Hellma® Analytics
High Precision in Spectro-Optics

Optical Reference Materials for UV/VIS Spectroscopy

PRODUCT CATALOGUE WITH HANDLING GUIDELINES

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

NIST
TRACEABLE

MADE IN GERMANY

Hellma®

RELIABLE AND SAFE MEASUREMENT RESULTS IN OPTICAL ANALYTICS

For over 100 years **Hellma** has been the world's leading supplier of UV|VIS|NIR spectroscopy cuvettes for laboratory analytics and is now also a solution specialist in process analytical technology for a wide range of applications in research and industry. Products and services of the **Hellma Analytics** and **Hellma Solutions** brands provide the basis for precise and reliable measurement results in laboratory and process and thus safe and high-quality end products in the chemical, pharmaceutical, life science, food and beverage, cosmetics, environment, energy, technology and research industries. Eight Hellma sales subsidiaries worldwide and over 200 international points of sales are available to customers for direct contact. Hellma is valued worldwide for its high performance, consulting competence and well-proven products. **100% Made in Germany.**

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Traceable to
NIST, PTB

DAkkS
accredited calibration laboratory*

ACCREDITED* ACCORDING TO DIN EN ISO/ IEC 17025

30 YEARS GUARANTEE
with regular recalibration

COMPLIANT
with the most important Pharmacopeias

* Accredited calibration laboratory by DAkkS according to DIN EN ISO/IEC 17025. The accreditation is valid only for the scope listed in the annex of the accreditation certificate D-K-18752-01-00.

Benefit from from over 100 years Experience.

EXPERTS TIP:

Where you see this icon you will find useful information to make your processes safer and more effective.

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* Accredited calibration laboratory by DAkkS according to DIN EN ISO/IEC 17025.
The accreditation is valid only for the scope listed in the annex of the accreditation certificate D-K-18752-01-00.

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, 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 must 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.
ENJOY READING!

FOR YOUR INFORMATION

Proven reliability completely documented.
The Hellma Analytics calibration laboratory is the only calibration laboratory in Germany accredited for the calibration of UV/Vis reference materials. The calibration laboratory is accredited by DAkkS according to DIN EN ISO/IEC 17025. The accreditation is valid only for the scope listed in the annex of the accreditation certificate D-K-18752-01-00.

1.1 Hellma Analytics calibration laboratory: Accredited* according to DIN EN ISO/IEC 17025

The Hellma calibration laboratory is accredited* by DAkkS according to DIN EN ISO/IEC 17025. The DIN EN ISO / IEC 17025 standard requires a comprehensive QM system that seamlessly connects to other systems such as ISO 9001. By achieving this accreditation*, we demonstrate proof-of-expertise in the calibration activities for the optical measurement variable radiometry. The accreditation* authorizes us to issue internationally recognized DAkkS* calibration certificates and is the key to the high quality of the measurements, the international comparability and confidence in the work of the calibration laboratory and the transparency of the results.

DAkkS
Deutsche
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D-K-18752-01-00

NIST
TRACEABLE

DIN EN ISO/IEC 17025*

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With the UV/Vis reference materials from Hellma Analytics, we create the basis for reliable measurements for our customers.

Birgit Kehl,
Compliance Manager
Calibration Laboratory

1.2 Traceable test equipment

Quality assurance and quality control regulations, such as ISO 9001, GLP, GMP and Pharmacopeias, require companies to verify the consistently accurate 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.

The production of the optical reference materials in the Hellma Analytics calibration laboratory, which is accredited by the DAkkS according to DIN EN ISO/IEC 17025, is based on the regulatory codes issued by NIST (National Institute of Standards and Technology), ASTM (American Society for Testing and Materials) and Pharmacopeias (Ph. Eur., USP). The accreditation is valid only for the scope listed in the annex of the accreditation certificate D-K-18752-01-00. All measurement results can be traced back to NIST (photometric accuracy) or to PTB (Physikalisch-Technische Bundesanstalt) (wavelength accuracy) standard reference materials (Photometric accuracy: NIST SRM® 930e, NIST SRM® 1930. Hellma 666S300; wavelength accuracy: Hellma 667005).

FOR YOUR INFORMATION

The UV/Vis reference materials from Hellma Analytics comply with the requirements stipulated by quality management systems and Pharmacopeias, meeting the highest quality requirements and ensuring the international comparability of measurement results.

Choose between filter glass-based optical reference materials, so called glass filters, and liquid-based optical reference materials, so called liquid filters:

1.3 Glass filter applications

666 at the beginning of the article number identifies our glass filters. Glass filters are reference materials made of glass manufactured specifically for calibration. They are, above all, extremely robust. All glass filters measured by Hellma Analytics are traceable to NIST primary standards. Glass filters are suitable for checking the following parameters of your spectrophotometer:

- **Wavelength accuracy**
- **Photometric accuracy (absorbance)**
- **Photometric linearity (Vis range)**

1.4 Liquid filter applications

667 at the beginning of the article number identifies our liquid filters. Liquid filters are liquid reference materials that are manufactured in compliance with Pharmacopeias and/or NIST standards and inserted 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 measurement conditions. Hellma Analytics liquid filters are suitable for checking the following parameters of your spectrophotometer:

- **Wavelength accuracy**
- **Photometric accuracy (absorbance)**
- **Photometric linearity in the UV range**
- **Stray light levels**
- **Spectral resolution**

