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## Calibration standards for spectrophotometers



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## Calibration of spectrophotometers

**UV/Vis-spectrophotometry is one of the most commonly used techniques of instrumental analysis. For example, spectrophotometry is used in clinical chemistry, pharmaceutical industry, research and very often in quality assurance.**

Regulations for quality control such as ISO 9000, Good Laboratory Practice (GLP), Good Manufacturing Practice

(GMP) or standard operating procedures in the pharmaceutical industry as required by the most important pharmacopeias (EP, DAB, USP), require a regular performance testing of the UV/Vis spectrophotometers used. These checks include the testing of the resolution, the wavelength accuracy as well as the testing of stray light and photometric accuracy.

### Liquid filter

With the Hellma liquid filters presented here for testing spectrophotometers it is possible to check the following in the wavelength region of 198 - 650 nm:

- Wavelength accuracy
- Photometric accuracy
- Stray light behaviour
- Resolution

### Solid filter

With the solid filters for testing spectrophotometers, the following parameters can be checked:

- Photometric accuracy  
in the visible spectral region
- Wavelength accuracy

The Hellma calibration standards and their compliance with the most important regulations

Material	Test	Region	Filter type	EP	DAB	USP	ASTM	Page
Holmium oxide (solution)	Wavelength	UV/Vis	Liquid filter	X	X	X	X	6
Holmium oxide glass	Wavelength	UV/Vis	Solid filter			X	X	7
Didymium glass	Wavelength	UV/Vis	Solid filter			X	X	8
Didymium glass	Photometric Accuracy	UV	Solid filter					13
Potassium dichromate (solution)	Photometric Accuracy	UV	Liquid filter	X	X	X	X	10
Neutral density glass	Photometric Accuracy	Vis	Solid filter			X	X	12
Toluene in Hexane (solution)	Resolution	UV	Liquid filter	X	X			17
Potassium chloride (solution)	Stray light	UV	Liquid filter	X	X		X	15
Sodium iodide (solution)	Stray light	UV	Liquid filter				X	15
Sodium nitrite (solution)	Stray light	UV	Liquid filter				X	15



## Traceability of the calibration and conformity to the regulations

The creation of the Hellma Calibration Standards is carried out in accordance with the regulations of the NIST (National Institute of Standards and Technology, USA), ASTM (American Society for Testing and Materials) and the European Pharmacopeia.

Liquid filters are filled under controlled conditions and permanently sealed. Solid filters are carefully cleaned and built into the holder in a stress-free manner. The manufacturing process is completed with the individual certification of the filters.

Hellma calibration standards are calibrated using a high-performance UV/Vis/NIR spectrophotometer. This instrument is used exclusively for calibrating purposes and is tested at regular intervals for its accuracy.

For testing our spectrophotometer, use is made, amongst others, of primary calibration standards of the NIST to which the measuring results can be referenced:

### Photometric accuracy

SRM 930e and SRM 1930 neutral density glass filter

### Wavelength accuracy

SRM 2034 Holmium oxide solution

The wavelength accuracy of the instrument is maintained by the utilisation of the emission lines of deuterium and mercury lamps. The linearity of the instrument is ensured by the double aperture method.



### Zertifikat der Kalibrierung Certificate of Calibration

Sekundär-Standards  
zur Kalibrierung von Spektralphotometern  
Kalibrierung der Ordinatenwerte

Messwerte der Kalibrierung der Neutralgläser als Sekundär-Standards

Secondary Spectrometric  
Calibration Standards  
Ordinate Calibration

Calibration data for neutral density glass secondary calibration standards:

Standard	Ident-Nr.	Ordinatenwerte (Absorption) bei den folgenden Wellenlängen / Ordinate reading (absorbance) at the following wavelengths					
		440.0 nm	458.0 nm	546.1 nm	580.0 nm	636.0 nm	
666-F2	1656	0.273	0.239	0.237	0.252	0.252	± 0.002
666-F3	1656	0.504	0.463	0.483	0.521	0.509	± 0.004
666-F4	1656	1.054	0.971	0.985	1.026	0.979	± 0.007

Die den Messwerten beigeordnete erweiterte Messunsicherheit ergibt sich aus der Standardmessunsicherheit durch Multiplikation mit dem Erweiterungsfaktor  $k = 2$ . Der Wert der Messgröße liegt mit einer Wahrscheinlichkeit von 95 % im zugeordneten Wertebereich.

The expanded uncertainty assigned to the measurement results is obtained by multiplying the standard uncertainty by the coverage factor  $k = 2$ . The value of the measurand lies within the assigned range of values with a probability of 95 %.

Datum der Kalibrierung:  
Neutralgläserfilter  
Messbedingungen bei der Kalibrierung der  
Filter

Für die in diesem Zertifikat angegebenen Werte wurden bei dem verwendeten Spektralphotometer folgende Einstellungen gewählt:  
Modus der Ordinatenwerte: Absorption  
Spaltbreite: 1 nm  
Integrationszeit: 3 s  
Es wurde das Wellenlängen-Wahl-Programm des Gerätes für die Messung der Absorptionswerte bei den oben angegebenen Wellenlängen verwendet.

Für die Kalibrierung dieser  
Standards

Conditions of Calibration of the neutral density glass filters  
The following settings were used on the spectrophotometer employed to obtain the calibration data quoted on this certificate:  
Ordinate mode: Abs  
Slit: 1 nm  
Integration time: 3 s

The

## Calibration standards for testing the wavelength accuracy

**Ideally, a standard for determining the wavelength accuracy makes use of small well-defined peaks of several wavelengths in the UV and visible spectra.**

In comparison to holmium oxide-liquid filters, the holmium oxide glass filter has a somewhat weaker defined spectrum. Especially in the deep UV region, the holmium peaks are superimposed by the absorption edge of the host glasses. A similar effect can be observed with the Didymium glass filter.

