbance values different from those which are specified in the Hellma catalogue? (e.g. Abs = 0.8 instead of 1.0)?

The absorbance values of the neutral density glass filters do depend on their thickness, and so they can be available on customer request.

Where do the greyish shadows on the holmium oxide filter come from? Do they affect the measurement?

The holmium oxide glass is slightly hygroscopic, so the covering is a kind of water film. Measurements are not affected. The filter may be wiped with alcohol and a soft tissue to remove the film. As a basic principle the filters should be stored in a dry place.

How often have the calibration filters to be recalibrated?

To check the correctness of the values given on the certificate, the calibration standards should be recalibrated at regular intervals. The periodicity of these intervals should be determined by the user and depends on the laboratory environment and the conditions of use, just like the total lifetime of the filters.

To define a statistical basis for establishing the recertification interval it is recommended to have all calibration standards recalibrated every 12 months in the first two years of use. Afterwards an interval of recalibration should be chosen which seems to be suitable based on the values obtained this way.

standards please see pages 18 and 19.

4 Handling Instructions

The calibration standards are to be considered measuring equipment in accordance with international standards and should be recalibrated at regular intervals (see EN ISO 9001, section 7.6 “Control of Inspection and Measuring Equipment“).

The calibration standards must be treated with special care if they are to retain their validity. Scratches, dirt, and corrosion on the optical surfaces can easily introduce substantial errors.

Based on our experience we recommend a recalibration of the solid filters every 12 months for the first two years of use and after that every 24 months, recalibration of the liquid filters not later than every 12 months. The periodicity of these intervals should be determined by the user and depends on the laboratory environment and the conditions of use.

Filters or sets of filters which are sent to us to be recalibrated will be cleaned and calibrated. They will then be returned to you with a new certificate.

Extensive handling instructions are provided with each set of filters.

All filters can also be ordered separately, e.g. as replacement parts, by their catalogue number. The filters are sequentially numbered and come complete with a certificate.

Influence of temperature

The uncertainty of the certified measurement results is only valid for measuring temperatures as stated on the certificate. It is recommended to store the filters after use at room temperature in their storage case in a dry, dust-free environment.

For reasons of safety and especially with the cells containing liquids, take care not to expose them to temperatures below 0 °C or above 50 °C when storing and during transportation.

Other factors influencing the measurement

Dirt and dust as well as damage (scratches, blemishes) to the polished surfaces can considerably affect the measured values. Store the filters in the case and avoid any contamination of the polished windows. There should always be a recalibration associated with the cleaning of the standards.

Safety instructions

The filters containing liquids carry a label on one side with the chemical formula of the substances contained inside. If a filter breaks, please follow the corresponding safety instructions (e.g. safety data sheet).

Preparing for a measurement

Because of the variety of possible designs of spectrophotometers the application of a calibration standard for checking an instrument can only be given in general terms

The spectrophotometer is switched on about half an hour before starting the measurements in order to achieve a constant instrument temperature (please follow the manufacturers’ instructions). The measurement should be carried out in a room with low humidity and the temperature which is given on the filters certificate.

The filters fit into cell holders for standard cells with 10 mm light path. They should always be placed in the cell holder in the same orientation, i.e. with the Hellma logo pointing towards the light source. The centre of the light beam should pass through the filter always at the same height in the lower third. When using single-beam photometers and especially diode array photometers connected to a separate cell holder via a fibre-optic cable, you should also consider that any stray radiation or vibrations (moving the fibre-optic cables) may affect the measurements.

Current versions of the safety data sheets for any used dangerous goods are available to download on the internet: www.hellma-worldwide.com.

For an overview of all Hellma calibration

5 Compliance References

Material	Test	Range	Configuration	EP	DAB	USP	ASTM
Holmium Oxide (Solution)	Wavelength	UV/Vis	Liquid Filter	✓	✓	✓	✓
Holmium Oxide Glass	Wavelength	UV/Vis	Solid Filter			✓	✓
Didymium Glass	Wavelength	UV/Vis	Solid Filter			✓	✓
Didymium Glass	Photometric Accuracy	UV/Vis	Solid Filter				
Potassium Dichromate (Solution)	Photometric Accuracy	UV	Liquid Filter	✓	✓	✓	✓
Potassium Dichromate (Solution)	Photometric Accuracy	Vis	Liquid Filter	✓			
Neutral Density Glass	Photometric Accuracy	Vis	Solid Filters			✓	✓
Toluene in Hexane (Solution)	Spectral Resolution	UV	Liquid Filter	✓	✓		
Potassium Chloride (Solution)	Stray Light	UV	Liquid Filter	✓	✓		✓
Sodium Iodide (Solution)	Stray Light	UV	Liquid Filter				✓
Sodium Nitrite (Solution)	Stray Light	UV	Liquid Filter				✓

6 Combination of the Liquid Filter Sets

667.000-UV Complete set for checking the spectrophotometer according to European Pharmacopeia

- 667.100-UV Set for checking for stray light according to European Pharmacopeia
  - 667-UV1 Potassium chloride in ultra pure water
  - 667-UV12 Ultra pure water (blank)
- 667.200-UV Set for checking the spectral resolution according to European Pharmacopeia
  - 667-UV6 Toluene in hexane
  - 667-UV9 Hexane (blank)
- 667.300-UV Set for Checking the photometric accuracy according to European Pharmacopeia
  - 667-UV7 Potassium dichromate in sulphuric acid
  - 667-UV17 Potassium dichromate in sulphuric acid (checking at 430 nm)
  - 667-UV8 Sulphuric acid (blank)
- 667-UV5 Checking the wavelength accuracy according to European Pharmacopeia (Holmium oxide in perchloric acid)