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

HELLMA ANALYTICS REFERENCE MATERIALS IN ACCORDANCE WITH THE MOST IMPORTANT REGULATIONS

MATERIAL	CHECKING OF	RANGE	Ph. Eur.	USP <857>	ASTM
GLASS FILTERS					
Holmium glass	Wavelength accuracy	UV/Vis	×	×	×
Didymium glass	Wavelength accuracy	UV/Vis	×	(×)	
Neutral density glass	Photometric accuracy / linearity	Vis	×	×	×
LIQUID FILTERS					
Holmium (solution)	Wavelength accuracy	UV/Vis	×	×	×
Rare Earth (Cerium-solution)	Wavelength accuracy	UV	×	×	
Didymium (solution)	Wavelength accuracy	UV/Vis	×	×	
Potassium dichro-mate (solution)	Photometric accuracy / linearity	UV/Vis	(×)	×	×
Nicotinic acid (Niacin-solution)	Photometric accuracy / linearity	UV	×	×	
Toluene in hexane (solution)	Spectral resolution	UV	×	×	
Potassium chloride (solution)	Stray light	UV	×	×	×
Potassium iodide (solution)	Stray light	UV	×	×	×
Sodium iodide (solution)	Stray light	UV	×	×	×
Sodium nitrite (solution)	Stray light	UV	×	×	×
Acetone (high purity)	Stray light	UV	(×)	×	×

(×) Not explicitly listed, but can be used according to the corresponding pharmacopoeia.

1.5 DAkkS calibration certificate*

After careful production, reference materials are measured in the Hellma Analytics calibration laboratory, which is accredited by DAkkS according to DIN EN ISO/IEC 17025, using a high-performance UV-Vis/NIR spectrophotometer. The accreditation is valid only for the scope listed in the annex of the accreditation certificate D-K-18752-01-00. Reference materials are only considered to be calibrated if they have been issued with a DAkkS calibration certificate and bear a calibration mark. Using the measurement values documented in the calibration certificate, users can check and calibrate their spectrophotometers accordingly.

* Accredited calibration laboratory by DAkkS according to DIN EN ISO/IEC 17025. The accreditation is valid only for the scope listed in the annex of the accreditation certificate D-K-18752-01-00.

Muster

Hellma Analytics
High Precision in Spectro-Optics

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

Kalibrierschein / Calibration Certificate

erstellt durch das Kalibrierlaboratorium
Issued by the calibration laboratory

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

Kalibrierzeichen
Calibration mark

94234
D-K-18752-01-00
2023-11

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*	**8005**
Auftraggeber / *Customer*	**Hellma Analytics GmbH Klosterrunsstr. 5 79379 Müllheim**
Auftragsnummer / *Order No.*	**712345**
Anzahl der Seiten des Kalibrierscheines / *Number of pages of the certificate*	**3**
Datum der Kalibrierung / *Date of calibration*	**14. November 2023** / *14 November 2023*

Dieser Kalibrierschein dokumentiert die metrologische Rückführbarkeit 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 metrological 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 of 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 des ausstellenden Kalibrierlaboratoriums. Kalibrierscheine sind bei Nennung des für die Freigabe Verantwortlichen in Klarschrift auch ohne Unterschrift gültig. Die dargestellten Ergebnisse beziehen sich nur auf die kalibrierten Gegenstände.
This calibration certificate may not be reproduced other than in full except with the permission of the issuing laboratory. Calibration certificates with the full name of the approval responsible person are valid without signature. The presented results relate only to the items calibrated.

Datum der Ausstellung / *Date of issue*	Freigabe des Kalibrierscheins durch / *Approval of the calibration certificate by*
14. November 2023 / *14 November 2023*	Birgit Kehl

FO-CERT-039
Rev.: 8 - 12.07.2023

1. Reference to the accreditation*
2. Name and address of issuing calibration laboratory
3. Description of calibrated item
4. Clear details of measured calibration item
5. Customer
6. Date of calibration measurement
7. Internal document reference, including date of issue and revision status
8. Date of issue of calibration certificate
9. Calibration certificate serial number
10. Hellma registration number at DAkkS*
11. Year and month of calibration
12. Approval by authorized person