It should be noted that with all standards for testing the wavelength accuracy a change of the slit width of the photometer can lead to a slight displacement of the absorption maximum.

Because of the small bandwidth of the peaks the height of the measured maximum varies strongly with a change of the slit width. Therefore standards for testing the wavelength accuracy can generally not be used for testing the photometric accuracy.

In principle, the calibration standards for testing the wavelength accuracy can also be measured for slit widths that deviate from the certificate.

At larger slit widths it is possible that weaker peaks cannot be detected anymore.

**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  $\pm 1$  nm for the ultraviolet range and  $\pm 3$  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.





## Wavelength accuracy

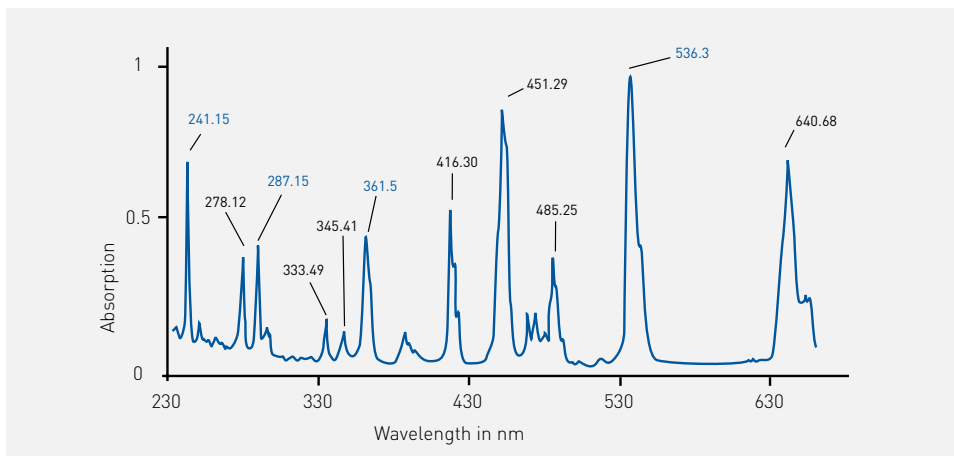
### Holmium oxide liquid filter

#### 667-UV5 holmium oxide dissolved in perchloric acid



The **667-UV5** filter holmium oxide dissolved in perchloric acid is well suited for testing the wavelength accuracy of photometers both in the UV- and Vis-range. There holmium oxide shows a

spectrum with many characteristic peaks. In their position and height the measured maxima depend on the spectral bandwidth and the selected slit width and vary accordingly.



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

The filter with the holmium perchlorate solution is placed in the measuring beam. The measurement is carried out with a small slit width (e.g. 1 nm). Optimally the average of several measurements is calculated in order to avoid errors in measurements. The exact position of peaks

at 241.15 nm, 287.15 nm, 361.5 nm and 536.3 nm is determined and compared with the values in the certificate. Deviations should not be greater than  $\pm 1$  nm in the ultra-violet and  $\pm 3$  nm in the visible range. If available the reference filter 667-UV14 liquid filter with perchloric acid can be

placed in the reference beam in order to better suppress the background. Ideally measurements should be carried out with the slit width shown in the certificate. If this cannot be done, then at least the smallest possible slit width should be used for measuring.

Catalogue number	667-UV5
Description	Holmium perchlorate filter, liquid filter certified
Use	Testing the wavelength accuracy in the UV and visible range between 240 nm and 650 nm for spectrophotometers with a spectral bandwidth (slit) between 0.1 nm and 3 nm
Material	Solution of holmium oxide in perchloric acid Filled into Hellma precision SUPASIL® quartz glass cuvettes
Theoretical Wavelength according to Pharm. Eur.	241.15 nm 287.15 nm 361.5 nm 536.3 nm

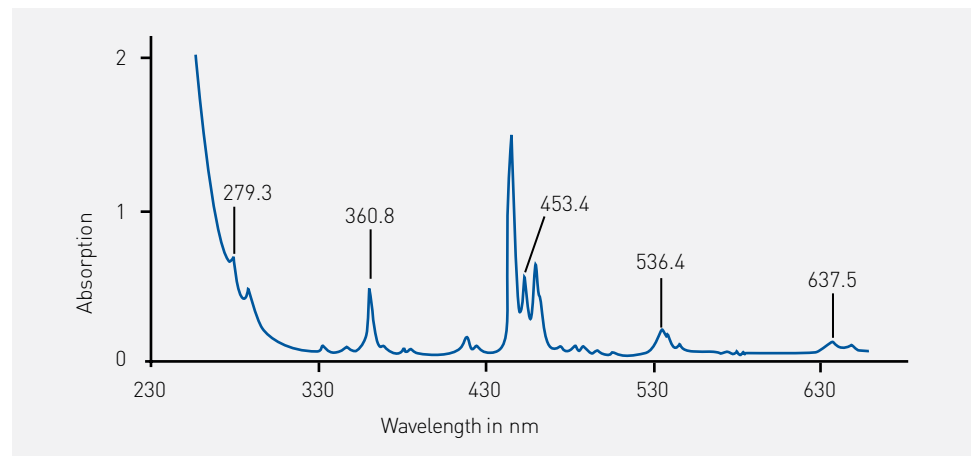


## Holmium oxide glass filter

### 666-F1 Holmium oxide doped glass



The light yellow filter marked F1 is a glass doped with holmium oxide ( $\text{Ho}_2\text{O}_3$ ). The chemical element holmium shows a series of small absorption peaks in UV and Vis range.



