Hellma Analytics
High Precision in Spectro-Optics

// EASY HANDLING
// RELIABLE MEASUREMENT RESULTS
// INTERNATIONAL COMPARABILITY

Accredited accordance to DIN EN ISO 17025

DAkkS
Deutsche Akkreditierungsstelle
D-K-18752-01-00

NIST TRACEABLE

Certified UV / Vis Reference Materials

GUIDELINES

// GLASS FILTERS
// LIQUID FILTERS
// CERTIFIED REFERENCE PLATES

Made in Germany
Certified Quality

CONTENTS

1. INTRODUCTION

Dear Readers,

Although checking measuring equipment to ensure that results are accurate has long been common practice for analytical balances, it still tends to take something of a backseat where spectrophotometers are concerned. Spectrophotometers are important instruments that play a major role in health care, the life sciences, environmental analysis and processes such as production control and ensuring product quality. Over the last two years, many laboratories have become considerably more aware of the need to check their spectrophotometers, making it all the more important to know that these precision tools are also subject to mandatory checks under DIN EN ISO 9001. The standard clearly stipulates that measuring equipment be calibrated or verified, either at regular intervals or before use, using measurement standards that can be traced back to international or national standards. For an overview of the measurement standards for UV-Vis spectrophotometers, please refer to our reference materials in this brochure. An increasing number of laboratories are turning to this easy method for ensuring high standards of work – not only to satisfy requirements in time for their next audit, but also to be safe in the knowledge that they are taking accurate measurements and thus basing their actions and responses on correct results. We are delighted that our products are helping to achieve this. Details of our product range, as well as usage guidelines, helpful tips, and recommendations, are all included in this Handbook. **WE VERY MUCH HOPE THAT YOU ENJOY READING IT!**

Birgit Kehl, Head of Hellma Analytics calibration laboratory

ACCREDITATION stands for trust – and trust is built on reliability
(in Latin accredere means giving credibility to something)

1.1 Hellma Analytics calibration laboratory: accredited to DIN EN ISO 17025

Our lab is a DAkkS calibration laboratory and is accredited to DIN EN ISO 17025, a comprehensive quality management system that acts as a seamless continuation of other systems such as ISO 9000. By achieving this accreditation, we have demonstrated proof of expertise in the calibration activities that we perform and are authorized to issue internationally recognized DAkkS calibration certificates. Accreditation is the key to high quality measurements, international comparability, and trust in both the work of the calibration laboratory and the transparency of results.

ACCREDITED TO DIN EN ISO 17025

DAkkS
Deutsche Akkreditierungsstelle
D-K-18752-01-00

DIN EN ISO 17025

1. INTRODUCTION

1.2 Certified test equipment

Quality assurance and quality control regulations, such as ISO 9000, GLP, GMP, and pharmacopoeias, require companies to verify the consistently excellent performance of any spectrophotometer in use. The two most important factors for obtaining precise spectrophotometer data are the photometric accuracy (absorbance accuracy) and wavelength accuracy of the spectrophotometer, which should be tested on a regular basis.

In the Hellma Analytics calibration laboratory, which is accredited to DIN EN ISO 17025, we manufacture certified reference materials based on the regulatory codes issued by NIST (National Institute of Standards and Technology), ASTM (American Society for Testing and Materials) and pharmacopoeias (Ph. Eur., DAB, USP). All certified measurement results can be traced back to NIST standard reference materials (SRMs). (Photometric accuracy: SRM® 930e and SRM® 1930, wavelength accuracy: SRM® 2034).

!

Hellma Analytics' DAkkS-certified reference materials comply with the provisions stipulated by quality management systems and pharmacopoeias, meeting the highest quality requirements and ensuring the international comparability of measurement results.

Choose between glass filters and liquid filters for your reference materials:

1.3 Glass filter applications

Glass filters are certified reference materials made of glass manufactured specifically for calibration. They are, above all, extremely robust. All glass filters certified by Hellma Analytics are traceable to NIST primary standards. Certified glass filters are suitable for checking the following parameters of your spectrophotometer:

- » Wavelength accuracy
- » Photometric accuracy (absorbance)

1.4 Liquid filter applications

Liquid filters are certified liquid reference materials that are manufactured in compliance with pharmacopoeias and/or NIST standards and filled into quartz glass cuvettes under controlled conditions. The cuvettes are then permanently sealed to become airtight. Liquid filters have the distinct advantage of equating to real measuring conditions. Certified Hellma Analytics liquid filters are suitable for checking the following parameters of your spectrophotometer:

- » Wavelength accuracy
- » Photometric accuracy (absorbance)
- » Stray light levels
- » Spectral resolution

You should regularly check your UV/Vis spectrophotometer for all of these factors, especially photometric and wavelength accuracy, while observing the relevant requirements in your device handbook. Thanks to their ease of use and long service life, certified Hellma Analytics reference materials provide an excellent aid for all routine checks.

HELLMA ANALYTICS REFERENCE MATERIALS IN ACCORDANCE WITH THE MOST IMPORTANT SET OF RULES AND REGULATIONS

MATERIAL	CHECKING OF	RANGE	PH.EUR.	DAB	USP 851	ASTM
GLASS FILTERS						
Holmium oxide glass	Wavelength accuracy	UV/Vis			X	X
Didymium glass filter	Wavelength accuracy	UV/Vis			X	X
Didymium glass filter	Photometric accuracy	UV				
Neutral density glass	Photometric accuracy	Vis			X	X
LIQUID FILTERS						
Holmium oxide solution	Wavelength accuracy	UV/Vis	X	X	X	X
Potassium dichromate solution	Photometric accuracy	UV/Vis	X	X	X	X
Toluene in hexane	Spectral resolution	UV	X	X		
Potassium chloride solution	Stray light	UV	X	X		X
Sodium iodide solution	Stray light	UV				X
Sodium nitrite solution	Stray light	UV				X

1.5 DAkkS calibration certificate

After careful production, reference materials are certified in the Hellma Analytics calibration laboratory (accredited to DIN EN ISO 17025) using a high-performance UV-Vis/NIR spectrophotometer. Reference materials are only considered to be certified if they have been issued with a DAkkS calibration certificate and bear a calibration mark. Using the measurement values documented and certified in the calibration certificate, users can check and calibrate their spectrophotometers accordingly.

DAkkS
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D-K-18752-01-00

! The DAkkS calibration certificate from the national accreditation body for the Federal Republic of Germany may only be issued by accredited partners. The Hellma Analytics calibration laboratory is the only such laboratory in Germany that is accredited to certify UV-Vis reference materials.

ACCURATE MEASUREMENT RESULTS GUARANTEED.

Hellma Analytics
Hellma GmbH & Co. KG
Klosterrunsstr. 5, 79379 Müllheim, Germany
Telefon / Phone: +49 7631 182 0

Sample

akkreditiert durch die / accredited by the
Deutsche Akkreditierungsstelle GmbH
als Kalibrierlaboratorium im / as calibration laboratory in the
Deutschen Kalibrierdienst DKD

Kalibrierschein / Calibration certificate
Kalibrierzeichen / Calibration mark: 21112 / D-K-18752-01-00 / 2014-12

Gegenstand / Object	Neutralglasfilter-Satz / Set of Neutral Density Glass Filters	
Hersteller / Manufacturer	Hellma GmbH & Co. KG	
Typ / Type	666S000 (666-F2 / 666-F3 / 666-F4)	
Fabrikat/Serien-Nr. / Serial number	1234	
Auftraggeber / Customer	Hellma Analytics GmbH, Klosterrunsstr. 5, 79379 Müllheim	
Auftragsnummer / Order No.	666666	
Anzahl der Seiten des Kalibrierscheines / Number of pages of the certificate	3	
Datum der Kalibrierung / Date of calibration	22. Dezember 2014 / 22 December 2014	

Dieser Kalibrierschein dokumentiert die Rückführung auf nationale Normale zur Darstellung der Einheiten in Übereinstimmung mit dem Internationalen Einheitensystem (SI). Die DAkkS ist Unterzeichner der multilateralen Übereinkommen der European Co-operation for Accreditation (EA) und der International Laboratory Accreditation Cooperation (ILAC) zur gegenseitigen Anerkennung der Kalibrierscheine. Für die Einhaltung einer angemessenen Frist zur Wiederholung der Kalibrierung ist der Benutzer verantwortlich.

This calibration certificate documents the traceability to national standards, which realize the units of measurement according to the International System of Units (SI). The DAkkS is signatory to the multilateral agreements of the European co-operation for Accreditation (EA) and the International Laboratory Accreditation Cooperation (ILAC) for the mutual recognition of calibration certificates. The user is obliged to have the object recalibrated at appropriate intervals.

Dieser Kalibrierschein darf nur vollständig und unverändert weiterverbreitet werden. Auszüge oder Änderungen bedürfen der Genehmigung sowohl der Deutschen Akkreditierungsstelle GmbH als auch des ausstellenden Kalibrierlaboratoriums. Kalibrierscheine ohne Unterschrift haben keine Gültigkeit.
This calibration certificate may not be reproduced other than in full except with the permission of both the Deutsche Akkreditierungsstelle GmbH and the issuing laboratory. Calibration certificates without signature are not valid.

Datum / Date	Leiter des Kalibrierlaboratoriums / Head of the calibration laboratory	Bearbeiter / Person in charge
22. Dezember 2014 / 22 December 2014	Birgit Kehl	Timo Rapp

FO-Labor-062, Rev.:5– 17.12.14

1. Name of accreditation body
2. Name and address of issuing laboratory
3. Description of calibrated item
4. Clear details of measured item
5. Customer
6. Date of measurement
7. Internal document reference, including date of issue and last modified date
8. Date of issue of calibration certificate
9. Calibration certificate serial numbers
10. DAkkS registration number
11. Year and month of calibration
12. Authorized persons

Seite / Page 2 / 3 — 21112 / D-K-18752-01-00 / 2014-12

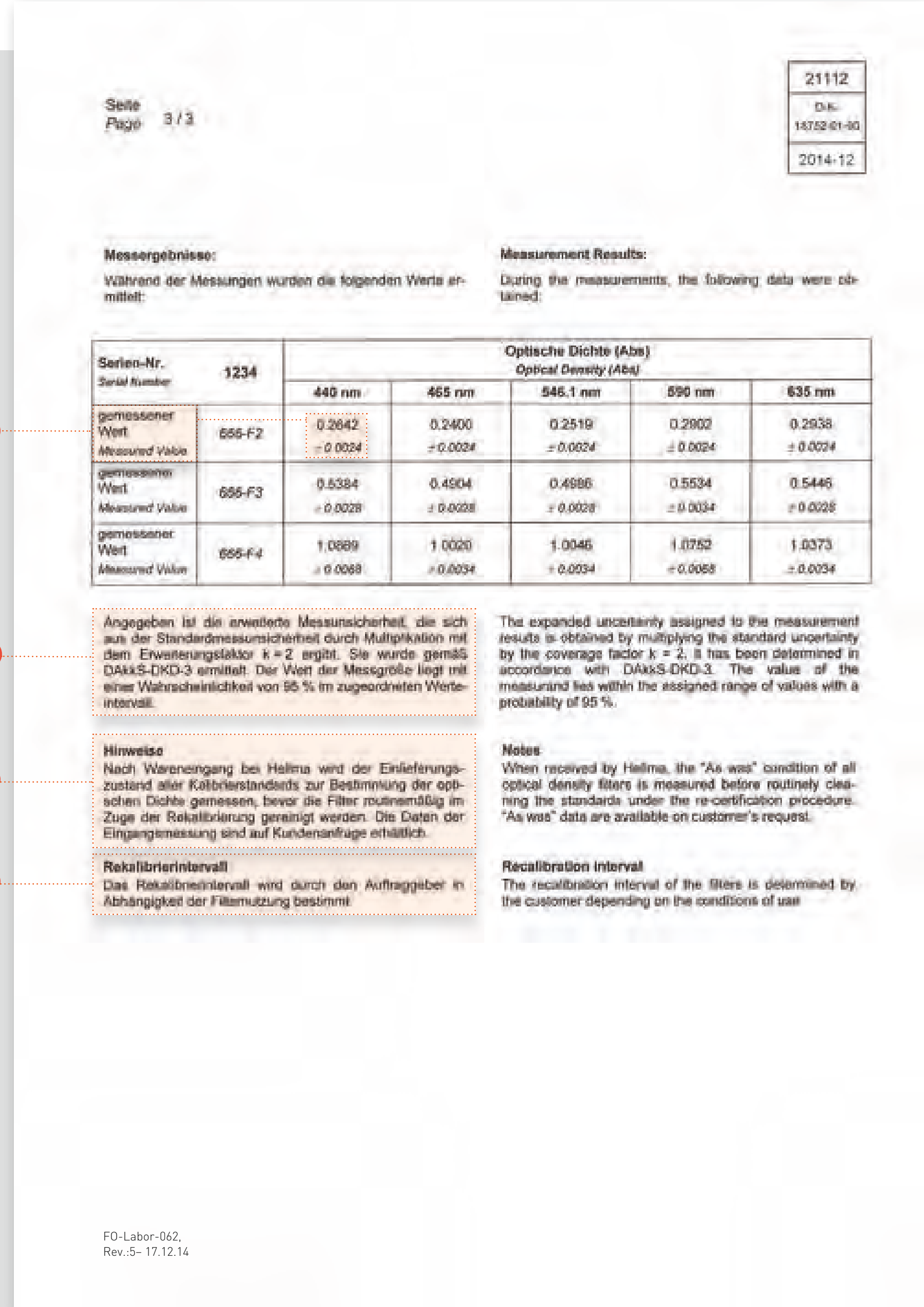
Kalibriergegenstand: Kalibrierstandard-Satz, bestehend aus drei Neutralglasfiltern NG11, NG5 und NG4.	**Calibration Object:** Set of calibration filters, consisting of three neutral density glass filters NG11, NG5 and NG4.
Kalibrierverfahren: Messung der optischen Dichte. Diese Kalibrierstandards wurden gegen Luft als Referenz gemessen.	**Calibration Method:** Measurement of optical density. These calibration standards were measured using air as reference.
Messtechnische Bedingungen bei der Kalibrierung: Die in diesem Kalibrierschein angegebenen Werte wurden mit dem verwendeten Spektralphotometer und den nachfolgenden Einstellungen ermittelt:	**Conditions of Calibration:** The following settings were used on the spectrometer employed to obtain the data quoted on this calibration certificate:
UV/VIS Modus der Ordinatenskala: Optische Dichte (Abs); Spaltbreite: 1,00 nm; Spaltmodus: Fix; Integrationszeit: 3,0 s	**UV/VIS** Ordinate mode: Optical density (Abs); Slit: 1.00 nm; Slit Mode: Fix; Integration time: 3.0 s
Für die Kalibrierung dieses Kalibriergegenstandes wurde ein UV/VIS/NIR-Spektralphotometer Varian Cary-5000 mit der Seriennummer UV1101M202 eingesetzt. Dieses Gerät wird regelmäßig auf die Einhaltung seiner Spezifikationen überprüft. Datum der letzten technischen Überprüfung: 27. November 2014	This calibration object was calibrated on a UV/VIS/NIR spectrophotometer Varian Cary-5000 with serial number UV1101M202. This instrument is regularly checked for the compliance with its specifications. Most recently technical check: 27 November 2014
Für die regelmäßige Überprüfung der photometrischen Richtigkeit werden die Bezugsnormale des NIST SRM 930e Filter Nr. 2115, gültig bis März 2015 und SRM 1930 Filter Nr. 202, gültig bis März 2016 eingesetzt.	A set of NIST SRM 930e Filter No. 2115, valid until March 2015 and SRM 1930 Filter No. 202, valid until March 2016 standard reference materials is used to regularly check the photometric accuracy of the spectrophotometer.
Zur regelmäßigen Überprüfung der Wellenlängenrichtigkeit wurde das intrinsische Bezugsnormal Hellma UV5 S.Nr. 0861 / 87450-PTB-14, gültig bis Dezember 2015 eingesetzt.	The intrinsic standard reference material Hellma UV5 serial no. 0861 / 87450-PTB-14, valid until December 2015 is used to regularly check the wavelength accuracy.
Zusätzlich werden die Emissionslinien von Deuterium, Quecksilber und Argon zur Überprüfung der Wellenlängenrichtigkeit verwendet.	In addition, the emission lines of deuterium, mercury and argon are used to check the wavelength accuracy.
Umgebungsbedingungen: Die Messungen wurden bei einer Umgebungstemperatur von 22°C ± 2°C und einer relativen Luftfeuchtigkeit von 30% bis 65% durchgeführt.	**Environmental Conditions:** Measurements were performed at an ambient temperature of 22°C ± 2°C and a relative humidity of 30% to 65%.