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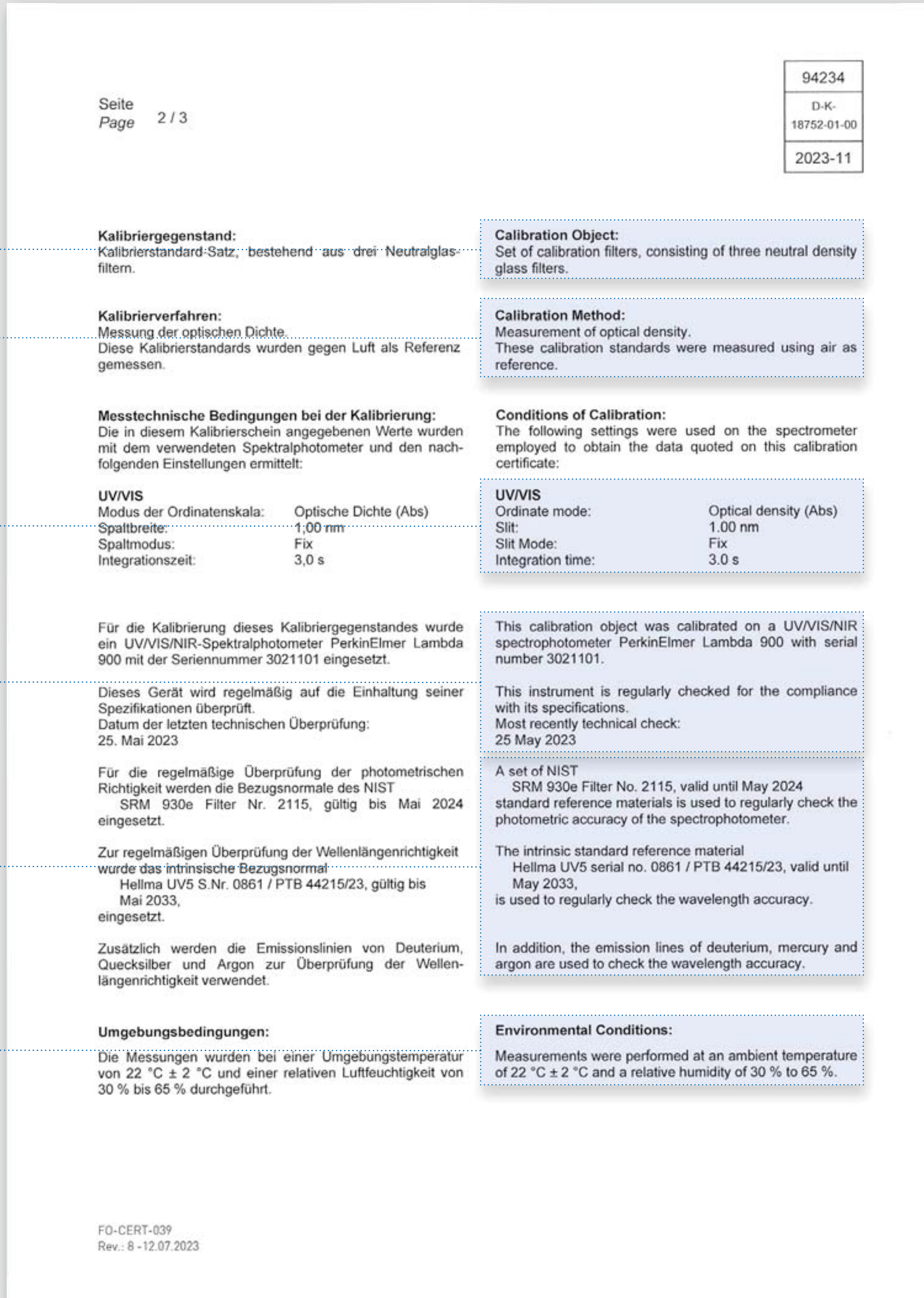
IMPORTANT INFORMATION

Only if the DAkkS calibration certificate* has been issued and the calibration mark has been affixed, do the reference materials become traceable reference materials.

* Accredited calibration laboratory by DAkkS according to DIN EN ISO/IEC 17025. The accreditation is valid only for the scope listed in the annex of the accreditation certificate D-K-18752-01-00.



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2023-11

1 **Kalibriergegenstand:** Kalibrierstandard-Satz, bestehend aus drei Neutralglasfiltern.	**Calibration Object:** Set of calibration filters, consisting of three neutral density glass filters.
2 **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:
3 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
4 Für die Kalibrierung dieses Kalibriergegenstandes wurde ein UV/VIS/NIR-Spektralphotometer PerkinElmer Lambda 900 mit der Seriennummer 3021101 eingesetzt. Dieses Gerät wird regelmäßig auf die Einhaltung seiner Spezifikationen überprüft. Datum der letzten technischen Überprüfung: 25. Mai 2023	This calibration object was calibrated on a UV/VIS/NIR spectrophotometer PerkinElmer Lambda 900 with serial number 3021101. This instrument is regularly checked for the compliance with its specifications. Most recently technical check: 25 May 2023
5 Für die regelmäßige Überprüfung der photometrischen Richtigkeit werden die Bezugsnormale des NIST SRM 930e Filter Nr. 2115, gültig bis Mai 2024 eingesetzt. Zur regelmäßigen Überprüfung der Wellenlängenrichtigkeit wurde das intrinsische Bezugsnormal Hellma UV5 S.Nr. 0861 / PTB 44215/23, gültig bis Mai 2033, eingesetzt. Zusätzlich werden die Emissionslinien von Deuterium, Quecksilber und Argon zur Überprüfung der Wellenlängenrichtigkeit verwendet.	A set of NIST SRM 930e Filter No. 2115, valid until May 2024 standard reference materials is used to regularly check the photometric accuracy of the spectrophotometer. The intrinsic standard reference material Hellma UV5 serial no. 0861 / PTB 44215/23, valid until May 2033, is used to regularly check the wavelength accuracy. In addition, the emission lines of deuterium, mercury and argon are used to check the wavelength accuracy.
6 **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-CERT-039
Rev.: 8 - 12.07.2023

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

1.5 DAkkS Calibration Certificate*

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 recalibration intervals (according to DIN EN ISO/IEC 17025). Exceptions may be made if requested by the customer or required by legislation.
5. Provision of the filters by the customer or by Hellma in the case of new filters.