Typical spectrum of a holmium oxide glass filter

Catalogue number	666-F1
Description	Holmium oxide glass filter, solid filter certified
Use	Testing the wavelength accuracy in the UV and Vis spectrum (279 nm to 638 nm) at spectral bandwidths up to 2 nm
Material	Holmium oxide doped glass
Filter thickness	2.1 mm
Wavelength at	279.3 nm 360.8 nm 453.4 nm 536.4 nm 637.5 nm



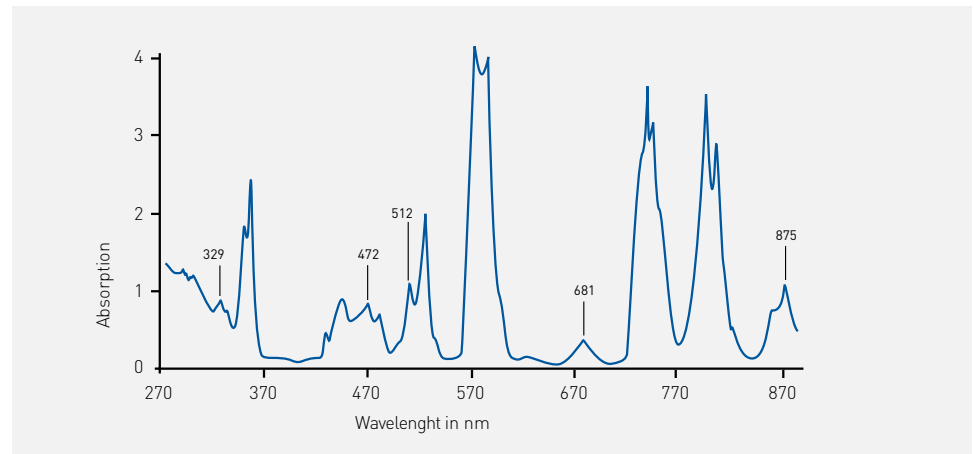
## Wavelength accuracy

### Didymium glass filter

666-F7 Neodymium oxide and Praseodymium oxide doped glass



The Didymium glass filter marked with F7 made of Schott BG 36 material can be used for testing the wavelength accuracy in the UV and Vis spectrum.



Typical spectrum of a Didymium glass filter

Didymium glass shows a spectrum with a number of characteristic peaks. The measured maxima depend on their position and height of the spectral width and the selected slit width and vary correspondingly.

In addition the use of the filter as absorption standard is possible at wavelengths of 270 nm, 280 nm, 300 nm, 320 nm and 340 nm.

Catalogue number	666-F7
Description	Didymium glass filter, solid filter certified
Use	Testing the wavelength accuracy in the UV and Vis region (329 nm to 875 nm) at a spectral bandwidth to 2 nm
Material	Glass BG 36 of the SCHOTT Glaswerke
Filter thickness	Approx. 1.2 mm
Wavelength at	329 nm 472 nm 512 nm 681 nm 875 nm

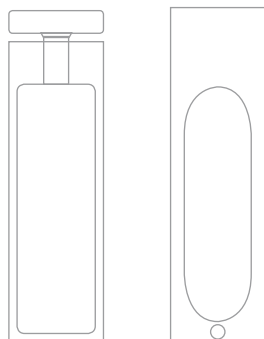




## Calibration standards for testing the photometric accuracy

**Various standards are available for determining the photometric accuracy:**

Neutral density glass filters are suitable for the visible spectrum; in the UV region, the testing with Potassium-dichromate is a proven method. Typical standards have broad peaks and broad valleys.





## Photometric accuracy

### Potassium dichromate liquid filter

667-UV60 0.006 % Potassium dichromate dissolved in perchloric acid

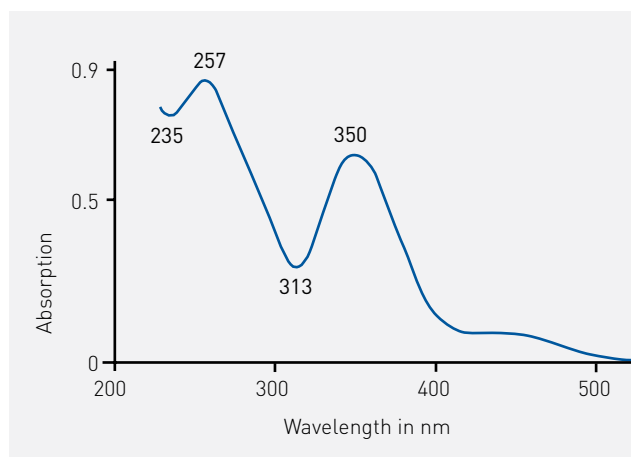


667-UV60 Potassium dichromate dissolved in perchloric acid is suitable for testing the photometric accuracy (absorbance) of spectrophotometers in the UV spectrum. Here, potassium dichromate shows a spectrum with characteristic peak maxima at 257 nm and 350 nm, minima at 235 nm and

313 nm. The potassium dichromate solution is kept in an airtight precision cuvette with a 10 mm light path. The absorption values of the cuvette are measured at the wavelengths 235 nm, 257 nm, 313 nm and 350 nm and recorded in the certificate.