FO-Labor-062, Rev.:5– 17.12.14

1. Description of calibrated item
2. Measurement conditions
3. Type of device used to carry out measurements
4. Type, serial number and validity of calibrated NIST/PTB reference standards used to regularly check reference photometers; details of additional checking methods
5. Ambient conditions during measurement

1. INTRODUCTION

1. Measurement value and smallest attributed measurement uncertainty that can be specified. This value only refers to Hellma Analytics measurements and applies solely to the company's specific measurement conditions. In justified cases, calibration certificates may also show measurement results that do not fall within the calibration laboratory's scope of accreditation. These must be clearly labeled as such on the calibration certificate.
2. Notes on determining expanded measurement uncertainty.
3. Notes on initial measurements of filters used to determine optical density. Initial measurements are not taken for filters used to determine wavelength accuracy.
4. Calibration certificates shall not contain recommended recertification intervals (in accordance with DAkkS-DKD-5). Exceptions may be made if requested by the customer or required by legislation.



Seite
Page 3 / 3

21112
D-K-
18752-01-00
2014-12

Messergebnisse:
Während der Messungen wurden die folgenden Werte ermittelt:

Measurement Results:
During the measurements, the following data were obtained:

Serien-Nr. *Serial Number*	1234	Optische Dichte (Abs) *Optical Density (Abs)* 440 nm	465 nm	546.1 nm	590 nm	635 nm
gemessener Wert *Measured Value*	666-F2	0.2642 ± 0.0024	0.2400 ± 0.0024	0.2519 ± 0.0024	0.2902 ± 0.0024	0.2938 ± 0.0024
gemessener Wert *Measured Value*	666-F3	0.5384 ± 0.0028	0.4904 ± 0.0028	0.4986 ± 0.0028	0.5534 ± 0.0034	0.5446 ± 0.0028
gemessener Wert *Measured Value*	666-F4	1.0889 ± 0.0068	1.0020 ± 0.0034	1.0046 ± 0.0034	1.0752 ± 0.0068	1.0373 ± 0.0034

Angegeben ist die erweiterte Messunsicherheit, die sich aus der Standardmessunsicherheit durch Multiplikation mit dem Erweiterungsfaktor k = 2 ergibt. Sie wurde gemäß DAkkS-DKD-3 ermittelt. Der Wert der Messgröße liegt mit einer Wahrscheinlichkeit von 95 % im zugeordneten Werteintervall.

The expanded uncertainty assigned to the measurement results is obtained by multiplying the standard uncertainty by the coverage factor k = 2. It has been determined in accordance with DAkkS-DKD-3. The value of the measurand lies within the assigned range of values with a probability of 95 %.

Hinweise
Nach Wareneingang bei Hellma wird der Einlieferungszustand aller Kalibrierstandards zur Bestimmung der optischen Dichte gemessen, bevor die Filter routinemäßig im Zuge der Rekalibrierung gereinigt werden. Die Daten der Eingangsmessung sind auf Kundenanfrage erhältlich.

Notes
When received by Hellma, the "As was" condition of all optical density filters is measured before routinely cleaning the standards under the re-certification procedure. "As was" data are available on customer's request.

Rekalibrierintervall
Das Rekalibrierintervall wird durch den Auftraggeber in Abhängigkeit der Filternutzung bestimmt.

Recalibration interval
The recalibration interval of the filters is determined by the customer depending on the conditions of use.

FO-Labor-062,
Rev.:5– 17.12.14

1.6 Extended absorption range – from 0 to < 3.1 for UV/Vis reference materials

Under the previous accreditation, the company was certified to manufacture UV-Vis reference materials for wavelength accuracy and optical density up to an absorbance of 2.05 A. However, pharmaceutical applications, biotechnology and spectrophotometer manufacturers all increasingly require reference materials with a certified absorbance up to 3 A.

Since NIST (National Institute of Standards and Technology) is no longer producing the relevant SRM 2930 filter set, Hellma Analytics has manufactured an equivalent reference material, which has been officially certified by NIST, and is available as their 666S300 filter set.

Extended DAkkS accreditation for certified UV-Vis reference materials

ARTICLE NO.	666S300
APPLICATON	Filter set for testing the photometric accuracy
CONTENT	F390: Glass filter (0.04 abs) F301: Neutral densitiy glass filter (2.5 abs) F303: Neutral densitiy glass filter (3.0 abs)
STANDARD CERTIFICATION	**Wavelengths:** 440, 465, 546.1, 590, 635 nm **slit width:** 1 nm

2. GLASS FILTERS

WAVELENGTH ACCURACY

2.1 Holmium oxide glass filter for checking wavelength accuracy

APPLICATION

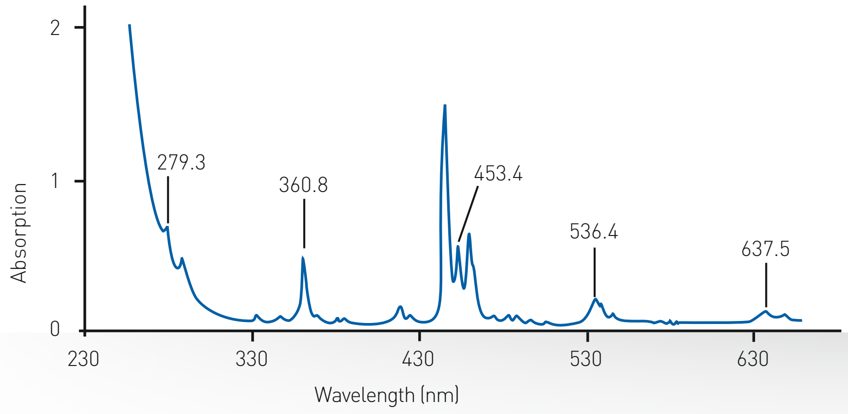
To measure wavelength accuracy, the filter absorbs the light beam of the spectrophotometer to a greater extent at certain wavelengths creating absorbance peaks. Ideally, any reference materials used to determine wavelength accuracy should have narrow, well-defined peaks at a variety of wavelengths in the UV and visible range.

PRODUCT DESCRIPTION

Holmium oxide glass filter 666-F1 has a range of narrow, well-defined peaks in the UV and visible range, making holmium an excellent choice for checking the wavelength scale of spectrophotometers. In comparison to filters that use holmium oxide solutions, the holmium oxide glass filter has a somewhat weaker spectrum with fewer peaks. In the low UV range in particular, the absorbance behavior of the glass matrix is superimposed on the holmium peaks. The main advantage of using a glass filter over a liquid filter is that it is more robust.

NOTES

The positions of holmium oxide peaks may vary slightly depending on the glass batch used. This is why Hellma Analytics certifies each holmium oxide glass filter individually.



Typical spectrum for a holmium oxide glass filter

ARTICLE NO.	666F1-339
APPLICATION	Testing the wavelength accuracy in the UV and Vis range [279 nm to 638 nm] at a spectral bandwidth up to 2 nm.
CONTENT	Holmium oxide glass filter with metal frame
STANDARD CERTIFICATION	**Wavelength accuracy at:** 279; 361; 453; 536; 638 nm **slit width:** 1 nm
POSSIBLE CERTIFICATION	**Additional possible wavelengths:** 287; 418; 445; 460 nm **slit width:** all up to 2 nm

2.2 Didymium glass filter for checking wavelength accuracy

APPLICATION

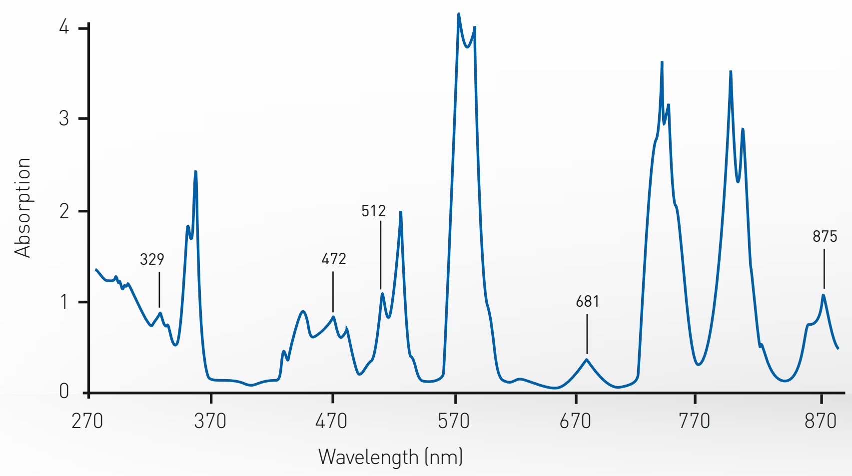
Please see 2.1 for more details of the application.

PRODUCT DESCRIPTION

Didymium glass filter 666-F7W is made from material specially manufactured by Schott AG. Like holmium oxide glass, didymium glass has a variety of characteristic peaks in the ultraviolet and visible range and is therefore typically used for checking wavelength accuracy. Its peaks are not as narrow as those of holmium oxide glass filters, however. The filter's absorbance behavior in the ultraviolet range also makes it suitable as an absorbance filter for checking photometric accuracy (see 2.3).

NOTES

The positions of didymium glass peaks may vary slightly depending on the glass batch used. This is why Hellma Analytics certifies each didymium glass filter individually.



Typical spectrum for a didymium glass filter

ARTICLE NO.	666F7W-323 or 666F7-323
APPLICATION	Testing the wavelength accuracy in the UV and Vis range [379 nm to 875 nm] at a spectral bandwidth up to 2 nm.
CONTENT	Didymium glass filter with metal frame
STANDARD CERTIFICATION	**Wavelength accuracy at:** 379; 472; 512; 681; 875 nm **slit width:** 1 nm
POSSIBLE CERTIFICATION	**Additional possible wavelengths:** 291; 302; 430; 446; 482; 626 nm **slit width:** all recommended up to 2 nm

2.3 Didymium glass filter for checking photometric accuracy

APPLICATION

To measure photometric accuracy (absorbance), the filter reduces the light beam from the spectrophotometer. An absorbance value (Abs) can be deduced from the light extinction caused by the filter.

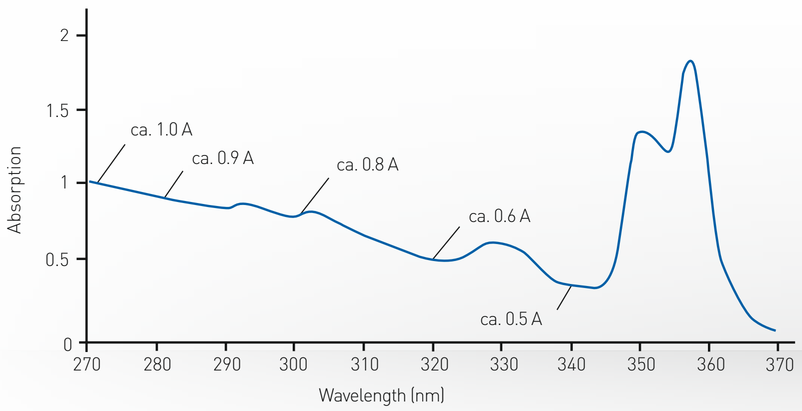
PRODUCT DESCRIPTION

Didymium glass filter 666-F7A is made from material specially manufactured by Schott AG. The didymium glass filter's absorbance behavior in the ultraviolet range also makes it suitable for use as an absorbance filter. Didymium glass filters are therefore suitable for checking wavelength accuracy in the UV-Vis range as well as checking photometric accuracy in the UV range.