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2023-11

Messergebnisse:

Während der Messungen wurden die folgenden Werte ermittelt:

Measurement Results:

During the measurements, the following data were obtained:

	Serien-Nr. *serial number* Filter Typ *filter type*	Optische Dichte (Abs) ± MU(*) (Abs) *Optical Density (Abs) ± MU(*) (Abs)*				
		440 nm	465 nm	546.1 nm	590 nm	635 nm
gemessener Wert *Measured Value*	8005 *666-F2*	0.2707 *± 0.0024*	0.2432 *± 0.0024*	0.2528 *± 0.0024*	0.2970 *± 0.0024*	0.3035 *± 0.0024*
gemessener Wert *Measured Value*	8005 *666-F3*	0.5378 *± 0.0028*	0.4941 *± 0.0028*	0.5074 *± 0.0028*	0.5671 *± 0.0034*	0.5664 *± 0.0034*
gemessener Wert *Measured Value*	8005 *666-F4*	1.0190 *± 0.0034*	0.9454 *± 0.0034*	0.9589 *± 0.0034*	1.0253 *± 0.0034*	0.9932 *± 0.0034*

(*) MU: Messunsicherheit – Measurement Uncertainty

Angegeben ist die erweiterte Messunsicherheit, die sich aus der Standardmessunsicherheit durch Multiplikation mit dem Erweiterungsfaktor $k = 2$ ergibt. Sie wurde gemäß EA-4/02 M:2022 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 EA-4/02 M:2022. The value of the measurand lies within the assigned range of values with a probability of 95 %.

Ort der Durchführung der Labortätigkeiten
Hellma GmbH & Co. KG
Klosterrunsstr. 5,
79379 Müllheim, Deutschland
Telefon: +49 7631 182-0

Location of performance of the laboratory activities
Hellma GmbH & Co. KG
Klosterrunsstr. 5,
79379 Müllheim, Germany
Phone: +49 7631 182-0

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 gegen Gebühr auf Anfrage erhältlich.
Die Beistellung der oben aufgeführten Kalibriergegenstände erfolgte durch den Auftraggeber.

Notes
Upon delivery at Hellma, the "as received" condition of all optical density filters is measured before routinely cleaning the standards under the recertification procedure. "As received" data is available for a fee upon request.

The customer provided the calibration objects mentioned above.

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.

Ende des Kalibrierscheins.

End of the calibration certificate.

FO-CERT-039
Rev.: 8 -12.07.2023

1. Measured value and assigned measurement uncertainty
2. Note on measurement uncertainty
3. Information
4. Recalibration interval
5. Providing the filters

1.6 Warranty – 30-year manufacturer's warranty on all Hellma Analytics reference materials

We're confident of our quality and you can be confident of reliable measurement results! All Hellma Analytics reference materials come with a 30-year warranty, provided that they are regularly recalibrated – at least every two years – at the Hellma Analytics calibration laboratory.

Reference materials sent in for recalibration are carefully cleaned and recalibrated before being sent back with a new calibration certificate and calibration mark. Damaged filters and filters that deviate significantly from nominal values are usually replaced in consultation with the customer.

Details of recalibration can be found on pages 34 and 35.

All our reference materials come with a 30-year warranty, provided that they are regularly recalibrated (at least every two years) at the Hellma Analytics calibration laboratory.

2. GLASS FILTER

Wavelength Accuracy

2.1 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.

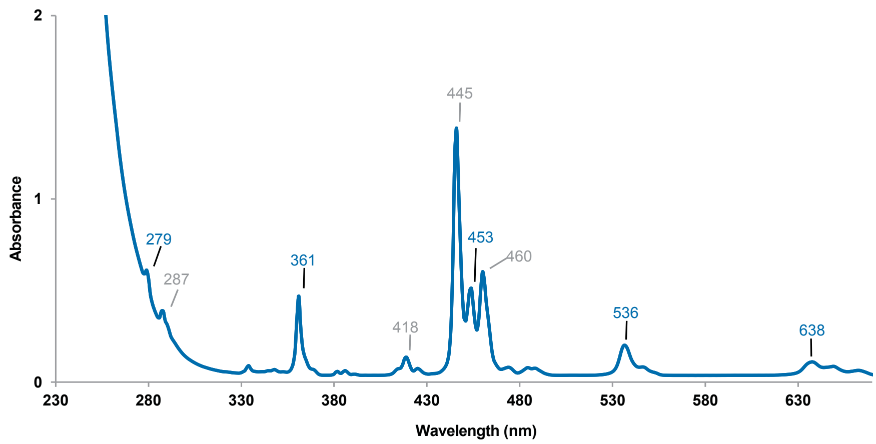
2.1.1 Holmium glass filter

PRODUCT DESCRIPTION

The holmium 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 solution, the holmium 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.

NOTE

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



Typical spectrum of a holmium glass filter

ARTICLE NO.	666F1-339
APPLICATION	Checking the wavelength accuracy in the UV and Vis range (270 nm to 640 nm)
CONTENT	Holmium glass filter with metal frame
STANDARD MEASUREMENT	**Wavelengths:** 279, 361, 453, 536, 638 nm **Slit width:** 1 nm
POSSIBLE MEASUREMENTS	**Wavelengths:** 279; 287; 361; 418; 445; 453; 460; 536; 638 nm **Slit width:** 1-2 nm (0.1-0.9 nm only on request)