With the measuring values care must be taken that the measurements are carried out with filter 667-UV14 (0.001 N perchloric acid) in the reference beam. The measuring values of the reference filter are shown separately in the certificate.

The individually measured absorption values are free of systematic errors when preparing the solution and in the light path. As the minimum and maximum in the absorption spectra are relatively broad they can be measured with a correspondingly large slit width, e.g. 2 nm. In this way, the influence of noise on the measuring-values can be kept small.



Typical spectrum of a 0.006 % potassium dichromate solution

Catalogue number	667-UV60
Description	Potassium dichromate filter, liquid filter certified
Use	Testing the absorption behaviour in the UV spectrum (235 nm to 350 nm) at a spectral bandwidth of 2 nm or less
Material	0.006 % Potassium dichromate dissolved in perchloric acid Filled into Hellma precision SUPRASIL® quartz glass cuvettes
Wavelength	235 nm, 257 nm, 313 nm, 350 nm

## Potassium dichromate liquid filter (430 nm)

667-UV600 0.06% Potassium dichromate dissolved in perchloric acid

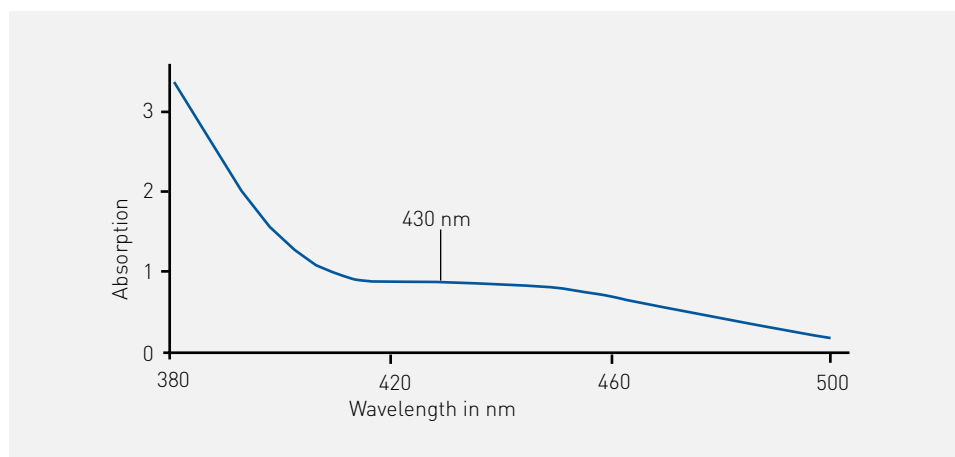


The European Pharmacopeia requires, besides the testing of the photometric accuracy in the UV spectrum, also a testing at a wavelength in the VIS spectrum (430 nm) with the aid of a 0.06 % solution of Potassium dichro-

mate in perchloric acid.

The absorption value of this solution at 430 nm is determined and recorded in the certificate. For this measuring value, care must also be taken that the measurement is carried out with

filter 667-UV14 (0.001 N perchloric acid) in the reference beam. The measuring value of the reference filter is shown separately in the certificate.



Typical spectrum of a 0.06 % potassium dichromate solution

Catalogue number	667-UV600
Description	Potassium dichromate filter, liquid filter certified
Use	Testing the absorption behaviour in the Vis spectrum (measuring wavelength 430 nm) at a spectral bandwidth of 2 nm or less
Material	0.06 % Potassium dichromate dissolved in perchloric acid filled into Hellma precision SUPASIL <sup>®</sup> quartz glass cuvettes.
Wavelength	430 nm



## Photometric accuracy

### Neutral density glass filter

666-F2, 666-F3, 666-F4



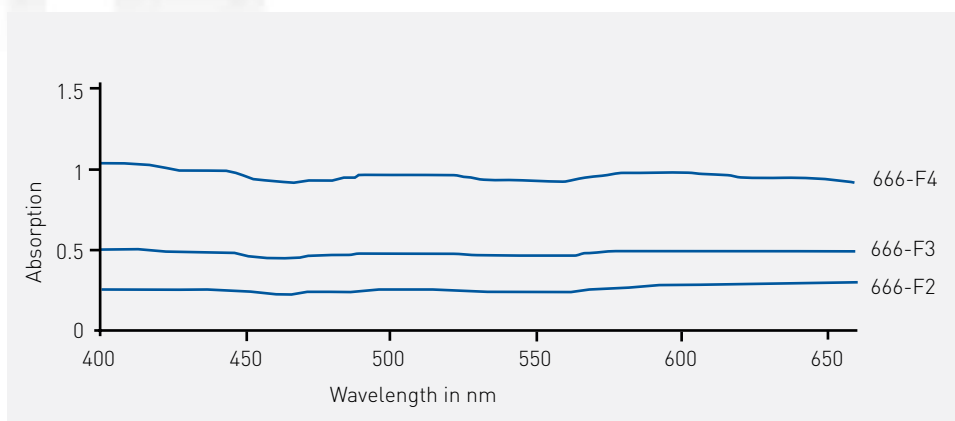
For decades SCHOTT neutral density glass filters have been used in the Vis spectrum for testing the photometric accuracy and linearity.

They provide a relatively constant transmission over the visible wavelength spectrum and are calibrated at the following wavelengths: 440.0 nm, 465.0 nm, 546.1 nm, 590.0 nm, 635.0 nm

If desired, other wavelengths are also possible.

The thickness of the filters is determined such that there is a stepping of the absorption values from approx. 0.25 A, 0.5 A and 1 A.