Absorbance behavior in the UV range can be checked at 270 nm, 280 nm, 297 nm, 320 nm, and 340 nm. Filters are routinely set at a thickness that produces a nominal optical density of 0.5 Abs at 340 nm. This results in increasingly larger absorbances the shorter the wavelengths become.

NOTES

These absorbance values vary greatly depending on the glass batch used, however, and can only be compared for filters derived from the same glass melting process. This is why all didymium glass filters are certified individually.



Selected spectrum for a didymium glass filter between 270 nm and 370 nm

ARTICLE NO.	666F7A-323 or 666F7-323
APPLICATION	Testing the photometric accuracy in the UV range (270 nm to 340 nm)
CONTENT	Didymium glass filter with metal frame
STANDARD CERTIFICATION	**Photmetric accuracy:** approx. 0.5 to 1 abs. **at wavelengths:** 270; 280; 297; 320; 340 nm **slit width:** 1 nm
POSSIBLE CERTIFICATION	**Additional possible wavelengths:** from 270 to 290 nm and 310 to 320 nm **slit width:** all possible up to 5nm

2.4 Neutral density glass filter for checking photometric accuracy

APPLICATION

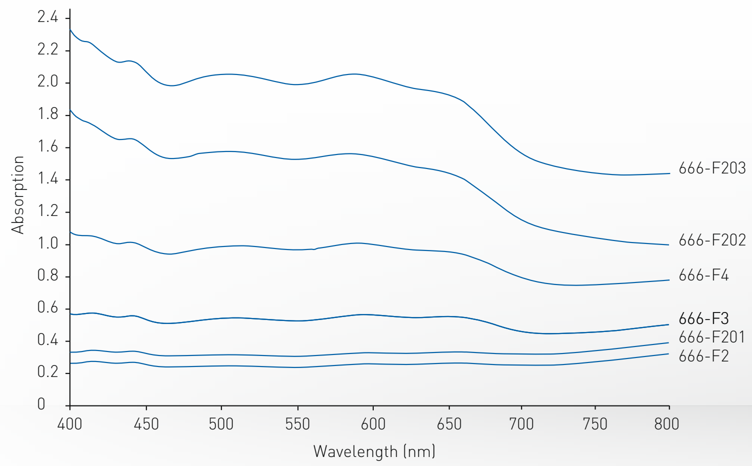
To measure photometric accuracy (absorbance), the filter reduces the light beam from the spectrophotometer. An absorbance value (Abs) can be deduced from the light extinction caused by the filter.

PRODUCT DESCRIPTION

Hellma Analytics neutral density glass filters are made from filter materials produced by Schott AG, which were selected on account of their homogeneity and stability. Thanks to a relatively constant transmittance within the wavelength range of 405 nm to 800 nm, they have been used to check photometric accuracy and linearity in the visible wavelength range (> 405 nm) for decades.

NOTES

If you have several neutral density glass filters with different nominal absorbances, you can check the linearity of your absorbance scale by plotting the absorbance values measured for each wavelength against the measurement values on the DAkkS calibration certificate in a diagram.



Typical spectrum for neutral density glass filters, measured with a slit width of 1 nm

ARTICLE NO.	666F2-39, 666F3-38, 666F4-37, 666F201-39, 666F202-36, 666F203-36
APPLICATION	Testing the photometric accuracy in the Vis range (405 nm to 890 nm)
CONTENT	**F2:** neutral density glass filter NG 11 (0.25 abs), **F3:** neutral density glass filter NG5 (0.5 abs), **F4:** neutral density glass filter NG4 (1.0 abs), **F201:** neutral density glass filter NG11 (0.3 abs) **F202:** neutral density glass filter NG3 (1.5 abs), **F203:** neutral density glass filter NG3 (2.0 abs)
STANDARD CERTIFICATION	**Wavelengths:** 440; 465; 546.1; 590; 635 nm **slit width:** 1 nm
POSSIBLE CERTIFICATION	**all wavelengths possible from 405 to 890 nm.** Also possible above 890 nm, with Hellma Analytics calibration certificate **slit width:** all possible up to 5 nm

2. GLASS FILTERS

SETS

2.5 Glass filter sets

Specifically created to meet customer requirements, Hellma Analytics glass filter sets consist of existing individual filters suitable for standard or custom validation procedures.

To ensure that filters can be easily identified, the set number is engraved on each filter frame. The absorbance/peak position values measured for each filter can be found on the DAkkS calibration certificate provided.

ARTICLE NO.	666S000	666S001
APPLICATION	Complete Glass Filter Set for testing the photometric accuracy und the wavelength accuracy of the spectrophotometer	Glass Filter Set for testing the wavelength accuracy and the photometric accuracy of the spectrophotometer
CONTENT	F1: holmium oxide glass filter F2: neutral density glass filter NG 11 (0.25 abs) F3: neutral density glass filter NG 5 (0.5 abs) F4: neutral density glass filter NG 4 (1.0 abs) F0: filter frame without glass (reference filter)	F3: neutral density glass filter NG 5 (0.5 abs) F4: neutral density glass filter NG 4 (1.0 abs) F7: didymium glass filter (0.5-1.0 abs)
STANDARD CERTIFICATION	**F1: holmium oxide glass filter:** **Wavelength accuracy at:** 279; 361; 453; 536; 638 nm **slit width:** 1 nm **F2, F3, F4: neutral density glass filter:** **wavelengths:** 440; 465; 546.1; 590; 635 nm **slit width:** 1 nm	**F3, F4: neutral density glass filter:** **wavelengths:** 440; 465; 546.1; 590; 635 nm **slit width:** 1 nm **F7: Didymium glass filter:** **wavelength accuracy at:** 329; 472; 512; 681; 875 nm **photometric accuracy:** from 0.5 to 1 abs **slit width:** 1 nm
POSSIBLE CERTIFICATION	**Wavelength accuracy;** **possible wavelengths:** 287; 418; 445; 460 nm **slit width:** up to 2 nm recommended **Photometric accuracy:** **wavelengths:** all possible from 405 to 890 nm Also possible above 890 nm, with Hellma Analytics calibration certificate **slit width:** all possible up to 5 nm	**F3, F4: neutral density glass filter:** **wavelength accuracy:** all possible from 405 to 890 nm. Also possible above 890 nm, with Hellma Analytics calibration certificate **slit width:** all possible up to 5 nm **didymium glass filter:** **wavelength accuracy:** **possible wavelengths:** 291; 302; 430; 446; 482; 626 nm **slit width:** up to 2 nm recommended **photometric accuracy:** **possible wavelengths:** from 270 to 290 nm and from 310 to 320 nm **slit width:** all possible up to 5 nm

For further sets, please see our product overview on pages 48 to 50.

2.6 General usage guidelines for glass filters

Glass filters are made of glass doped with metal ions/rare earth metals, which are assembled stress-free in black anodized precision frames made of aluminum. They are designed to fit into all spectrophotometers equipped with a holder for standard cuvettes with a 10-mm optical path length. To ensure that filters can be easily identified, each filter frame is engraved with the filter type and serial number. Details of the absorbance and peak position values measured for each filter can be found on the respective calibration certificate. Please ensure that you do not touch the glass surfaces of the filter. Dirt, dust, and damage can significantly impair the accuracy of measurement results. Anodized aluminum holders should not come into contact with acids or alkalis.

STORAGE
After use, we strongly recommend storing the filters at room temperature, in their packaging, and in a dry, dust-free area.

OTHER FACTORS THAT MAY INFLUENCE MEASUREMENTS
Dirt (e.g. fingerprints) and dust on, or damage (scratches, corrosion) to, polished optical surfaces can significantly impair the accuracy of measurement results. Always store the filters in their original packaging and protect the optical windows from contamination. Only handle the filters by their frames.

CLEANING
Dirt often accumulates on optical surfaces as a result of regular use. This is best removed using a lint-free cloth and alcohol or Hellmanex III cleaning solution.

INFLUENCE OF TEMPERATURE ON MEASUREMENTS
Temperature has a very small influence on certified measurement values, and temperatures between 20°C und 24°C fall within the measurement uncertainty stated on the calibration certificate. Measurements should therefore be taken in this range to keep any potential temperature influence on the results to a minimum.

Hellmanex III Cleaning concentrate

- // The cleaning concentrate significantly lowers surface tension and ensures optimum surface wetting even when used on complex shapes or on very large surfaces
- // It is extremely easy to rinse off, leaving no trace of the cleaner on the glass surface
- // It uses special surfactants that cause no absorption above 288 nm, and does not interfere with subsequent measurements in the UV/Vis range
- // It is extremely compatible with materials, reduces corrosion of glass and surfaces, and preserves optical surface quality

ARTICLE NO.	PRODUCT
9-307-011-4-507	Hellmanex III, 1 l (1.3 kg)
9-307-011-5-507	Hellmanex III, 10 l (14 kg)
9-307-011-6-507	Hellmanex III, 25 l (35 kg)

2. GLASS FILTERS

2.7 Calibration with glass filters

PREPARATIONS

There are many different makes, models and designs of spectrophotometer, these steps are only general guidelines. Please refer to your manufacturer's information to get more details.

STEPS TO TAKE BEFORE PERFORMING CALIBRATION WITH GLASS FILTERS

1. Warm up the spectrophotometer until the correct operating temperature has been reached and remains constant (e.g. for one hour), taking care to observe the device manufacturer's guidelines.
2. Make sure that you use a stable cuvette holder for 10 mm standard cuvettes to measure the filters, as this is the only way to guarantee the best positioning of the filters in the light path. Check that the holder is secure and stable in the sample compartment.
3. To begin with, carry out a baseline correction with an empty sample compartment.
4. Check that the filter is correctly positioned in the light path by first placing empty filter holder F0 in the cuvette holder. The F0 marking must be visible from above. Ensure that all filter frames are always positioned in the same way, i.e. with serial numbers facing the light source.
5. Check that the device's display has not changed. In spectrophotometers with very large beams, the measurement beam may touch the filter frame (beam clipping). If this is the case, you will notice a change in the device's display.
   » If necessary, adjust the height of the cuvette holder until the light beam shines through the aperture unimpeded. To help, you can switch the device's measurement beam to visible i.e. by adjusting the monochromator to 500 nm. There may be other ways of doing this depending on the device.
   » If the light beam touches the sides of the aperture, adjust the horizontal position of the cuvette holder until the light beam shines through the center of the aperture. The filter frame is correctly positioned if the display values, from the zero adjustment performed in step 3 (baseline correction), do not change. In rare cases where the zero reading can not be retained after inserting the empty filter frame and carrying out the above procedures it is permissible to re-zero the instrument with the empty filter frame in place and then continue with the filter measurements.
6. Carry out the filter measurement in a closed sample compartment as carefully as you would carry out a sample measurement (open sample compartments produce incorrect results).
7. Please note that, if you are using a diode array spectrophotometer with a stand-alone cuvette holder connected via a fiber-optic cable, extraneous light and vibrations (e.g. movement of fiber-optic cables) may also impair the accuracy of measurement results.

We look forward to hearing any comments, criticism or suggestions you may have:
feedback@hellma.com

Watch a video of the individual steps here.

2. GLASS FILTERS

2.7 Calibration with glass filters

WAVELENGTH ACCURACY

Watch a video of the individual steps here.

There are many different makes, models and designs of spectrometer, these steps are only general guidelines. Please refer to your manufacturer's information to get more details.

STEPS FOR CHECKING WAVELENGTH ACCURACY WITH A HOLMIUM OXIDE GLASS OR DIDYMIUM GLASS FILTER

1. First of all, follow the 'steps to take before performing calibration with glass filters'.
2. Run the scan program on your spectrophotometer, observing the guidelines in the user manual. Select a scanning range that covers all of the peaks listed on the filter's calibration certificate.
3. Set your spectrophotometer to the measurement parameters that appear on the calibration certificate provided. Select the slowest scanning speed and a small data interval.
4. If possible, carry out a baseline correction.
5. Measurements are taken using an air blank which means, which means that the reference cuvette holder remains empty in double beam photometers, while a reference measurement is taken using the empty cuvette holder in single beam photometers.
6. Insert the holmium oxide glass or didymium glass filter into the cuvette holder. Ensure that the filter is inserted into the holder as far as it will go, and that the filter ID can be seen from above. The filters must always be positioned in the cuvette holder in the same way, i.e. with the serial number facing the light source.
7. Start the measurement.
8. Calculate the positions of the peaks at the wavelengths stated on the calibration certificate. (Take several measurements and then use the mean of the measured values to avoid errors).
9. Compare your measurement values with the certified ones.

MEASUREMENT PARAMETERS FOR CHECKING WAVELENGTH ACCURACY

Ensure that you have selected the correct measurement parameters before plotting the absorbance curve to calculate peak positions. Incorrect parameters may distort the absorbance curve and thus shift the actual positions of peaks. Please use the settings stated on the accompanying calibration certificate. It should be noted that changing the slit width of the spectrophotometer can cause the absorption maxima to shift slightly. Ignore any influence that the spectral bandwidth from 1 nm to 2 nm has on peak positions. Peak heights, however, may vary greatly following changes to the slit width due to their narrow nature. As a result, filters for checking wavelength accuracy are usually unsuitable for checking absorbance accuracy.

TIP

Thomas Brenn, Product Manager

»Generally speaking, filters can also be measured using a slit width that differs from the information provided on the calibration certificate. However, please note that large slit widths will prevent weaker peaks from being resolved. In cases of doubt, it is therefore advisable to choose as small a slit width as possible. We recommend taking several measurements and then using the mean value to avoid errors during evaluation.«

2. GLASS FILTERS

2.7 Calibration with glass filters

PHOTOMETRIC ACCURACY

There are many different makes, models and designs of spectrophotometer, these steps are only general guidelines. Please refer to your manufacturer's information to get more details.