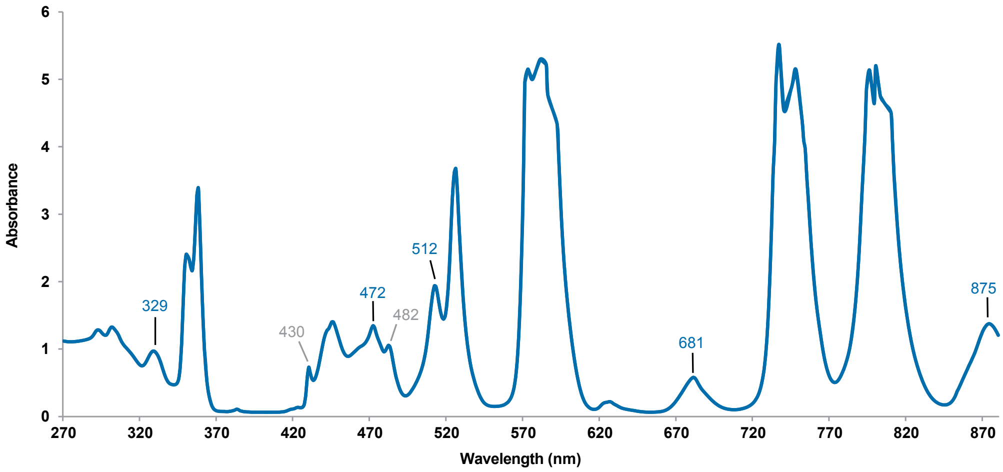
2.1.2 Didymium glass filter

PRODUCT DESCRIPTION

The didymium glass filter 666-F7W is made from material specially manufactured by Schott AG. Like holmium glass, didymium glass has a variety of characteristic peaks in the ultraviolet and visible range and is therefore typically used for checking wavelength accuracy. However, its peaks are not as narrow as those of holmium glass filters.

NOTE

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



Typical spectrum of a didymium glass filter

ARTICLE NO.	666F7W-323
APPLICATION	Checking the wavelength accuracy in the UV and Vis range (320 nm to 880 nm)
CONTENT	Didymium glass filter with metal frame
STANDARD MEASUREMENT	**Wavelengths:** 329, 472, 512, 681, 875 nm **Slit width:** 1 nm
POSSIBLE MEASUREMENTS	**Wavelengths with 1 nm slit width:** 329; 430; 472; 482; 512; 681; 875 nm **Wavelengths with 2 nm slit width:** 430; 472; 482; 512; 875 nm

Photometric Accuracy

2.2 Checking the photometric accuracy

APPLICATION

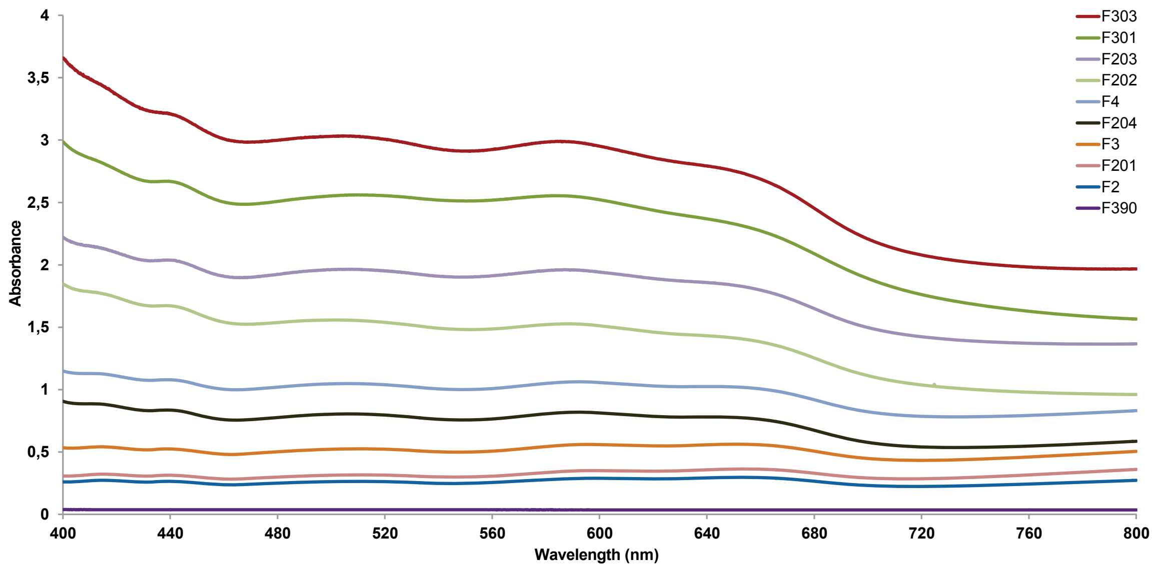
To measure photometric accuracy (absorbance), the filter reduces the light beam from the spectrophotometer. An absorbance value (Abs) can be deduced from the light extinction caused by the filter.

> **NOTE**
>
> 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 calibration certificate in a diagram.

2.2.1 Neutral density glass filter

PRODUCT DESCRIPTION

Hellma Analytics neutral density glass filters are made from filter materials produced by Schott AG, which are selected on account of their homogeneity and stability. Thanks to a relatively constant transmittance within the wavelength range of 405 nm to 890 nm, they have been used to check photometric accuracy and linearity in the visible wavelength range (over 405 nm) for decades. Please note, the thickness of the filter is set at the manufacturing so that the specified nominal optical density (values from 0.04 to 3.0 Abs) is obtained at 546.1 nm. This results in slightly increasing absorbances at shorter wavelengths.