Also other thicknesses are available on request.



Typical spectrum of the neutral density glass filter, taken with a slit width of 1 nm

Catalogue number	666-F2 666-F3 666-F4
Description	Neutral density glass filter, solid filter certified
Use	Testing the absorption behaviour in the visible spectrum (440 nm to 635 nm)
Material	Filter materials NG 11, NG 5, NG 4 from SCHOTT Glaswerke

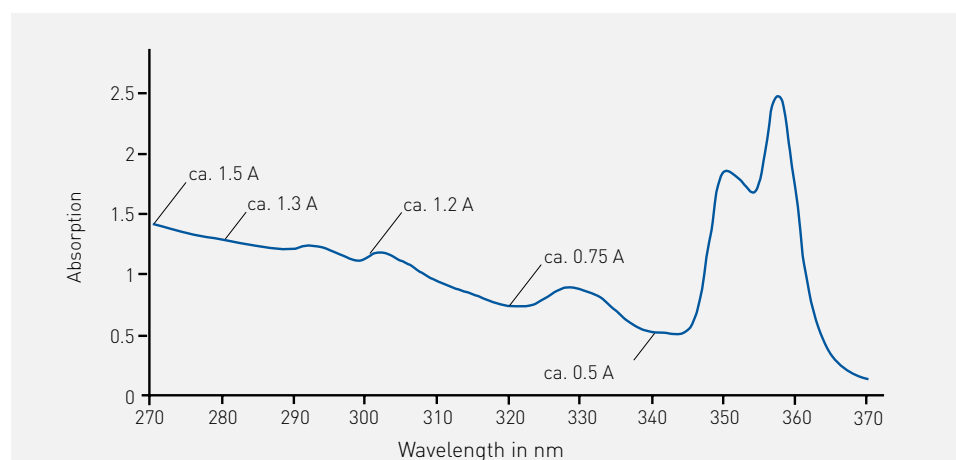
## Didymium glass filter

666-F7



The Didymium glass filter marked F7 is usually a standard for testing the wavelength accuracy in the UV/Vis spectrum.

It can also be used for testing the photometric accuracy.



Typical spectrum of a Didymium glass filter between 270 nm and 370 nm

The testing of the absorption behaviour in the UV spectrum is possible for wavelengths of 270 nm, 280 nm, 300 nm, 320 nm and 340 nm. As standard the thickness of the filter is set during production

such that at 340 nm there is an absorption of approximately 0.5 A. With reductions in the wavelength the absorption values increase up to approx. 1.5 A at 270 nm.

Catalogue number	666-F7
Description	Didymium glass filter, solid filter certified
Use	Testing the photometric accuracy in the UV spectrum (270 to 340 nm)
Material	Glass BG 36 from SCHOTT Glaswerke
Designation	Didymium glass filter, certified
Filter thickness	Approx. 1.2 mm
Nominal value of the absorption	Depending on wavelength
Absorption value at	270 nm 280 nm 300 nm 320 nm 340 nm



## Stray light

### Calibration standards for testing stray light

**Stray light (false light) is light that is detected but does not belong to the selected measuring wavelength. Causes of this defect are light scattering, diffraction or a poor adjustment of the measuring instrument.**

**Stray light** is problematic as it reduces the region of the measurable absorption and degrades the linear relationship of concentration and absorption.

**Stray light** can become a problem at any wavelength but the smaller the energy throughput, e.g. when measuring in the UV range, the more the stray light influences the measurement value.

If it is desired to test the instrument for stray light, then filters are required that have a very sharply limited spectrum (so called cut-off filters). Below a fixed wavelength (cut-off wavelength) Hellma stray light-filters do not transmit. The transmission values in this wavelength region are thus due to stray light.



**In the European Pharmacopeia the testing for stray light is described as follows:**

"The stray light can be measured at a defined wavelength with suitable filters or solutions:

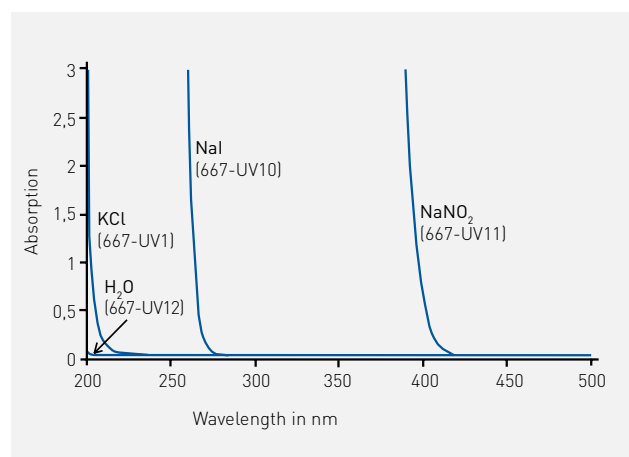
For example, the absorption of a solution of potassium chloride R (12 g/l) between 220 nm and 200 nm in a light path of 1 cm must rise steeply and at a wavelength of 198 nm must be greater than 2, measured against water R as a compensation liquid."

In order to realistically estimate the amount of stray light, a filter must be selected whose cut-off wavelength is as near as possible above the required wavelength. The following table shows the offered solutions for stray light measurements for the cut-off wavelengths 200 nm, 259 nm and 385 nm or below.