STEPS FOR CHECKING PHOTOMETRIC ACCURACY WITH NEUTRAL DENSITY GLASS OR DIDYMIUM GLASS FILTERS

1. First of all, follow the 'steps to take before performing calibration with glass filters'.
2. Run the wavelength selection program on your spectrophotometer, observing the guidelines in the user manual. Select the wavelengths provided on the calibration certificate.
3. Set your spectrophotometer to the measurement parameters that appear on the calibration certificate provided.
4. Adjust to zero.
5. Measurements are taken using an air blank which means that the reference cuvette holder remains empty in double beam spectrophotometers, while a reference measurement is taken using the empty cuvette holder in single beam spectrophotometers.
6. Insert the neutral density glass or didymium glass filter into the cuvette holder. Ensure that the filter is inserted into the holder as far as it will go, and that the filter ID can be seen from above. The filters must always be positioned in the cuvette holder in the same way, i.e. with the serial number facing the light source.
7. Start the program for measuring the absorbance values at the wavelengths stated on the calibration certificate. (Take several measurements and then use the mean of the measured values to avoid errors).
8. Compare your measurement values with the certified ones.

Watch a video of the individual steps here.

Carola Steinger, Chemical Laboratory Technician

»In practice it is easier to simply add up uncertainty contributions than to combine their statistics. However, the method used to determine measurement uncertainty depends on the specifications of your quality system and your measurement accuracy requirements.«

See the FAQs for more details.

2. GLASS FILTERS

2.7 Calibration with glass filters

INTERPRETING MEASUREMENT RESULTS

INTERPRETING THE MEASUREMENT RESULTS OF GLASS FILTERS FOR CHECKING PHOTOMETRIC AND WAVELENGTH ACCURACY

The measurement uncertainties that appear on the calibration certificate only refer to measurements conducted by Hellma Analytics and apply solely to the measurement conditions at the company (spectrophotometer used, environmental influences such as temperature, air humidity, user influence, and reference materials used).

The smallest possible measurement uncertainty can then be derived by statistically combining the measurement uncertainty stated on the calibration certificate and all of the user's uncertainty contributions. These include the wavelength scale tolerance of the spectrophotometer used and other influences on measurement accuracy (environmental factors such as temperature, air humidity, user influence, etc.). For further literature on correctly calculating measurement uncertainty, please refer to chapter 8 of this user manual.

3. LIQUID FILTERS
WAVELENGTH ACCURACY

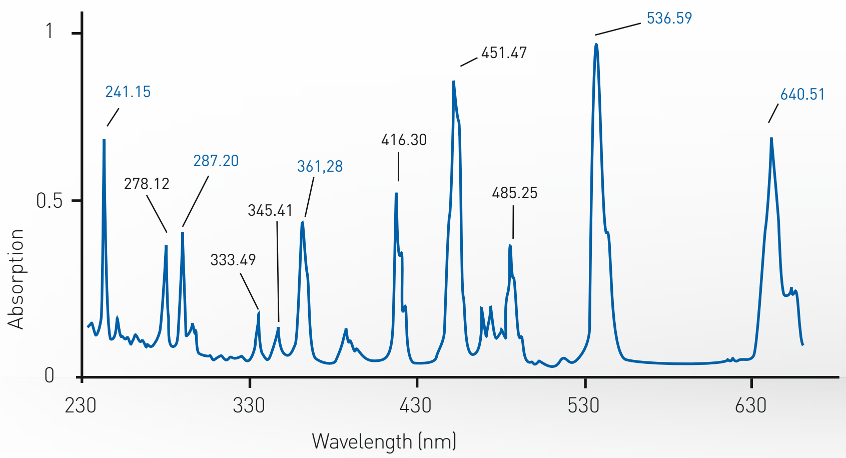
3.1 Holmium oxide liquid filter for checking wavelength accuracy

APPLICATION

To measure wavelength accuracy, the filter reduces the light beam of the spectrophotometer to a greater extent at certain wavelengths (peaks). Ideally, any standards used to determine wavelength accuracy should have narrow, well-defined peaks at a variety of wavelengths in the UV and visible range.

PRODUCT DESCRIPTION

The holmium oxide liquid filter consists of a solution of holmium oxide dissolved in perchloric acid. This filter is ideally suited to checking the wavelength accuracy of spectrophotometers in the UV and visible range. It has a spectrum with a variety of characteristic, very well-defined peaks in the range between 240 nm and 650 nm.



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

We would be interested to hear about your experiences with reference materials.
Write to us at feedback@hellma.com

ARTICLE NO.	667005
APPLICATION	Testing the wavelength accuracy according to the European Pharmacopoeia in the UV/ Vis range
CONTENT	Holmium oxide in perchloric acid
STANDARD CERTIFICATION	**wavelengths:** 241; 287; 361; 536; 640 nm **slit width:** 1 nm
POSSIBLE CERTIFCATION	**additional wavelengths:** 250; 278; 333; 345; 386; 416; 451; 468; 485 nm **slit width:** all up to 2 nm, above peaks become indistinct

LIQUID FILTERS

3. LIQUID FILTERS
PHOTOMETRIC ACCURACY

3.2 Potassium dichromate liquid filter for checking photometric accuracy

APPLICATION

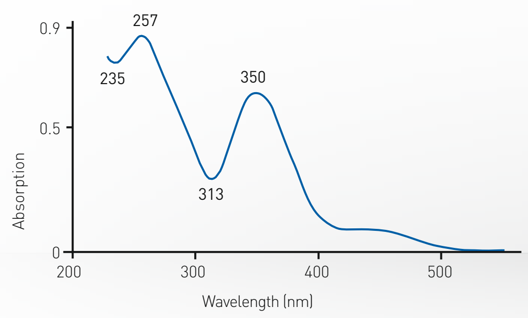
Photometric accuracy (absorbance) is measured by shining a light beam from the spectrophotometer through the inserted filter. An absorbance value (Abs) can be deduced from the light extinction caused by the filter.

PRODUCT DESCRIPTION

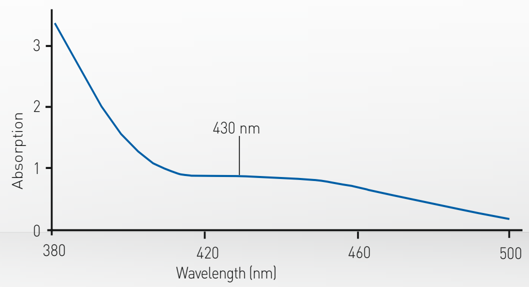
Potassium dichromate in perchloric acid is very suitable for checking the photometric accuracy of spectrophotometers. In the UV range, the potassium dichromate spectrum has characteristic maxima at 257 nm and 350 nm and characteristic minima at 235 nm and 313 nm. The spectrum reaches a plateau at 430 nm, which is used to determine photometric accuracy in the visible range. Hellma Analytics purchases the reference material for this filter directly from NIST (SRM® 935a "Potassium Dichromate"). The filter solutions are manufactured in strict compliance with NIST requirements and filled under controlled conditions. The cuvettes are then immediately fused to become airtight.

NOTES

As the filters are certified individually, measurement results are free from systematic errors made when preparing solutions and with regards to the optical path length of the cuvette. The measurement values of reference filter UV14 (perchloric acid measured against an air blank) appear separately on the DAkkS calibration certificate. To check absorbance linearity, take measurements using potassium dichromate filters with different concentrations. Plot the absorbance values measured for each filter and wavelength against the measurement values that appear on the DAkkS calibration certificate in a graph.



Typical spectrum of a 0.006 % potassium dichromate solution



Typical spectrum of a 0.06 % potassium dichromate solution

ARTICLE NO.	667020, 667040, 667060, 667080, 6670100, 667600, 667014 (reference filter)
APPLICATION	Testing the photometric accuracy in the UV range (235 to 350 nm) und Vis range (measurement wavelength 430 nm) at a spectral bandwidth of 2 nm or less
CONTENT	UV20, 20mg potassium dichromate in HCL04 (0.25 abs) UV40, 40mg potassium dichromate in HCL04 (0.5 abs) UV60, 60mg potassium dichromate in HCL04 (0.75 abs), in accordance with the European Pharmacopoeia UV80, 80mg potassium dichromate in HCL04 (1.0 abs) UV0100, 100mg potassium dichromate in HCL04 (1.25 abs) UV600, 600mg potassium dichromate in HCL04 (1.0 abs) in accordance with the European Pharmacopoeia UV14, perchloric acid (HCL04), reference filter
STANDARD CERTIFICATION	**at UV20 - UV100 wavelengths:** 235; 257; 313; 350 nm **at UV600 wavelength:** 430 nm **slit width:** 2 nm
POSSIBLE CERTIFCATION	**wavelength:** fixed **slit width:** all up to 2 nm

3. LIQUID FILTERS
STRAY LIGHT

3.3 Liquid filters for checking for stray light

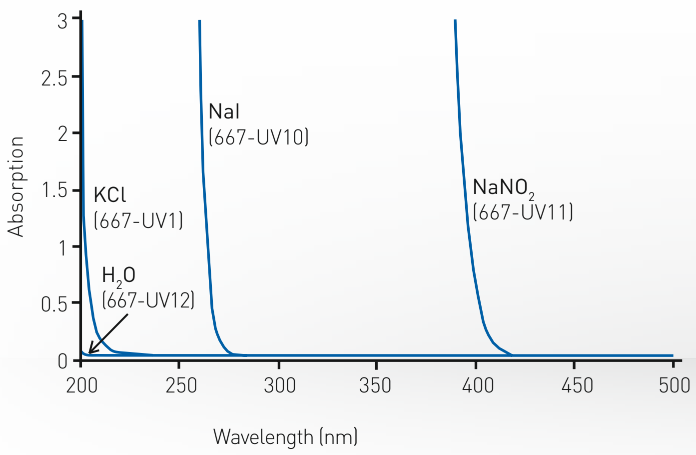
APPLICATION

In a spectrophotometer, stray light is light that passes by the sample and falls directly on the detector. This can lead to incorrect measurement results. Stray light may be caused by scattering or diffraction, by poor optical alignment, the use of incorrect or damaged cuvettes, incorrectly fitted sampling accessories or damaged seals around a light-tight sample chamber. Stray light is problematic, as it reduces the range of measurable absorbance and impairs the linearity between concentration and absorbance. Cut-off filters (filters with a strictly defined spectrum) are required to check the device for stray light.

PRODUCT DESCRIPTION

Hellma Analytics stray light filters do not allow light to pass through them below a certain wavelength (cut-off wavelength). Any transmittance values displayed in the cut-off wavelength range therefore represent stray light.

Due to their strictly defined spectrum, potassium chloride filters, sodium iodide filters, and sodium nitrite filters are ideally suited to qualifying the stray light level of spectrophotometers in compliance with pharmacopoeias. The steps are the same for all stray light filters.



ARTICLE NO.	667001, 667010, 667011, 667012 (reference filter)
APPLICATION	Testing for stray light in the UV range (UV1 according to the European Pharmacopoeia) (at wavelengths from 198 nm to 370nm, depending on the filter selected)
CONTENT	UV1, potassium chloride in pure water UV10, sodium iodide in pure water UV11, sodium iodide in pure water UV12, pure water (reference filter)
STANDARD CERTIFICATION	**UV1:** cut-off at 200 nm **UV10:** cut-off at 259 nm **UV11:** cut-off at 385 nm **slit width:** 2 nm
POSSIBLE CERTIFCATION	**wavelengths:** fixed **possible slit widths:** all up to 5 nm

3. LIQUID FILTERS
SPECTRAL RESOLUTION LIQUID FILTERS

3.4 Liquid filters for checking spectral resolution

APPLICATION

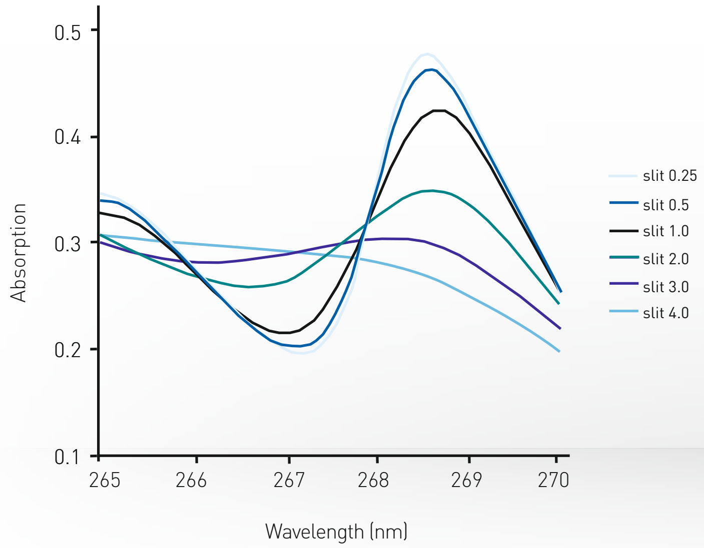
Regularly checking the spectral resolution of spectrophotometers ensures, for example, that neighbouring peaks are resolved and not superimposed on the peaks of bordering wavelengths. This also prevents absorbance errors.

PRODUCT DESCRIPTION

The toluene in hexane liquid filter has a prominent point in its spectrum, which is excellent for determining the spectral resolution and/or actual slit width of spectrophotometers in compliance with the European Pharmacopoeia.

NOTES

A spectrophotometer's spectral resolution is very closely connected to the correct slit width setting and characterized by its ability to resolve (recognize) two very closely related peaks. The smaller the slit and corresponding spectral bandwidth, the higher the resolution.



Typical spectra of the liquid filter toluene, measured with different slit widths

Timo Rapp, Chemical Laboratory Technician

»As a rule of thumb, the slit width should be no more than 10% of the peak width at half maximum in order to be able to determine its absorbance with an accuracy of 99.5%. Two peaks are deemed to be resolved separately if the minimum absorbance between them amounts to less than 80% of the maximum peak. Impairments to the spectrophotometer's spectral resolution will cause two different peaks to be shown as a combined peak, leading to inaccurate measurement results.«

ARTICLE NO.	667006, 667009
APPLICATION	Testing the resolution accoding to the European Pharmacopoeia
CONTENT	UV6, toluene in n-hexane UV9, n-hexane (reference filter)
STANDARD CERTIFICATION	**wavelength:** scan from 265 to 270 nm **slit width:** 0.5; 1.0; 2.0 nm with Hellma Analytics Calibration Certificate (no DAkkS Calibration Certificate)
POSSIBLE CERTIFCATION	**wavelength:** fixed **possible slit widths:** from 0.5 to 3 nm

3. LIQUID FILTERS

SETS

3.5 Liquid filter set compliant with the European Pharmacopoeia

The complete liquid filter set 667003 was compiled on the basis of European Pharmacopoeia requirements and contains all filters required to carry out a complete spectrophotometer check.