Typical spectrum of a neutral glass filter with a slit width of 1 nm

ARTICLE NO.	**666F390-25, 666F2-39, 666F201-39, 666F3-38, 666F204-37, 666F4-37, 666F202-36, 666F203-36, 666F301-361, 666F303-361**
APPLICATION	Checking the photometric accuracy in the Vis range (405 – 890 nm)
CONTENT	**Neutral density glass filters:** F390 (0.04 Abs); F2 (0.25 Abs); F201 (0.3 Abs); F3 (0.5 Abs); F204 (0.7 Abs); F4 (1.0 Abs); F202 (1.5 Abs); F203 (2.0 Abs); F301 (2.5 Abs); F303 (3.0 Abs)
STANDARD MEASUREMENT	**Wavelengths:** 440, 465, 546.1, 590, 635 nm **Slit width:** 1 nm
POSSIBLE MEASUREMENTS	All wavelengths from 405 to 890 nm possible. Over 890 nm is also possible with Hellma Analytics calibration certificate (since the accredited* range ends at 890 nm). **Slit widths:** all up to 5 nm

* Accredited calibration laboratory by DAkkS according to DIN EN ISO/IEC 17025.The accreditation is valid only for the scope listed in the annex of the accreditation certificate D-K-18752-01-00

Sets

2.3 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 calibration certificate provided

Handling instructions for glass filters can be found on the pages 46 to 51.

FILTER-SET	CONTENT	WAVELENGTH nm	ARTICLE NO.
Glass filter sets for checking photometric accuracy, photometric linearity and wavelength accuracy			
666-S000	Glass filter set: F0, F1, F2, F3, F4 (Abs: 0.25, 0.5, 1.0)	A: 440; 465; 546.1; 590; 635 W: 279; 361; 453; 536; 638	666S000
666-S002	Glass filter set: F2, F3, F4 (Abs: 0.25, 0.5, 1.0)	A: 440; 465; 546.1; 590; 635	666S002
666-S004	Glass filter set: F0, F201, F202, F203 (Abs: 0.3, 1.5, 2.0)	A: 440; 465; 546.1; 590; 635	666S004
666-S005	Glass filter set: F0, F1, F3, F4 (Abs: 0.5, 1.0)	A: 440; 465; 546.1; 590; 635 W: 279; 361; 453; 536; 638	666S005
666-S006	Glass filter set: F0, F2, F3, F4 (Abs: 0.25, 0.5, 1.0)	A: 440; 465; 546.1; 590; 635	666S006
666-S010	Glass filter set: F1, F390, F2, F3, F4 (Abs: 0.04, 0.25, 0.5, 1.0)	A: 440; 465; 546.1; 590, 635 W: 279; 361: 453; 536; 638	666S010
666-S200	Glass filter set: F2, F4, F203 (Abs: 0.25, 1.0, 2.0)	A: 440; 465; 546.1; 590; 635	666S200
666-S300	Glass filter set: F390, F301, F303 (Abs: 0.04, 2.5, 3.0)	A: 440; 465; 546.1; 590; 635	666S300

3. LIQUID FILTERS

Wavelength Accuracy

3.1 Checking wavelength accuracy

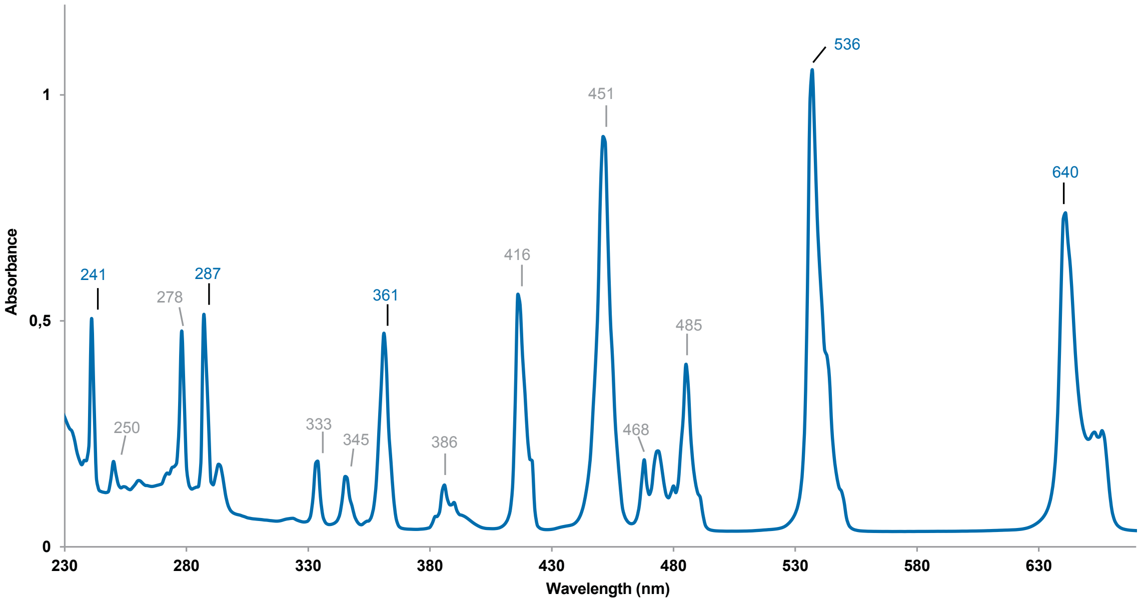
APPLICATION

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.

3.1.1 Holmium liquid filter

PRODUCT DESCRIPTION

The holmium 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. This filter is recommended in both the Ph. Eur. and USP for checking wavelength accuracy in the 240 – 650 nm range.