Catalogue Number	Content	Cut-Off Wavelength	Certified Wavelengths
667.100-UV	667-UV1 Aqueous potassium chloride solution	200 nm	198 nm, 200 nm
	667-UV12 Purified water (reference)		
667.101-UV	667-UV10 Aqueous sodium iodide solution	259 nm	220 nm
	667-UV12 Purified water (reference)		
667.102-UV	667-UV11 Aqueous sodium nitrite solution	385 nm	340 nm, 370 nm
	667-UV12 Purified water (reference)		
667-UV12	667-UV12 Purified water filter		198 nm, 200 nm, 300 nm, 400 nm



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



These calibration standards show a very high absorption below defined wavelengths (cut-off-wavelengths) in the UV spectrum. In this way the stray radiation can be tested. The testing for stray light is carried out with a reference standard of purified water that is provided with every calibration filter set. The procedure is the same for all stray light filters.

### Application

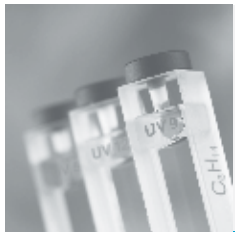
The photometer is set to a starting wavelength that is 30 nm above the cut-off wavelength of the filter to be used. If, for example, potassium chloride is used then the start is at 230 nm. Then a scan is carried out down towards shorter wavelengths and the absorption below the cut-off wavelength is observed. The transmission that is measured below the cut-off wavelength is stray light.

### Testing the maximum range of the photometer

The filter **667-UV12** is filled with purified water, whose absorption is defined above 200 nm to the NIR practically only by the reflection losses at the two glass/air transitions. With the certified values at 198 nm, 200 nm, 300 nm and 400 nm it is possible to test the maximum range of the instrument for very low absorption values. For clear deviations from the certified values, es-

pecially when the measured values are smaller than 0.02 A, contact should be made with the customer service of the instrument manufacturer.

Catalogue number	667-UV1 667-UV10 667-UV11
Description	Liquid filter with sharp cut-offs of transmission at certain wavelength, certified
Use	Testing for stray light in the UV spectrum (at wavelengths from 198 nm to 385 nm, depending on the selected filter)
Material	Aqueous solutions in Hellma precision SUPRASIL <sup>®</sup> quartz glass cuvettes
Reference filter	667-UV12 purified water filter



## Resolution

### Calibration standards for testing the 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.**

The resolution for modern photometers depends directly on the slit width. The smaller the slit and the associated spectral bandwidth, the higher the resolution.

For wavelengths and absorption measurements, therefore, the resolution of the monochromator must be taken into account. However, when a small spectral bandwidth leads to a reduction in the detectable measured light, then the signal-noise relationship reduces.

For high resolution photometers, the spectral bandwidth should not be more than 10 % of the natural bandwidth of the peak to be investigated.

The regular testing of the photometer for resolution ensures that, for instance, neighbouring peaks can be resolved and do not merge with the peaks of the neighbouring wavelength. In addition the absorption errors will be prevented.



## Toluene liquid filter

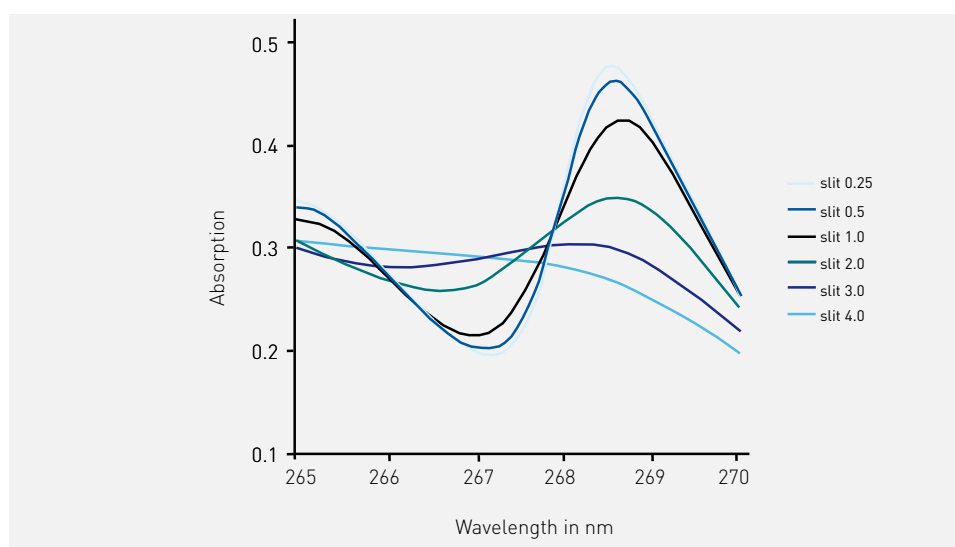
### 667-UV6 Toluene in n-Hexane



In order to determine the resolution of a spectrophotometer, the behaviour of the absorption of a maximum at  $\lambda_{\text{max}}$  (269 nm) to absorption of a minimum at  $\lambda_{\text{min}}$  (266 nm) is shown. From this, one obtains details of the spectral bandwidth of the instrument used.

Measurements of the resolution are carried out using hexane **reference filter 667-UV9** which is provided with every filter set. The execution of the measurement is the same for all bandwidths. The wavelength spectrum from 265 nm to 270 nm is scanned and the

peak maximum and peak minimum are determined. Then the relationship between the two is calculated.