- ✔ **Checking photometric accuracy (UV60, UV600, UV14)**
- ✔ **Checking wavelength accuracy (UV5)**
- ✔ **Checking for stray light (UV1 and UV12)**
- ✔ **Checking spectral resolution (UV6 and UV9)**

All liquid filters consist of reference materials that are filled into precision Hellma cuvettes made of quartz glass (SUPRASIL®). These cuvettes are permanently sealed, and the complete set is delivered in a high-quality storage box. To ensure easy identification, each filter is engraved with its serial number. The calibration values measured for each filter can be found on the DAkkS and Hellma Analytics calibration certificates provided.

ARTICLE NO.	667003
APPLICATION	Complete filter set for testing the spectrophotometer according to the European Pharmacopoeia Photometric accuracy, wavelength accuracy, stray light and resolution
CONTENT	UV1, potassium chloride in pure water UV12, pure water (reference filter) UV5, holmium oxide in HClO4 UV6, toluene in n-hexane UV9, n-hexane (reference filter) UV60, 60mg potassium dichromate in HClO4 UV600, 600mg potassium dichromate in HClO4 UV14, perchloric acid (reference filter)
STANDARD CERTIFICATION	**UV1/UV12: wavelength:** 200 nm (cut-off) **UV5: wavelenght:** 241; 287; 361; 536; 640 nm; **slit width**: 1 nm **UV6/UV9: wavelength:** scan from 265 to 270 nm**; slit width:** 0.5; 1.0 ; 2.0 nm **UV60:** approx. 0.75 abs; **wavelengths:** 235; 257; 313; 350 nm; **slith width:** 2 nm **UV600:** approx. 1.0 abs; **wavelength:** 430 nm; **slit width:** 2 nm
POSSIBLE CERTIFICATION	**UV1/UV12: wavelength:** fixed; **slit width:** all up to 5 nm **UV5: wavelenght:** 250, 278, 333, 345, 386, 416, 451, 468, 485 nm; **slit width:** all up to 2 nm; above, peaks become indistinct **UV6/UV9: wavelength:** fixed; **slit width:** 0.5 to 3 nm **UV60: wavelength:** fixed; **slit width:** all up to 2 nm **UV600: wavelength:** fixed; **slit width:** all up to 2 nm

3.6 Liquid filter set compliant with the United States Pharmacopeia

ARTICLE NO.	667004
APPLICATION	Filter set for testing the spectrophotometer according to USP 851, photometric accuracy and wavelength accuracy
CONTENT	F2: neutral density glass filter (0.25 abs) F3: neutral density glass filter (0.5 abs) F4: neutral density glass filter (1.0 abs) F0: filter frame without glass (reference filter) UV60: 60mg potassium dichromate in HClO4 UV14: perchloric acid (HClO4, reference filter) UV5: holmium oxide in HClO4
STANDARD CERTIFICATION	**F2, F3 and F4: wavelengths:** 440, 465, 546.1, 590, 635; **slit width:** 1 nm **UV60: wavelengths:** 235, 257, 313, 350; **slit width:** 2 nm **UV5: wavelengths:** 241, 250, 278, 287, 333, 345, 361, 385, 416, 452, 468, 485, 536, 640 nm; **slit width:** 1 nm
POSSIBLE CERTIFICATION	**F2, F3, F4: wavelengths:** all possible from 405 to 890 nm also possible above 890 nm with Hellma Analytics Calibration Certificate **slit width:** all possible up to 5 nm **UV60: wavelength:** fixed; **slit width:** up to 2 nm **UV5: wavelength:** fixed, **slit width:** all up to 2 nm; above, peaks become indistinct

3.7 General usage guidelines for liquid filters

Liquid filters bear a marking on one side showing the chemical formula of the substance contained in the cuvette. If a filter breaks, please observe the codes of conduct and safety instructions that apply to this substance. This information can be found in the safety instructions. Up-to-date safety instructions for all substances used to manufacture liquid filters are available at **www.hellma-analytics.com/download**.

!

Always take great care when placing liquid filters in the sample holder of your spectrophotometer. Wherever possible, only touch filters by their caps or matt sides. Take care not to touch the polished surfaces. The filters are fragile and should be handled with the utmost care.

STORAGE

After use, we strongly recommend storing the filters at room temperature, in their packaging, and in a dry, dust-free area. Liquid filters must not be exposed to temperatures below 4°C or above 40°C. This also applies when transporting and delivering liquid filters for recertification.

OTHER FACTORS THAT MAY INFLUENCE MEASUREMENTS

Dirt (e.g. fingerprints) and dust on, or damage (scratches, corrosion) to, polished surfaces can significantly impair the accuracy of measurement results. Always store the filters in their original packaging and protect the optical windows from contamination. Only handle the filters by their caps or matt surfaces.

CLEANING

Dirt often accumulates on optical surfaces as a result of regular use. This is best removed using a lint-free cloth and alcohol or Hellmanex III cleaning solution.

For further information, please see page 17

INFLUENCE OF TEMPERATURE ON MEASUREMENTS

Temperature has a very small influence on certified measurement values, and temperatures of between 20°C und 24°C fall within the measurement uncertainty stated on the calibration certificate. Measurements should therefore be taken in this range to keep any potential temperature influence on the results to a minimum.

LIQUID FILTERS

3. LIQUID FILTERS

3.8 Calibration with liquid filters

PREPARATIONS

There are many different makes, models and designs of spectrophotometer, these steps are only general guidelines. Please refer to your manufacturer's information to get more details.

STEPS TO TAKE BEFORE PERFORMING CALIBRATION WITH LIQUID FILTERS

1. Warm up the spectrophotometer until the correct operating temperature has been reached and remains constant (e.g. for one hour), taking care to observe the device manufacturer's guidelines.
2. Make sure that you use a stable cuvette holder for 10 mm standard cuvettes to measure the liquid filters, as this is the only way to guarantee the best positioning of the filters in the light path. Check that the holder is secure and stable in the sample compartment.
3. The filters should always be positioned in the cuvette holders in the same way, i.e. with the Hellma lettering facing the light source. The light beam must pass through the part of the filter filled with liquid (solution).
4. Carry out the filter measurement in a closed sample compartment as carefully as you would carry out a sample measurement (open sample compartments produce incorrect results).
5. Please note that, if you are using a diode array spectrophotometer with a stand-alone cuvette holder connected via a fiber-optic cable, extraneous light, extraneous light and vibrations (e.g. movement of fiber-optic cables) may also impair the accuracy of measurement results.

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3. LIQUID FILTERS

3.8 Calibration with liquid filters

WAVELENGTH ACCURACY

There are many different makes, models and designs of spectrophotometer, these steps are only general guidelines. Please refer to your manufacturer's information to get more details.

STEPS FOR CHECKING WAVELENGTH ACCURACY WITH A HOLMIUM OXIDE LIQUID FILTER

1. First of all, follow the 'steps to take before performing calibration with liquid filters'.
2. Run the scan program on your spectrophotometer, observing the guidelines in the user manual. Select a scanning range that covers all of the peaks listed on the filter's calibration certificate.
3. Set your spectrophotometer to the measurement parameters that appear on the calibration certificate provided. Select the slowest scanning speed and a small data interval.
4. If possible, carry out a baseline correction.
5. Measurements are taken against the Perchloric Acid blank UV14, which means that the reference cuvette holder remains empty in double beam photometers, while a reference measurement is taken using the empty cuvette holder in single beam photometers.
6. Insert the holmium oxide liquid filter into the cuvette holder, observing the general usage guidelines for liquid filters. The filters should always be positioned in the cuvette holders in the same way, i.e. with the Hellma lettering facing the light source.
7. Start the measurement.
8. Calculate the positions of the peaks at the wavelengths stated on the calibration certificate. (Take several measurements and then use the mean of the measured values to avoid errors).
9. Compare your measurement values with the certified ones.

MEASUREMENT PARAMETERS FOR CHECKING WAVELENGTH ACCURACY

Ensure that you have selected the correct measurement parameters before plotting the absorbance curve to calculate peak positions. Incorrect parameters may distort the absorbance curve and thus shift the actual positions of peaks. Please use the settings stated on the accompanying calibration certificate. It should be noted that changing the slit width of the spectrophotometer can cause the absorption maxima to shift slightly. Ignore any influence that the spectral bandwidth from 1 nm to 2 nm has on peak positions. Peak heights, however, may vary greatly following changes to the slit width due to their narrow nature. As a result, filters for checking wavelength accuracy are usually unsuitable for checking absorbance accuracy.

Carola Steinger, Chemical Laboratory Technician

»Generally speaking, filters can also be measured using a slit width that differs from the information provided on the calibration certificate. However, please note that large slit widths will prevent weaker peaks from being resolved. In cases of doubt, it is therefore advisable to choose as small a slit width as possible. We recommend taking several measurements and then using the mean value to avoid errors during evaluation.«

See the FAQs for more details.

LIQUID FILTERS

PHOTOMETRIC ACCURACY

There are many different makes, models and designs of spectrophotometer, these steps are only general guidelines. Please refer to your manufacturer's information to get more details.

STEPS FOR CHECKING PHOTOMETRIC ACCURACY WITH A POTASSIUM DICHROMATE LIQUID FILTER

1. First of all, follow the 'steps to take before performing calibration with liquid filters'.
2. Run the wavelength selection program on your spectrophotometer, observing the guidelines in the user manual. Select the wavelengths provided on the calibration certificate.
3. Set your spectrophotometer to the measurement parameters that appear on the calibration certificate provided.
4. Adjust to zero.
5. As a general rule, measurements are taken using a reference filter filled with perchloric acid observing the general usage guidelines for liquid filters. The filters should always be positioned in the cuvette holders in the same way, i.e. with the Hellma Analytics lettering facing the light source.
6. Measurements in a single beam spectrophotometer: Carefully insert the perchloric acid reference filter provided into the cuvette holder. Start the measurement. Next, measure the certified reference material, which contains potassium dichromate dissolved in perchloric acid. Then subtract the reference measurement values from the measurement values of the certified reference material. Or set zero/blank the instrument with the Perchloric Acid reference filter UV14 fitted, then replace with the certfied reference material and read the value directly from the display.
7. Measurements in a double beam spectrophotometer: Carefully insert the certified reference material, which contains potassium dichromate dissolved in perchloric acid, into the sample holder, and the perchloric acid reference filter into the reference sample holder.
8. Start the program for measuring the absorbance values at the wavelengths stated on the calibration certificate. (Take several measurements and then use the mean of the measured values to avoid errors).
9. Compare your measurement values with the certified ones.

INTERPRETING MEASUREMENT RESULTS

MEASUREMENT PARAMETERS FOR CHECKING PHOTOMETRIC ACCURACY

As the difference between the maxima and minima in the absorbance spectrum is relatively large, the potassium dichromate liquid filters may also be measured with a slit width that differs from the one on the calibration certificate. However, please note that using large slit widths (> 2 nm) may result in slight deviations from the values stated on the calibration certificate. In cases of doubt, it is therefore advisable to choose the slit width quoted on the calibration certificate. We recommend taking several measurements and then using the mean value to avoid errors during evaluation.

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INTERPRETING THE MEASUREMENT RESULTS OF LIQUID FILTERS FOR CHECKING PHOTOMETRIC AND WAVELENGTH ACCURACY

The measurement uncertainties that appear on the calibration certificate only refer to measurements conducted by Hellma Analytics and apply solely to the measurement conditions at the company (spectrophotometer used, environmental influences such as temperature, air humidity, user influence, and reference materials used).

The smallest possible measurement uncertainty that can be achieved by the user can then be derived by statistically combining the measurement uncertainty stated on the calibration certificate with all the user's uncertainty contributions, such as the wavelength scale tolerance of the spectrophotometer used and other influences on measurement accuracy (environmental factors such as temperature, air humidity, user influence, etc.). For further literature on correctly calculating measurement uncertainty, please refer to chapter 8 of this user manual.

TIP

Birgit Kehl, Head
Hellma Analytics calibration laboratory

»In practice it is easier to simply add up uncertainty contributions than to combine their statistics. However, the method used to determine measurement uncertainty depends on the specifications of your quality system and your measurement accuracy requirements.«

See the FAQs for more details.

3. LIQUID FILTERS

3.8 Calibration with liquid filters

STRAY LIGHT LEVEL

There are many different makes, models and designs of spectrophotometer, these steps are only general guidelines. Please refer to your manufacturer's information to get more details.

STEPS FOR CHECKING THE STRAY LIGHT LEVEL

1. First of all, follow the 'steps to take before performing calibration with liquid filters'.
2. Run the scan program on your spectrophotometer, observing the guidelines in the user manual. Select a scanning range that covers all of the values listed on the filter's calibration certificate.
3. Set your spectrophotometer to the measurement parameters quoted on the calibration certificate provided
4. Set the spectrophotometer to a wavelength of approx. 20 nm above the cut-off wavelength for the stray light filter used (for potassium chloride (UV1), for example, start at 220 nm) and scan up to the wavelength for which you wish to determine the stray light level.
5. If possible, carry out a baseline correction.
6. As a general rule, take the measurements using a reference filter filled with water (UV12) observing the general usage guidelines for liquid filters. The filters should always be positioned in the cuvette holders in the same way, i.e. with the Hellma Analytics lettering facing the light source.
7. Measurements in a single beam spectrophotometer: Carefully insert the reference filter provided into the cuvette holder. Start the measurement. Next, measure the certified reference material. Then subtract the reference measurement values from the measurement values of the certified reference material.
8. Measurements in a double beam spectrophotometer: Carefully insert the certified reference material into the sample holder and the reference filter into the reference sample holder. Start the measurement.
9. Scan up to the wavelength for which you wish to determine the stray light level.
10. The light level (remaining transmittance value) measured below the cut-off wavelength represents stray light.