667.101-UV Set for checking for stray light

- 667-UV10 Sodium Iodide in Ultra Pure Water
- 667-UV12 Ultra Pure Water (Blank)

667.102-UV Set for checking for stray light

- 667-UV11 Sodium nitrite in ultra pure water
- 667-UV12 Ultra pure water (blank)

667.301-UV Set for checking the photometric accuracy

- 667-UV13 Potassium dichromate in perchloric acid
- 667-UV14 Perchloric acid (blank)

667.400-UV Set for checking the wavelength accuracy

- 667-UV5 Holmium oxide in perchloric acid
- 667-UV14 Perchloric acid (blank)

*All filters and filter sets are also available individually

standards please see pages 18 and 19.

7.1 Liquid Filters Overview

Catalogue-No.	Use	Consisting of	Substance	Wavelength nm
667.000-UV	Complete set for checking the spectrophotometer according to Ph. Eur.	667.100-UV 667.200-UV 667.300-UV 667-UV5	Potassium chloride + blank Toluene in hexane + blank Potassium dichromate in H_2SO_4 + blank Holmium oxide in $HClO_4$	198, 200 (certified) 266, 269 235, 257, 313, 350, 430 241.15, 287.15, 361.5, 536.3

Available in particular sets or individually

667.100-UV	Set for checking for stray light according to Ph. Eur.	667-UV1 667-UV12	Potassium chloride Ultra pure water (blank)	200 (cut-off) 198, 200 (certified)
667.200-UV	Set for checking the spectral resolution according to Ph. Eur.	667-UV6 667-UV9	Toluene in hexane Hexane (blank)	266, 269
667.300-UV	Set for checking the photometric accuracy according to Ph. Eur.	667-UV7 667-UV17 667-UV8	Potassium dichromate in H_2SO_4 Potassium dichromate in H_2SO_4 (430 nm) Sulphuric acid (blank)	235, 257, 313, 350 430
667-UV5	Checking the wavelength accuracy according to Ph. Eur.	667-UV5	Holmium oxide in perchloric acid	241.15, 287.15, 361.5, 536.3
667.101-UV	Set for checking for stray light	667-UV10 667-UV12	Sodium iodide Ultra pure water (blank)	259 (cut-off) 220 (certified)
667.102-UV	Set for checking for stray light	667-UV11 667-UV12	Sodium nitrite Ultra pure water (blank)	385 (cut-off) 340, 370 (certified)
667.301-UV	Set for checking the photometric accuracy	667-UV13 667-UV14	Potassium dichromate in perchloric acid Perchloric acid (blank)	235, 257, 313, 350
667.400-UV	Set for checking the wavelength accuracy	667-UV5 667-UV14	Holmium oxide in perchloric acid Perchloric acid (blank)	241.15, 287.15, 361.5, 536.3

7.2 Solid Filters Overview

Catalogue-No.	Use	Consisting of	Material	Wavelength nm
666.000	Complete set for checking the wavelength accuracy and the photometric accuracy	666-F1 666-F2 666-F3 666-F4 666-F0	Holmium oxide glass filter Neutral density glass filter NG 11 Neutral density glass filter NG 5 Neutral density glass filter NG 4 Empty filter mount	279, 361, 454, 536, 638 440, 465, 546.1, 590, 635 440, 465, 546.1, 590, 635 440, 465, 546.1, 590, 635

Available individually

666-F1	Checking the wavelength accuracy	Holmium oxide glass filter	279, 361, 454, 536, 638
666-F2	Checking the photometric accuracy	Neutral density glass filter NG 11	440, 465, 546.1, 590, 635
666-F3	Checking the photometric accuracy	Neutral density glass filter NG 5	440, 465, 546.1, 590, 635
666-F4	Checking the photometric accuracy	Neutral density glass filter NG 4	440, 465, 546.1, 590, 635
666-F7	Checking the photometric accuracy	Didymium glass filter BG 36	270, 280, 300, 320, 340
666-F7	Checking the wavelength accuracy	Didymium glass filter BG 36	329, 472, 512, 681, 875
666-F0	Empty filter mount		

Do You Need More Information?

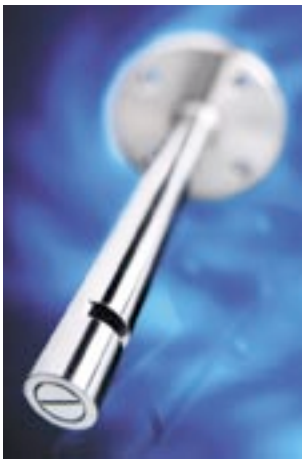
If so, just order our general catalogue or product brochures and fact sheets by using the enclosed fax information sheet.

If you have questions about anything, please ask us. Our specialists will gladly take the time to discuss and answer your questions you might have.

Your unsolved problems are our challenge!

If you have measuring projects and Hellma‘s standard product range has not yet been able to offer you the right solution: talk to us. In collaboration with our customers we will develop individual and tailor made solutions.

Visit us on the internet: www.hellma-worldwide.com. Up-to-date information about our company and about Hellma‘s products can be found on the web.

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