Typical spectra of the Toluene liquid filters with varying slit widths

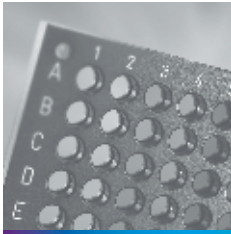
Slit width in nm	Ratio (Recommended values)
0.25	2.3
0.5	2.2
1.0	2.0
2.0	1.4
3.0	1.1
4.0	1.0

For determining the resolution, the spectra of the given slit widths are measured, the ratios are calculated and the values are compared with the certificate. If the ratios deviate mar-

kedly downwards (e.g. 15 %) then contact should be made with the manufacturer of the instrument. However, it must be remembered that the results are also dependent on the measurement

conditions. Thus, especially for small slit widths, a sufficiently large integration time must be selected.

Catalogue number	667.200-UV
Description	Toluene liquid filter, certified
Use	Testing the resolution of spectral bandwidths
Material	Solution of toluene in hexane, filled into Hellma precision cuvettes of SUPRASIL <sup>®</sup> quartz glass
Liquid filter set	667-UV6 Toluene in n-hexane 667-UV9 Hexane (reference standard)
Wavelength	667-UV6 266 nm, 269 nm



# Calibration standards for microplate readers

## 666.013 Calibration standards for microplate readers

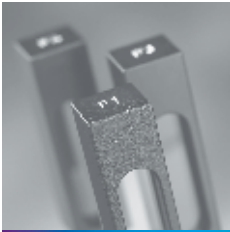
With the calibration standard 666.013 the photometric accuracy of microplate readers can be tested. It has the dimensions of a microplate, whose 96 wells, have diameters of 6.6 mm.

Neutral density glass strips are located behind each 16 windows having varying absorption values (see table). Each calibration standard is provided with a certificate of the calibration.

For determining the wavelength accuracy of microplate readers, standard calibration plates are available in which the neutral density glass strips are replaced by holmium oxide glass strips or other glass (on request).



Catalogue number	666.013
Designation	Calibration standard for microplate readers NG 11, NG 5, NG 4, NG 3 certified
Nominal value of the absorption	0.25 0.5 1.0 1.5 2.5
Absorption values at	405 nm 450 nm 490 nm 650 nm



# Solid filters

## 666.000 Calibration filter set

The set 666.000 consists of neutral density glass filters for testing the absorption values and a holmium oxide glass filter for testing the wavelength accuracy. The glass filters are mounted in precision frames of aluminium. They are designed such that they fit into the holders for standard cuvettes with a 10 mm light path with which the spectrophotometer is equipped. The set is delivered together with an empty filter frame as reference in a sturdy box.

For purposes of identification, the set number is engraved on each filter frame. The measured values of the absorption and the wavelengths are shown for each filter in the calibration certificate supplied. For purposes of working in the laboratory, the measurement values are also shown on the inside of the lid of the box.



Catalogue number	666.000
Designation	Solid filter set for testing the photometric accuracy and the wavelength accuracy
Consisting of	666-F1 Holmium oxide glass filter 666-F2 Neutral density glass filter NG 11 666-F3 Neutral density glass filter NG 5 666-F4 Neutral density glass filter NG 4 666-F0 Filter holder, empty as reference
Peakposition	666-F1 279.3 nm, 360.8 nm, 453.4 nm, 536.4 nm, 637.5 nm
Absorption value certified at	666-F2 440 nm, 465 nm, 546.1 nm, 590 nm, 635 nm 666-F3 440 nm, 465 nm, 546.1 nm, 590 nm, 635 nm 666-F4 440 nm, 465 nm, 546.1 nm, 590 nm, 635 nm

All sets are also available individually!



## Complete sets

### Liquid filters

#### 667.003-UV calibration filter set

The set 667.003-UV consists of a total of eight liquid filters and permits the complete testing of the photometers, as required of the European Pharmacopeia. All liquid filters consist of calibration solutions that are filled into Hellma SUPRASIL® precision

quartz cuvettes. The cuvettes are permanently sealed. The set is supplied in a sturdy box. For purposes of identification, a number is engraved on each filter. The measured values are shown for each filter in the calibration certificate.



Catalogue number	667.003-UV
Designation	Liquid filter set for testing photometers according to Ph. Eur.
Consisting of	667.100-UV Potassium chloride and reference filter 667.200-UV Toluene in hexane and reference filter 607.305-UV Potassium dichromate in perchloric acid and reference filter 667-UV5 Holmium oxide in perchloric acid
Wavelengths at which measurements are taken	667.100-UV 198 nm, 200 nm (certified) 667.200-UV 266 nm, 269 nm 607.305-UV 235 nm, 257 nm, 313 nm, 350 nm, 430 nm 667-UV5 241.15 nm, 287.15 nm, 361.5 nm, 536,3 nm

All filters are also available individually!



## Liquid filters

### Make up of the sets

Catalogue number	Purpose	Contents
667.003-UV Complete set for testing photometers according to the European Pharmacopeia	667.100-UV Set for testing for stray light according to the European Pharmacopeia	667-UV1 Potassium chloride in purified water
		667-UV12 Purified water reference filter
	667.200-UV Set for testing the resolution according to the European Pharmacopeia	667-UV6 Toluene in n-hexane
		667-UV9 n-hexane reference filter
	667.305-UV Set for testing the photometric accuracy according to the European Pharmacopeia	667-UV60 Potassium dichromate in perchloric acid
		667-UV600 Potassium dichromate in perchloric acid (testing at 430 nm)
		667-UV14 Perchloric acid reference filter
	667-UV5 Filter for testing the wavelength accuracy according to the European Pharmacopeia	667-UV5 Holmium oxide in perchloric acid
	667.101-UV Set for testing for stray light	667-UV10 Sodium iodide in purified water
		667-UV12 Purified water reference filter
	667.102-UV Set for testing for stray light	667-UV11 Sodium nitrite in purified water
		667-UV12 Purified water reference filter
	667.307-UV Linearity filter set for testing the absorption and linearity	667-UV20 0.002 % Potassium dichromate in perchloric acid
		667-UV40 0.004 % Potassium dichromate in perchloric acid
		667-UV60 0.006 % Potassium dichromate in perchloric acid
		667-UV80 0.008 % Potassium dichromate in perchloric acid
		667-UV100 0.01 % Potassium dichromate in perchloric acid
		667-UV14 Perchloric acid reference filter
	667.400-UV Set for testing the wavelength accuracy	667-UV5 Holmium oxide in perchloric acid
		667-UV14 Perchloric acid reference filter