MEASUREMENT PARAMETERS FOR CHECKING STRAY LIGHT LEVEL

For a realistic calculation of the stray light level, choose a filter with a cut-off wavelength as close above the required wavelength as possible. This enables the stray light test to be carried out at the wavelength at which the stray light filter can fully absorb light. The remaining transmittance displayed by the device at the measurement wavelength represents the stray light level. Since this value differs depending on the properties of the measuring system, filters can only be certified with regard to their suitability for use as a stray light filter. Certification therefore demonstrates that filters have virtually full absorbance in the measuring range and steep peaks at high transmittance values.

!

Please note that the reference for the measuring system to be tested is not provided by the entire transmittance characteristic of the stray light filter, but solely by the transmittance value measured in the range of virtually full absorbance.

TIP

You can check the lower absorbance range of your spectrophotometer using reference filter 667-UV12, which is filled with ultrapure water. The filter's absorbance characteristics from 200 nm to NIR are practically only determined by the reflection losses on the two air/glass surfaces. You can check your device's display at very low absorbance values against the certified values at 198 nm, 200 nm, 300 nm and 400 nm. If your results differ significantly from the certified values, particularly if the measured values are smaller than 0.02 A, you should contact the device manufacturer.

INTERPRETING MEASUREMENT RESULTS WHEN CHECKING FOR STRAY LIGHT

To estimate the sample measurement error due to stray light, compare the calculated stray light level to the signal strength from the sample measurement. For example, a stray light value of 0.1% transmittance and a sample with an absorbance of around 1 Abs would equate to a measurement error due to stray light of around 0.4%. If you have calculated a stray light level that is considerably higher than the level stated in the device specifications, check whether extraneous light could have interfered with this result. If you can rule out extraneous light, please contact a service technician.

SPECTRAL RESOLUTION

There are many different makes, models and designs of spectrophotometer, these steps are only general guidelines. Please refer to your manufacturer's information to get more details.

STEPS FOR CHECKING SPECTRAL RESOLUTION

1. First of all, follow the 'steps to take before performing calibration with liquid filters'.
2. Run the scan program on your spectrophotometer, observing the guidelines in the user manual. Select a scanning range that covers both of the required peaks.
3. Set your spectrophotometer to the measurement parameters stated on the calibration certificate provided.
4. If possible, carry out a baseline correction.
5. Take the measurement using a reference filter filled with hexane – if the spectrum is corrected to zero at 300 nm, measurements can also be taken using air – observing the general usage guidelines for liquid filters. The filters should always be positioned in the cuvette holders in the same way, i.e. with the Hellma Analytics lettering facing the light source.
6. Measurements in a single beam spectrophotometer: Carefully insert the hexane reference filter provided into the cuvette holder. Start the measurement. Next, measure the certified reference material, which contains toluene in hexane. Then subtract the reference measurement values from the measurement values of the certified reference material.
7. Measurements in a double beam spectrophotometer: Carefully insert the toluene in hexane liquid filter into the sample holder and the hexane reference filter into the reference sample holder. Start the measurement.
8. Measure the actual minimum of the absorbance values at 266 nm and the actual maximum at 269 nm. (Take several measurements and then use the mean of the measured values to avoid errors).
9. If possible, carry out a baseline correction each time you change the slit width.
10. Determine the ratio using both of the measured values, as stated on the calibration certificate provided.

INTERPRETING THE MEASUREMENT RESULTS

MEASUREMENT PARAMETERS WHEN CHECKING SPECTRAL RESOLUTION

When measuring spectral resolution, the liquid filter absorbs the light beam from the spectrophotometer to significantly different extents in a narrow wavelength range (5 nm). The filter will show a clear maximum and minimum within the narrow range. After placing the liquid filter in the spectrophotometer, run the scan program in the defined wavelength range and divide the maximum peak measured at Ðmax = 269 nm by the minimum peak measured at Ðmin 266 nm. The resulting ratio represents the absorbance ratio, which is directly linked to the slit width. If the ratio is considerably lower (e.g. 15%), please contact the device manufacturer. Please note, however, that the result also depends on the measurement conditions. Therefore, please make sure that you select a sufficiently long integration time, particularly if using a small slit width.

INTERPRETING MEASUREMENT RESULTS WHEN CHECKING SPECTRAL RESOLUTION

Regulatory codes or internal applications and measuring procedures may place requirements on the ratios that must be achieved. In addition, comparing calculated ratios with certified values may provide an indication of the actual slit width of the device used.

Share your experience of our products by writing to:
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!

Please note that the filter set for determining spectral resolution does not fall within our scope of accreditation, and therefore cannot be issued with a DAkkS calibration certificate or calibration mark.

ABSORBANCE RATIO OF MAXIMUM/MINIMUM PEAK IN RELATION TO SLIT WIDTH

SLIT WIDTH	ABSORBANCE RATIO
0,25	2,3
0,5	2,2
1,0	2,0
2,0	1,4
3,0	1,1
4,0	1,0

(see Standards and Best Practice in Absorption Spectrometry, edited by C. Burgess & T. Frost)

4. CERTIFIED REFERENCE PLATES

PHOTOMETRIC ACCURACY

4.1 Reference plates for checking photometric accuracy

APPLICATION

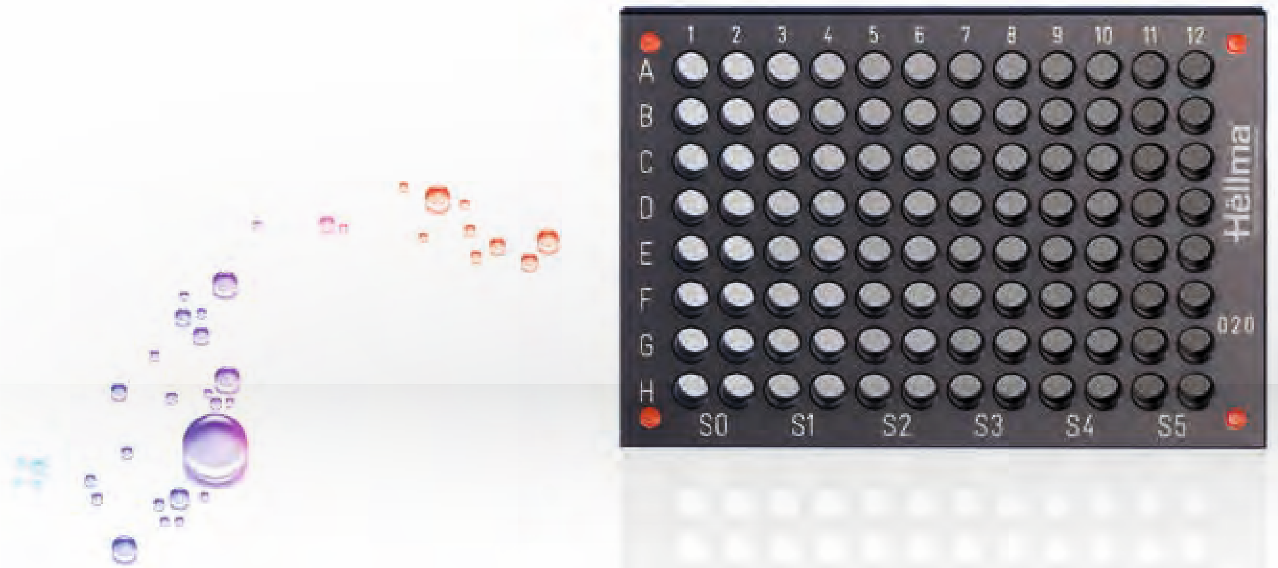
The Hellma Analytics reference plate 666R013 can be used to check the photometric accuracy of microtitre plate readers.

PRODUCT DESCRIPTION

The reference plate dimensions are suitable for a 96-well microtitre plate with a diameter of 6.6 mm per window (H 14.5 mm x D 125 mm x L 85.5 mm). Each of the five neutral density glass filters (fields S1–S5) in the reference plate can measure the absorbance value for 16 windows. The other 16 windows do not contain glass (S0) and serve as references.

NOTES

The reference plate has five neutral density glass filters with different nominal absorbance values, allowing you to check the linearity of your absorbance scale by plotting the absorbance values measured for each wavelength against the measurement values on the DAkkS calibration certificate in a diagram.



ARTICLE NO.	666R013
APPLICATION	Reference plate for microplate readers for testing the photometric accuracy
CONTENT	neutral density glass filter NG 11 (0.25 abs) neutral density glass filter NG5 (0.5 abs) neutral density glass filter NG 4 (1.0 abs) neutral density glass filter NG 3 (1.5 abs) neutral density glass filter NG 3 (2.5 abs) frame without glass (reference filter)
STANDARD CERTIFICATION	**photometric accuracy certified at wavelengths:** 405; 450; 490; 650 nm **at 8 points in a row**
POSSIBLE CERTIFICATION	**wavelengths:** all possible from 405 to 890 nm Also possible above 890 nm, but only with a Hellma Analytics Calibration Certificate **slit widths:** all possible up to 5 nm

4. CERTIFIED REFERENCE PLATES

PHOTOMETRIC AND WAVELENGTH ACCURACY

4.2 Reference plate for checking photometric and wavelength accuracy

APPLICATION

The Hellma Analytics reference plate 666R113 can be used to check the photometric and wavelength accuracy of microtitre plate readers.

PRODUCT DESCRIPTION

The reference plate dimensions are suitable for a 96-well microtest plate with a diameter of 6.6 mm per window (H 14.5 mm x D 125 mm x L 85.5 mm). Each of the four neutral density glass filters used (fields S1–S4) can measure the absorbance value for 16 windows. Holmium oxide glass (S5) is used to test the wavelength accuracy in 16 windows while a further 16 windows (S0) do not contain glass and serve as references.

NOTES

The reference plate has four neutral density glass filters with different nominal absorbance values, allowing you to check the linearity of your absorbance scale by plotting the absorbance values measured for each wavelength against the measurement values on the DAkkS calibration certificate in a diagram.

ARTICLE NO.	666R113
APPLICATION	Reference plate for microplate readers for testing the wavelength accuracy and the photometric accuracy
CONTENT	neutral density glass filter NG5 (0.5 abs) neutral density glass filter NG 4 (1.0 abs) neutral density glass filter NG 3 (1.5 abs) neutral density glass filter NG 3 (2.0 abs) holmium oxide glass filter frame without glass (reference filter)
STANDARD CERTIFICATION	**photometric accuracy certified at 8 points in row at wavelengths:** 405; 450; 490; 650 nm **wavelength accuracy certified at:** 279; 361; 453; 536; 638 nm **slit width:** 1 nm
POSSIBLE CERTIFICATION	**photometric accuracy:** additional wavelengths: all possible from 405 to 890nm also possible above 890 nm, but only with a Hellma Analytics Calibration Certificate **slit widths:** all possible up to 5 nm **wavelength accuracy:** **additional possible wavelengths:** 287; 418; 445; 460 nm **slit widths:** all up to 2 nm recommended

CERTIFIED REFERENCE PLATES

4. CERTIFIED REFERENCE PLATES FOR MICROTITRE PLATE READERS

4.3 General usage guidelines for reference plates

Reference plates are made of glass doped with metal ions or rare earth metals, which is annealed and assembled in black anodized precision frames made of aluminum. They are designed to fit into all microtitre plate readers. To ensure easy identification, each reference plate is engraved with the reference plate type and serial number. Details of the absorbance and peak position values measured for each filter can be found on the respective calibration certificate. Please ensure that you do not touch the glass surfaces of the filter. Dirt, dust, and damage can significantly impair the accuracy of measurement results. Anodized aluminum frames should not come into contact with acids or alkalis.

STORAGE

After use, we recommend storing reference plates at room temperature, in their packaging, and in a dry, dust-free area.

OTHER FACTORS THAT MAY INFLUENCE MEASUREMENTS

Dirt (e.g. fingerprints) and dust on, or damage (scratches, corrosion) to, polished surfaces can significantly impair the accuracy of measurement results. Always store reference plates in their original packaging and protect the optical windows from contamination. Only handle reference plates by their frames.

CLEANING

Dirt often accumulates on optical surfaces as a result of regular use. This is best removed using a lint-free cloth and alcohol.

INFLUENCE OF TEMPERATURE ON MEASUREMENTS

Temperature has a very small influence on certified measurement values. Measurements taken at temperatures between 20°C und 24°C fall within the measurement uncertainty stated on the calibration certificate. Measurements should therefore be taken in this range to keep any potential temperature influence on the results to a minimum.

4. CERTIFIED REFERENCE PLATES

4.4 Calibration with reference plates

PREPARATIONS

There are many different makes, models and designs of spectrometer, these steps are only general guidelines. Please refer to your manufacturer's information to get more details.

STEPS TO TAKE BEFORE PERFORMING CALIBRATION WITH REFERENCE PLATES

1. Warm up the microtitre plate reader until the correct operating temperature has been reached and remains constant (e.g. for one hour), taking care to observe the device manufacturer's guidelines.
2. To begin with, carry out a baseline correction with an empty sample compartment.
3. Check that the reference plate is correctly positioned in the light path by first measuring the windows without glass (usually rows 1 and 2). The label showing the reference plate type must be visible from above.
4. Check that the device's display has not changed. In microtitre plates with very large beams, the measurement beam may touch the window frame. If this is the case, you will notice a change in the device's display.
   » If necessary, adjust the position of the reference plate holder until the light beam shines through the empty window unimpeded.
   » The reference plate is correctly positioned if the display values from the zero adjustment performed in step 2 (baseline correction) do not change.
5. Carry out the filter measurement in a closed sample compartment as carefully as you would carry out a sample measurement (open sample compartments produce incorrect results).

Ideas, criticism, experiences?
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4. CERTIFIED REFERENCE PLATES

4.4 Calibration with reference plates

PHOTOMETRIC ACCURACY

There are many different makes, models and designs of spectrophotometer, these steps are only general guidelines. Please refer to your manufacturer's information to get more details.