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

ARTICLE NO.	667005
APPLICATION	Checking the wavelength accuracy according to **Ph. Eur.** in the UV and Vis range
CONTENT	Holmium oxide in perchloric acid
STANDARD MEASUREMENT	**Wavelengths:** 241, 287, 361, 451, 485, 536, 640 nm **Slit width:** 1 nm
POSSIBLE MEASUREMENTS	**Wavelengths:** 241, 250, 278, 287, 333, 345, 361, 386, 416, 451, 468, 485, 536, 640 nm **Slit width:** 1-2 nm (0.1- 0.9 nm only on request), above peaks become indistinct

ARTICLE NO.	667005USP
APPLICATION	Checking the wavelength accuracy according to **USP <857>** in the UV and Vis range
CONTENT	Holmium oxide in perchloric acid
STANDARD MEASUREMENT	**Wavelengths:** 241, 250, 278, 287, 333, 345, 361, 386, 416, 451, 468, 485, 536, 640 nm **Slit width:** 1 nm
POSSIBLE MEASUREMENTS	**Slit width:** 1-2 nm

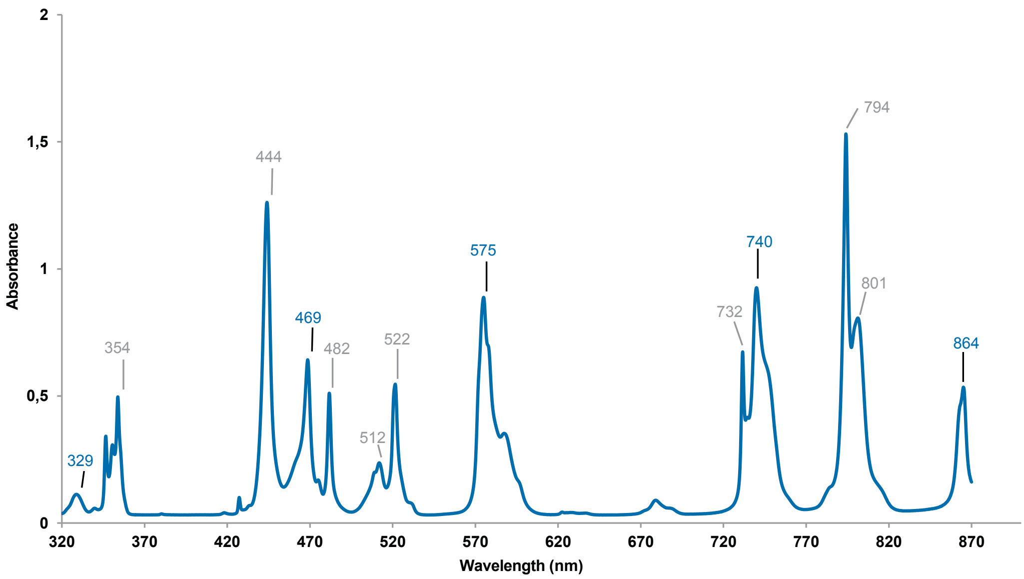
NOTE ON WAVELENGTH MEASUREMENT ACCORDING TO Ph. Eur.

The Ph. Eur. requires that all measurements be measured against the corresponding solvent as a reference for background correction. This means that to check the wavelength accuracy using holmium oxide in perchloric acid (UV5), perchloric acid (UV14), the corresponding solvent, must be used as a reference.

3.1.2 Didymium liquid filter

PRODUCT DESCRIPTION

The didymium liquid filter consists of praseodymium and neodymium, dissolved in perchloric acid. This filter is ideally suited for 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 320 nm and 870 nm. This filter is recommended in both Ph. Eur. and USP <857> for checking wavelength accuracy above 640 nm.



Typical spectrum of didymium dissolved in perchloric acid at a slit width of 1 nm

ARTICLE NO.	667025
APPLICATION	Checking the wavelength accuracy in the UV and Vis range
CONTENT	Didymium in perchloric acid
STANDARD MEASUREMENT	**Wavelengths:** 329, 469, 575, 740, 864 nm **Slit width:** 1 nm
POSSIBLE MEASUREMENTS	**Wavelengths:** 329, 354, 444, 469, 482, 512, 522, 575, 732, 740, 794, 801, 864 nm **Slit width:** 1-2 nm (0.1- 0.9 nm only on request), above peaks become indistinct

ARTICLE NO.	667025EPUSP
APPLICATION	Checking the wavelength accuracy according to **Ph. Eur.** and **USP <857>**
CONTENT	Didymium in perchloric acid
STANDARD MEASUREMENT	**Wavelengths:** 512, 732, 740, 794, 801, 864 nm **Slit width:** 1 nm
POSSIBLE MEASUREMENTS	**Slit width:** 1-2 nm