All filters and filter sets are also available individually.



## Product overview

### Liquid filters

#### Overview

Catalogue Number	Use	consisting of	Content	Wavelength
667.003-UV	Complete set for testing photometers according to Ph. Eur.	667.100-UV	Potassium chloride and reference filter	198 nm; 200 nm (certified)
		667.200-UV	Toluene in hexane and reference filter	266 nm; 269 nm
		667.305-UV	Potassium dichromate in HClO <sub>4</sub> and reference filter	235 nm; 257 nm; 313 nm; 350 nm; 430 nm
		667-UV5	Holmium oxide in perchloric acid	241.15 nm; 287.15 nm; 361.5 nm; 536.3 nm
667.100-UV	Set for testing to stray light according to Ph. Eur.	667-UV1	Potassium chloride	200 nm (cut-off)
		667-UV12	Purified water (reference filter)	198 nm; 200 nm (certified)
667.200-UV	Set for testing the resolution according to Ph. Eur.	667-UV6	Toluene in hexane	266 nm; 269 nm
		667-UV9	Hexane (reference filter)	
667.305-UV	Set for testing the photometric accuracy according to Ph. Eur.	667-UV60	Potassium dichromate in HClO <sub>4</sub>	235 nm; 257 nm; 313 nm; 350 nm
		667-UV600	Potassium dichromate in HClO <sub>4</sub> (430 nm)	430 nm
		667-UV14	Perchloric acid (reference filter)	
667.307-UV	Filter set for testing the absorption and linearity	667-UV20	0.002 % Potassium dichromate in perchloric acid	235 nm; 257 nm; 313 nm; 350 nm
		667-UV40	0.004 %	
		667-UV60	0.006 %	
		667-UV80	0.008 %	
		667-UV100	0.01 %	
		667-UV14	Perchloric acid reference filter	
667-UV5	Testing the wavelength accuracy according to Ph. Eur.	667-UV5	Holmium oxide in perchloric acid	241.15 nm; 287.15 nm; 361.5 nm; 536.3 nm
667.101-UV	Set for testing for stray light	667-UV10	Sodium iodide	259 nm (cut-off)
		667-UV12	Purified water (reference filter)	220 nm (certified)
667.102-UV	Set for testing for stray light	667-UV11	Sodium nitrite	385 nm (cut-off)
		667-UV12	Purified water (reference filter)	340 nm; 370 nm (certified)
667.400-UV	Set for testing the wavelength accuracy	667-UV5	Holmium oxide in perchloric acid	241.15 nm; 287.15 nm; 361.5 nm; 536.3 nm
		667-UV14	Perchloric acid (reference filter)	

All filters and filter sets are also available individually.



## Solid filters

### Overview

Catalogue number	Use	consisting of	Contents	Wavelength
666.000	Complete set for testing the photometric accuracy and the wavelength accuracy	666-F1	Holmium oxide glass filter	271.3 nm; 360.8 nm; 453.4 nm; 536.4 nm; 637.5 nm
		666-F2	Neutral density glass filter NG 11	440 nm; 465 nm; 546.1 nm; 590 nm; 635 nm
		666-F3	Neutral density glass filter NG 5	440 nm; 465 nm; 546.1 nm; 590 nm; 635 nm
		666-F4	Neutral density glass filter NG 4	440 nm; 465 nm; 546.1 nm; 590 nm; 635 nm
		666-F0	Filter holder, empty as reference	

### Individually available

666-F1	Testing the wavelength accuracy	Holmium oxide glass filter	271.3 nm; 360.8 nm; 453.4 nm; 536.4 nm; 637.5 nm
666-F2	Testing the photometric accuracy (Nominal value of the absorption 0.25 A)	Neutral density glass filter NG 11	440 nm; 465 nm; 546.1 nm; 590 nm; 635 nm
666-F3	Testing the photometric accuracy (Nominal value of the absorption 0.5 A)	Neutral density glass filter NG 5	440 nm; 465 nm; 546.1 nm; 590 nm; 635 nm
666-F4	Testing the photometric accuracy (Nominal value of the absorption 1 A)	Neutral density glass filter NG 4	440 nm; 465 nm; 546.1 nm; 590 nm; 635 nm
666-F7	Testing the photometric accuracy	Didymium glass filter BG 36	270 nm; 280 nm; 300 nm; 320 nm; 340 nm
666-F7	Testing the wavelength accuracy	Didymium glass filter BG 36	329 nm; 472 nm; 512 nm; 681 nm; 875 nm
666-F0	Filter holder, empty as reference		



## FAQ

### Frequently asked question

#### 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 to 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 re-certification 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.

## Handling

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.

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.

**Usually customer choose 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.

### 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](http://www.hellma-worldwide.com)**

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