STEPS FOR CHECKING PHOTOMETRIC ACCURACY WITH REFERENCE PLATES

1. First of all, follow the 'steps to take before performing calibration with reference plates'.
2. Run the wavelength selection program on your microtitre plate reader, observing the guidelines in the user manual. Select the wavelengths stated on the calibration certificate.
3. Set your microtest plate reader to the measurement parameters quoted on the calibration certificate provided.
4. Adjust to zero.
5. Place the reference plate in the plate holder. Ensure that the reference plate ID is visible from above. Reference plates must always be positioned in the plate holders in the same way.
6. Start the program for measuring absorbance values at the wavelengths stated on the calibration certificate – the positions measured are those where neutral density glass filters are inserted.
7. Take several measurements and then use the mean of the measured values to avoid errors.
8. Compare your measurement values with the certified ones.

MEASUREMENT PARAMETERS FOR CHECKING PHOTOMETRIC ACCURACY

Generally speaking, reference plates can also be measured using a slit width that differs from the information provided on the calibration certificate. However, please note that using large slit widths may result in slight deviations from the values stated on the calibration certificate. In cases of doubt, it is therefore advisable to choose as small a slit width as possible. We recommend taking several measurements and then using the mean value to avoid errors during evaluation.

MEASUREMENT PARAMETERS FOR CHECKING WAVELENGTH ACCURACY

Ensure that you have selected the correct measurement parameters before plotting the absorbance curve to calculate peak positions. Incorrect parameters may distort the absorbance curve and thus shift the actual positions of peaks. Please use the settings stated on the accompanying calibration certificate. It should be noted that changing the slit width of the microtitre plate reader can cause the absorbance maxima to shift slightly. Ignore any influence that the spectral bandwidth from 1 nm to 2 nm has on peak positions. Peak heights, however, may vary greatly following changes to the slit width due to their narrow nature. As a result, filters for checking wavelength accuracy are usually unsuitable for checking absorbance accuracy.

WAVELENGTH ACCURACY

There are many different makes, models and designs of spectrometer, these steps are only general guidelines. Please refer to your manufacturer's information to get more details.

STEPS FOR CHECKING WAVELENGTH ACCURACY WITH REFERENCE PLATES

1. First of all, follow the 'steps to take before performing calibration with reference plates'.
2. Run the scan program on your microtitre plate reader, observing the guidelines in the user manual. Select a scanning range that covers all of the peaks listed on the filter's calibration certificate.
3. Set your microplate reader to the measurement parameters quoted on the calibration certificate provided. Select the slowest scanning speed and a small data interval.
4. If possible, carry out a baseline correction.
5. Place the reference plate in the plate holder. Ensure that the filter ID is visible from above. Reference plates must always be positioned in the plate holders in the same way.
6. Start the measurement for the positions where holmium oxide glass filters are inserted (usually S5).
7. Calculate the positions of the peaks at the wavelengths stated on the calibration certificate.
8. Take several measurements and then use the mean of the measured values to avoid errors.
9. Compare your measurement values with the certified ones.

INTERPRETING MEASUREMENT RESULTS WITH REFERENCE PLATES FOR CHECKING PHOTOMETRIC AND WAVELENGTH ACCURACY

The measurement uncertainties stated on the calibration certificate only refer to measurements conducted by Hellma Analytics and apply solely to the measurement conditions at the company (spectrophotometer used, environmental influences such as temperature, air humidity, user influence, and reference materials used).

The smallest possible measurement uncertainty that can be achieved by the user can then be derived by statistically combining the measurement uncertainty stated on the calibration certificate with all the user's uncertainty contributions, such as the wavelength scale tolerance of the microtest plate reader used and other influences on measurement accuracy (environmental factors such as temperature, air humidity, user influence, etc.). For further literature on correctly calculating measurement uncertainty, please refer to chapter 8 of this user manual.

TIP

Birgit Kehl, Head
Hellma Analytics calibration laboratory

»In practice it is easier to simply add up uncertainty contributions than to combine their statistics. However, the method used to determine measurement uncertainty depends on the specifications of your quality system and your measurement accuracy requirements.«

See the FAQs for more details.

CERTIFIED REFERENCE PLATES

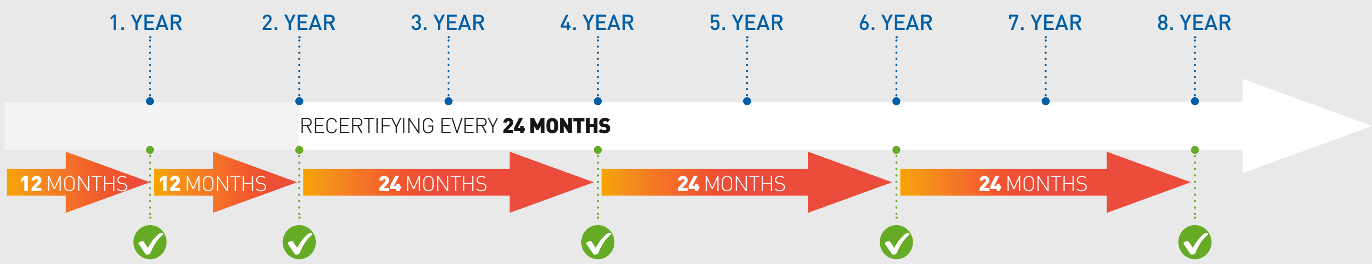
5. RECERTIFICATION

RECERTIFICATION INTERVALS FOR REFERENCE MATERIALS

As is the case for all measuring devices, the reference materials used to verify spectrophotometers must also be checked and recertified at regular intervals (see for example ISO 9001:2008 "Control of Monitoring and Measuring Equipment"). This allows you to ensure that you consistently fulfill your in-house quality requirements and guarantees high levels of accuracy and reliability in your measurements.

The length of intervals between the recertification of reference materials depends on how frequently materials are used, the wear associated with this, accuracy requirements, and the requirements of a company's internal auditing. Therefore, only clients themselves can determine these. In general, a recertification interval of 12 months is recommended for checking and recertifying glass filters during the first two years of use, with an interval of 24 months thereafter. We recommend verifying and recertifying liquid filters within a maximum of 12 months. Intervals should be specified individually in accordance with your QA system.

GLASS FILTERS



LIQUID FILTERS

RETURNING YOUR REFERENCE MATERIALS FOR RECERTIFICATION

01. Complete the return shipment form in full. When returning several filters or filter sets, please use one form for each.

02. Enclose a copy of the current calibration certificate.

03. Send your filter to the Hellma Analytics calibration laboratory via your local Hellma office. Use the address label printed on the rear of the return shipment form to do so.

04. Filters are cleaned and recertified in the Hellma Analytics calibration laboratory. If necessary, filters will also be repaired or exchanged following a consultation.

05. **You will receive your filter with a new DAkkS calibration certificate or a Hellma Analytics calibration certificate in the case of filters for verifying spectral resolution.**

30-YEAR WARRANTY

All of our reference materials come with a 30-year warranty, provided that they are regularly recertified (at least every two years) at the Hellma Analytics calibration laboratory. Certified reference materials (filters) sent for recertification are carefully cleaned and recertified before being sent back with a new DAkkS calibration certificate and calibration mark. Damaged filters and filters that deviate significantly from nominal values are usually replaced in consultation with the customer.

6.1 Why do holmium oxide glass filters become cloudy? Will this interfere with the measurement?

The glass material used for this filter is somewhat hygroscopic, which means that the filters become coated with a kind of water film. The film does not interfere with measurements or change the characteristic peak positions of holmium oxide. The filter can be easily wiped down using alcohol and a soft cloth. The filter should generally be stored in a dry place.

6.2 How long can a calibration standard be used for in total?

Depending on the conditions in which they are used and stored, as well as how they are maintained, filters usually last for many years. We recommend having filters regularly recertified so that any signs of deterioration can be recognized at an early stage.

6.3 How often should filters be recertified?

Certified reference materials should be recertified at regular intervals to check that the values stated on the calibration certificate are still valid. It is up to the user to decide on the regularity of these intervals, which should take into account the use, storage and usage conditions of the filter in the laboratory. To establish a statistical database for determining recertification intervals, we recommend having all reference materials recertified at least every 12 months during their first two years of use, and then selecting a suitable recertification interval based on the values measured. (Please see chapter 9).

6.4 What do the tolerances on the calibration certificate tell us and how can they be correctly interpreted?

The measurement uncertainties that appear on calibration certificates only refer to measurements conducted by Hellma Analytics and apply solely to the measurement conditions at the company (spectrophotometer used, environmental influences such as temperature, air humidity, user influence, reference materials used, etc.). Consequently, the measurement uncertainties of the NIST reference materials used to ensure traceability have been mathematically combined with the measurement uncertainty statistics calculated by Hellma Analytics. The value provided is therefore an expanded measurement uncertainty (double standard deviation, coverage factor k=2). This means that the actual value is 95% certain to fall within this range. To correctly calculate the measurement uncertainties valid for their measuring system, reference material users should follow the same steps, mathematically/statistically combining the measurement uncertainties provided with the measurement uncertainty statistics they have calculated themselves for a particular spectrophotometer and relevant conditions (see ISO/IEC Guide 98-3:2008 'Guide to the Expression of Uncertainty in Measurement').

6.5 What is a baseline correction?

Baseline corrections are carried out with an empty cuvette holder to compensate for the lamps. Since lamps emit light at different strengths at various wavelengths, baseline corrections (also known as auto zero) are carried out to determine a zero value. Baseline corrections are usually performed automatically when the spectrophotometer is started up, but can also be carried out manually.

6.6 What is background correction?

Background correction is carried out to eliminate any influences that extend beyond the sample's properties. In double beam photometers, background correction is performed by simultaneously measuring the comparison cuvette in the reference beam path. This comparison cuvette usually contains pure solvent. In single beam photometers, background correction is carried out before the actual sample measurement is taken by measuring the comparison cuvette. The values obtained for the comparison cuvette are then deducted from the values of the sample measurement.

6.7 Why does the calibration certificate for the filter set used to determine spectral resolution look different to other calibration certificates?

Determining spectral resolution does not fall within our scope of accreditation. The filter set for determining spectral resolution therefore cannot be issued with a DAkkS calibration certificate or calibration mark. That is why this calibration certificate looks different from other calibration certificates for filter sets.

6.8 Why does Hellma Analytics no longer offer potassium dichromate filters for checking photometric accuracy with sulfuric acid as a solvent, as described in the European Pharmacopoeia?

In the past, certified reference materials for checking photometric accuracy in the UV range contained a solution of potassium dichromate in sulfuric acid and were manufactured in strict compliance with European Pharmacopoeia requirements. Over a number of years, Hellma Analytics noticed a continuous decrease in the absorbance values of the 'potassium dichromate dissolved in sulfuric acid' filter during daily calibrations. We do not have a sufficient explanation for why this happens, but we assume that the comparatively high ionic strength of sulfuric acid causes mixed chromium (VI) complexes to form. To compensate for this behavior, over which we have no control, filters would need to be recertified much more regularly. Another possibility would be preparing new solutions every time the spectrophotometer is checked. As a simple alternative, we offer a liquid filter that uses 'potassium dichromate dissolved in perchloric acid'. This type of liquid filter for checking photometric accuracy has proven itself as a reliable and very stable standard for many years. No changes in absorbance properties comparable to those of the sulfuric acid model are known for this filter. Hellma Analytics cuvettes are permanently sealed, eliminating concerns about the toxicity of perchloric acid. Furthermore, the European Pharmacopoeia states that "suitable certified reference materials" may also be used, which undoubtedly applies to our perchloric acid solvent model. This model also contains a formulation described by NIST.

6.9 Why does the weight of potassium dichromate filters seem to change after every recertification?

Due to measurement uncertainties, measurement values may fall within a specific range. This leads to an apparent change in weight from qualification to qualification, as the initial weight is calculated directly from the measured absorbance values. Earlier versions of regulatory codes stipulated that filters for checking photometric accuracy had to contain 60.06 mg/l potassium dichromate, and allowed a tolerance of 0.01 Abs. More current versions of the European Pharmacopoeia have replaced this very strict provision, now accepting weights between 57.0 mg/l and 63.0 mg/l. The specific absorbance calculated (see European Pharmacopoeia, chapter 2.2.25) is now stated with a margin of tolerance.

6.10 Why are these peaks measured for certifying holmium oxide glass and didymium glass filters?

Measurement errors are low in medium to high transmittance ranges. As a result, peaks in the range from 0 Abs to 1.0 Abs (corresponds to 100% -T to 10% T) are preferred for certification.

6.11 How do I calculate my measurement uncertainty?

The measurement uncertainties stated on the calibration certificate only refer to measurements conducted by Hellma Analytics and apply solely to the measurement conditions at the company (spectrophotometer used, environmental influences such as temperature, air humidity, user influence, reference materials used, etc.). The smallest possible measurement uncertainty that can be achieved by the user can then be derived by statistically combining the measurement uncertainty stated on the calibration certificate with all the user's uncertainty contributions, such as the wavelength scale tolerance of the spectrophotometer used and other influences on measurement accuracy (environmental factors such as temperature, air humidity, user influence, etc.).

EXAMPLE OF CALCULATING STANDARD MEASUREMENT UNCERTAINTY FOR A NEUTRAL DENSITY GLASS FILTER (HIGHLY SIMPLIFIED): THE CALIBRATION CERTIFICATE STATES THE FOLLOWING MEASUREMENT VALUES AND MEASUREMENT UNCERTAINTIES:

SERIAL NUMBER	3524	OPTISCHE DICHTE (Abs) Optical Density (Abs)				
		440 nm	465 nm	546.1 nm	590 nm	635 nm
MEASURED VALUE	666-F2	0.2542 ± 0.0024	0.2254 ± 0.0024	0.2254 ± 0.0024	0.2415 ± 0.0024	0.2416 ± 0.0024

Here, a wavelength of 440 nm produces the following parameters:

Target measurement value (x_s) : 0,2542 Abs
Expanded measurement uncertainty: +/- 0,0024 Abs (coverage factor k=2)
Standard measurement uncertainty (x_a): +/- 0,0012 Abs

Next, you must calculate the measuring error specific to your spectrophotometer (xb) – refer to the operating instructions for more details – and define a value for the measuring error due to environmental influences at your company (xu) (such as temperature and air humidity).

Example of measuring error parameters:

Spectrophotometer (x_b): +/- 0,01 Abs
Environmental influences (x_u): +/- 0,001 Abs

Calculating standard measurement uncertainty (MU):

$MU = \sqrt{x_a^2 + x_b^2 + x_u^2} = 0,0101$

Expanded measurement uncertainty is calculated by multiplying this value by coverage factor k.

As shown here, in practice it is often easier to simply add up uncertainty contributions than to combine their statistics. However, the method used to determine measurement uncertainty depends on the specifications of your quality system and your measurement accuracy requirements. For further literature on correctly calculating measurement uncertainty, please refer to the recommendations for further reading in chapter 8 of this user manual.

7. GLOSSARY

Abbreviations:

A: absorbance
ASTM: American Society for Testing and Materials
BG: Specific term for Schott glass
DAB: Deutsches Arzneibuch (German Pharmacopoeia)
DAkkS: Deutsche Akkreditierungsstelle (National accreditation body for the Federal Republic of Germany)
DAR: Deutscher Akkreditierungsrat (German accreditation body)
DKD: Deutscher Kalibrierdienst (German calibration body)
Ph. Eur.: European Pharmacopoeia
FAQs: Frequently asked questions
GLP: Good laboratory practice
GMP: Good manufacturing practice
I: Intensity
I0: Original intensity
0: Original
k: Coverage factor for measurement uncertainty
λmax: Maximum peak at defined wavelength
λmin: Minimum peak at defined wavelength
NIR: Near-infrared
NIST: National Institute of Standards and Technology
PTB: Physikalisch-Technische Bundesanstalt (Germany's national metrology institute)
SRM®: Standard Reference Material (registered trademark of NIST)
USP: United States Pharmacopeia
UV: Ultraviolet
Vis: Visible (visible wavelength range)

Absorbance (Abs):
When light falls on or passes through a sample, the quantity of absorbed light is equal to the difference between the original intensity I0 and the intensity I after interaction with the sample. This is because part of the irradiated light is transferred to the molecules, causing the beam to have a smaller output when it exits the sample. The extent to which light is absorbed is determined by the principles of the Beer-Lambert law. The amount of absorbed light can be expressed as transmittance (see definition) or absorbance. Absorbance is defined as Abs = -logT. According to the relevant standard, this parameter is referred to as spectral optical density on transmittance ("optical density").

Optical density: see absorbance

Visible range: Part of the optical spectrum that stretches from 380 nm to 780 nm of the wavelength range of electromagnetic radiation. This range is generally referred to as light. This is the only range in which the human eye can 'see' electromagnetic radiation.

Spectral resolution: This refers to a measuring system's ability to separate individual wavelength ranges.

Spectral bandwidth: Wavelength range that appears with a continuum at the exit slit when the monochromator is exposed to irradiation. Spectral bandwidth is determined by the bandwidth of emitted radiation where the light has reached half the maximum intensity.

Spectral optical density on transmittance: see absorbance

Transmittance (T): When light falls on or passes through a sample, the quantity of absorbed light is equal to the difference between the original intensity I0 and the intensity I after interaction with the sample. This is because part of the irradiated light is transferred to the molecules, causing the beam to have a smaller output when it exits the sample. The extent to which light is absorbed is determined by the principles of the Beer-Lambert law. The amount of absorbed light can be expressed as transmittance (see definition) or absorbance. Transmittance is normally expressed as a fraction of 1 or as a percentage, and is defined as follows: T = I/I0 or %T = (I/I0) * 100.

Ultraviolet range (UV range): Also known as UV radiation, this is the short-wave part of the optical radiation spectrum. UV radiation has a wavelength range of 100 nm to 380 nm.

Wavelength: Wavelength is the distance between two identical, adjacent corresponding points of the same wave phase at a certain point in time.

8. RECOMMENDATIONS FOR FURTHER READING

Standards and Best Practice in Absorption Spectrometry; Edited by C. Burgess and T. Frost UVSG, ISBN 0-632-05313-5 Blackwell Service

Qualitätssicherung in der Analytischen Chemie; Werner Funk, Vera Dammann, Gerhild Donnevert; ISBN-10: 3-527-31112-2; Verlag: WILEY-VCH

ISO/IEC Guide 98-3:2008; **Evaluation of measurement data – Guide to the expression of uncertainty in measurement**

NIST Special Publication 260-54 Standard Reference Materials: **Certification and Use of Acidic Potassium Dichromate Solutions as an Ultraviolet Absorbance Standard – SRM 935**

NIST Special Publication 260-116 Standard Reference Materials: **Glass Filters as a Standard Reference Material for Spectrophotometry – Selection, Preparation, Certification, and Use of SRM 930 and SRM 1930**

NIST Special Publication 260-102: Standard Reference Materials: **Holmium Oxide Solution Wavelength Standard from 240 to 640 nm – SRM 2034**

European Pharmacopoeia (Ph.Eur.)

DKD3

9. PRODUCT OVERVIEW

Glass filters with DAkkS calibration certificates

TYPE	MATERIAL	WAVELENGTH nm	ARTICLE-NO.
Glass Filter for testing the wavelength accuracy			
666-F1	Holmium Oxide Glass Filter F1	279; 361; 453; 536; 638	666F1-339
666-F7W	Didymium Glass Filter F7W	329; 472; 512; 681; 875	666F7W-323
Glass Filter for testing the photometric accuracy			
666-F2	Neutral Density Glass Filter F2 (Nominal value of the absorption 0.25)	440; 465; 546,1; 590; 635	666F2-39
666-F201	Neutral Density Glass Filter F201 (Nominal value of the absorption 0.3)	440; 465; 546,1; 590; 635	666F201-39
666-F3	Neutral Density Glass Filter F3 (Nominal value of the absorption 0.5)	440; 465; 546,1; 590; 635	666F3-38
666-F4	Neutral Density Glass Filter F4 (Nominal value of the absorption 1.0)	440; 465; 546,1; 590; 635	666F4-37
666-F202	Neutral Density Glass Filter F202 (Nominal value of the absorption 1.5)	440; 465; 546,1; 590; 635	666F202-36
666-F203	Neutral Density Glass Filter F203 (Nominal value of the absorption 2.0)	440; 465; 546,1; 590; 635	666F203-36
666-F7A	Neutral Density Glass Filter F7A (Nominal value of the absorption approx. 0.5–1.0)	270; 280; 297; 320; 340	666F7A-323
Glass Filter for testing the wavelength accuracy and the photometric accuracy			
666-F7	Didymium Glass Filter F7	A: 270; 280; 297; 320; 340 W: 329; 472; 512; 681; 875	666F7-323
Empty filter mount			
666-F0	Aluminum frame		666F0-71

TYPE	CONSISTING OF	WAVELENGTH nm	ARTICLE-NO.
Sets for testing the wavelength accuracy and the photometric accuracy			
666-S000	Complete Glass Filter Set: F1, F2, F3, F4, F0	A: 440; 465; 546,1; 590; 635 W: 279; 361; 453; 536; 638	666S000
666-S001	Glass Filter Set: F3, F4, F7	A: 270; 280; 297; 320; 340; 440; 465; 546,1; 590; 635 W: 329; 472; 512; 681; 875	666S001
666-S002	Glass Filter Set: F2, F3, F4	A: 440; 465; 546,1; 590; 635	666S002
666-S004	Glass Filter Set: F201, F202, F203	A: 440;465; 546,1; 590; 635	666S004
666-S300	Glass Filter Set: F301, F303, F390 (Abs: 0.04; 2.5; 3.0)	A: 440;465; 546,1; 590; 635	666S300

A: Wavelengths for absorbance W: Wavelengths for wavelength accuracy

Complete Set 666-S000 — Single Filters

666-F0 666-F1 666-F2 666-F3 666-F4 666-F201 666-F202 666-F203 666-F7

Liquid filters with DAkkS calibration certificates

TYPE	CONTENT	WAVELENGTH nm	ARTICLE-NO.
Liquid Filter for testing the photometric accuracy			
667-UV20	20 mg potassium dichromate in $HClO_4$ (0.25 Abs)	235; 257; 313; 350	667020
667-UV40	40 mg potassium dichromate in $HClO_4$ (0.5 Abs)	235; 257; 313; 350	667040
667-UV60	60 mg potassium dichromate in $HClO_4$ (0.75 Abs)	235; 257; 313; 350	667060
667-UV80	80 mg potassium dichromate in $HClO_4$ (1.0 Abs)	235; 257; 313; 350	667080
667-UV0100	100 mg potassium dichromate in $HClO_4$ (1.25 Abs)	235; 257; 313; 350	6670100
667-UV600	600 mg potassium dichromate in $HClO_4$ (1.0 Abs)	430	667600
667-UV14	Perchloric acid ($HClO_4$), reference filter	235; 257; 313; 350	667014
667-UV301	Filter Set for UV range: UV60, UV14	235; 257; 313; 350	667301
667-UV304	Filter Set for Vis range: UV600, UV14	430	667304
667-UV305	Filter Set for UV/Vis range: UV60, UV600, UV14	235; 257; 313; 350; 430	667305
Liquid Filter Set for testing the linearity of the absorption			
667-UV307	Filter-Set: UV20, UV40, UV60, UV80, UV100, UV14	235; 257; 313; 350	667307
Liquid Filter for testing the wavelength accuracy			
667-UV5	Holmium oxide in perchloric acid	241; 287; 361; 536; 640	667005
667-UV400	Filter Set: UV05, UV14	241; 287; 361; 536; 640	667400
Liquid Filter for testing to stray light			
667-UV1	Potassium chloride in pure water	200 (cut-off)	667001
667-UV10	Sodium iodide in pure water	259 (cut-off)	667010
667-UV11	Sodium nitrite in pure water	385 (cut-off)	667011
667-UV12	Pure water (reference filter)	198; 200; 300; 400	667012
667-UV100	Filter Set UV-100: UV1, UV12	200 (cut-off)	667100
667-UV101	Filter Set UV-101: UV10, UV12	259 (cut-off)	667101
667-UV102	Filter Set UV-102: UV11, UV12	385 (cut-off)	667102
667-UV103	Filter Set UV-103: UV1, UV10, UV11, UV12	200; 259; 385 (cut-off)	667103
667-UV104	Filter Set UV-104: UV10, UV11, UV12	259; 385 (cut-off)	667104

A: Wavelengths for absorbance W: Wavelengths for wavelength accuracy S: Wavelengths for stray light R: Wavelengths for spectral resolution *with Hellma Analytics calibration certificate

Complete Set 667-UV003 — 667-UV100 — 667-UV200 — 667-UV305

667-UV 1 667-UV 12 667-UV 6 667-UV 9 667-UV 60 667-UV 600 667-UV 14 667-UV 5

Liquid filters with DAkkS calibration certificates

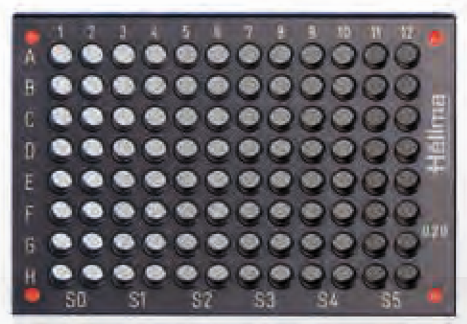
Liquid Filter for testing the resolution			
667-UV6*	Toluene in n-hexane	Scan: 265 – 270	667006
667-UV9*	n-hexane (reference filter)	Scan: 265 – 270	667009
667-UV200*	Filter Set UV-200: UV6, UV9	Scan: 265 – 270	667200

TYPE	CONTENT	WAVELENGTH nm	ARTICLE-NO.
Liquid Filter – Set according to USP 851			
667-UV004	F0: Aluminum frame, F2: Neutral Density Glass filter(0.25 Abs), F3: Neutral Density Glass filter (0.5 Abs), F4: Neutral Densitiy Glass filter (1.0 Abs), UV60: 60 mg potassium dichromate in $HClO_4$ UV 14: Perchloric acid ($HClO_4$, reference filter , UV 5: Holmium oxide in perchloric acid	A: 440; 465; 546.1; 590; 635 A: 440; 465; 546.1; 590; 635 A: 440; 465; 546.1; 590; 635 A: 235; 257; 313; 350 A: 235; 257; 313; 350 W: 241; 250; 278; 287; 333; 345; 361; 385; 416; 452; 468; 485; 536; 640	667004 New
Complete Filter Set for testing the photometer according to Ph.Eur.			
667-UV003	Complete Filter Set: UV1, UV12, UV6, UV9, UV60, UV600, UV14, UV5	A: 235; 257; 313; 350; 430 W: 241; 287; 361; 536; 640 S: 200 (cut-off) R: Scan 265 – 270	667003

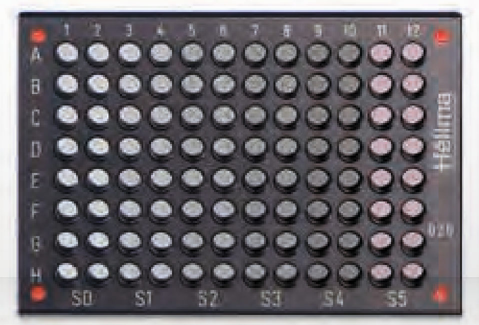
A: Wavelengths for absorbance W: Wavelengths for wavelength accuracy S: Wavelengths for stray light R: Wavelengths for spectral resolution *with Hellma Analytics calibration certificate

Reference plates for qualifying microplate readers with DAkkS calibration certificates

TYPE	USAGE	MATERIAL Nominal value of absorption	WAVELENGTH nm	ARTICLE-NO.
666-R013	to check photometric accuracy	Neutral Density Glass Filter NG 11 (0.25) , NG 5 (0.5), NG 4 (1.0), NG 3 (1.5), (2.5)	405, 450, 490, 650	666R013
666-R113	to check photometric accuracy and wavelength accuracy	Neutral Density Glass Filter NG 5 (0.5), NG 4 (1.0), NG 3 (1.5), (2.0) Holmium Oxide glass filter	405; 450; 490; 650 279; 361; 453; 536; 638	666R113



666-R013



666-R113

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