Wavelength Accuracy

3.1.3 HoDi liquid filter

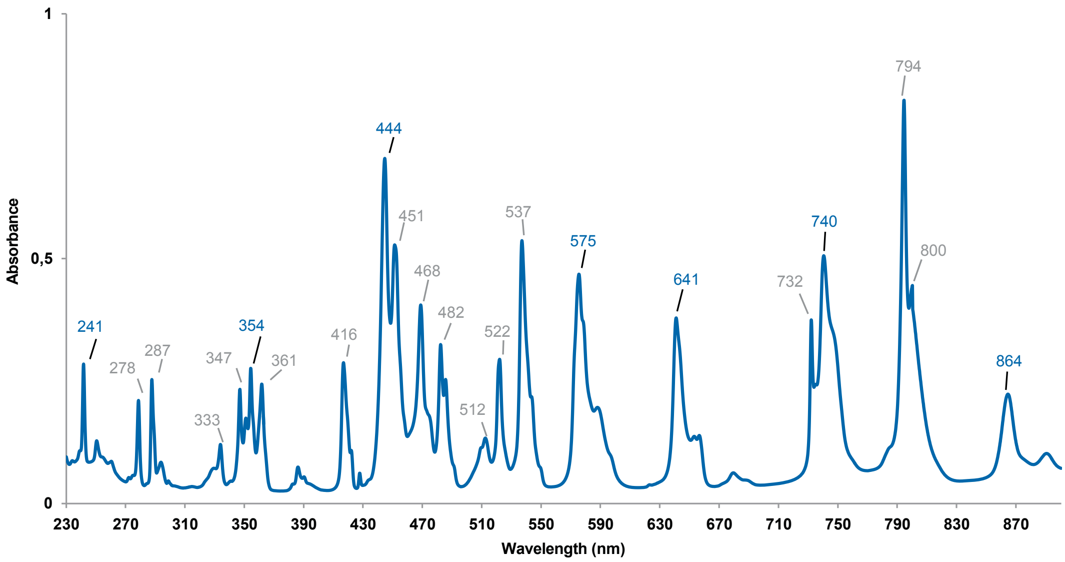
PRODUCT DESCRIPTION

The HoDi liquid filter consists of a solution of holmium oxide and didymium (praseodymium and neodymium) in perchloric acid. This filter features an especially broad wavelength spectrum and is therefore ideally suited to checking the wavelength accuracy of spectrophotometers in the UV and visible range. It has a broad spectrum with a variety of characteristic, very well-defined peaks in the range between 241 nm and 864 nm. Depending on the performance of the spectrophotometer used, up to 22 peaks can be detected at a slit width of 1 nm.

Checking the UV-Vis wavelength accuracy

Broad wavelength spectrum from 240 – 870 nm

Two filters in one: Holmium + Didymium = HoDi



Typical spectrum of HoDi filter measured at a slit width of 1 nm

ARTICLE NO.	667045
APPLICATION	Checking the wavelength accuracy in the UV and Vis range
CONTENT	Holmium oxide and didymium in perchloric acid
STANDARD MEASUREMENT	**Wavelengths:** 241, 354, 444, 575, 641, 740, 864 nm **Slit width:** 1 nm
POSSIBLE MEASUREMENTS	**Wavelengths:** 241, 278, 287, 333, 347, 354, 361, 416, 444, 451, 468, 482, 512, 522, 537, 575, 641, 732, 740, 794, 801, 864 nm **Slit width:** 1 nm (0.1-2 nm only on request)

ARTICLE NO.	667045EPUSP
APPLICATION	Checking the wavelength accuracy according to **Ph. Eur.** and **USP <857>**
CONTENT	Holmium oxide and didymium in perchloric acid
STANDARD MEASUREMENT	**Wavelengths:** 241, 287, 361, 451, 482, 512, 537, 641, 732, 740, 794, 801, 864 nm **Slit width:** 1 nm

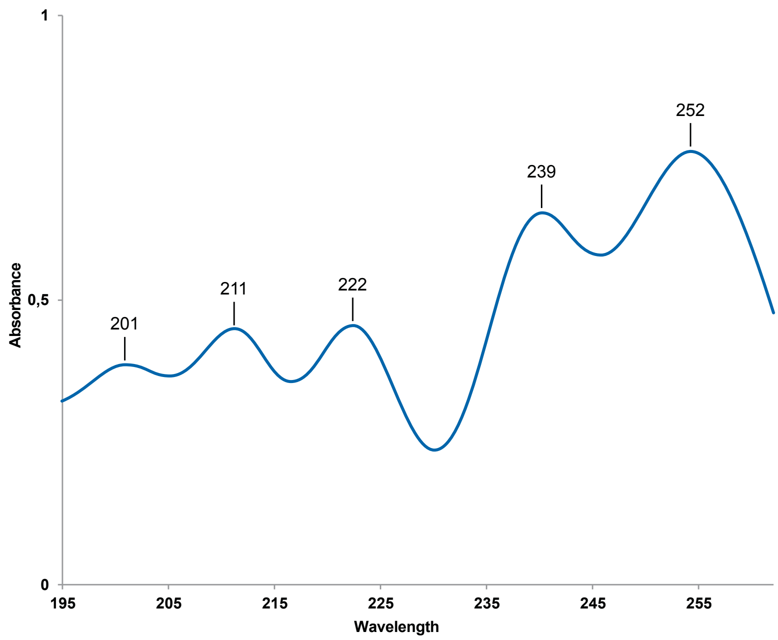
3.1.4 Rare Earth (cerium) liquid filter

PRODUCT DESCRIPTION

The rare earth liquid filter consists of cerium dissolved in perchloric acid. This filter is ideally suited for checking the wavelength accuracy of spectrophotometers in the low UV range. It has a spectrum with five characteristic peaks in the range from 200 nm to 260 nm. This filter is recommended in both Ph. Eur. and USP <857> for checking wavelength accuracy in the 200 – 260 nm range.

Specially developed for checking wavelength accuracy in the low UV range

Wavelength spectrum from 200 – 260 nm



Typical spectrum of a rare earth filter measured at a slit width of 1 nm

ARTICLE NO.	667035
APPLICATION	Checking the wavelength accuracy in the low UV range according to **Ph. Eur.** and **USP <857>**
CONTENT	Cerium in perchloric acid
STANDARD MEASUREMENT	**Wavelengths:** 201, 211, 222, 239, 252 nm **Slit width:** 1 nm

Photometric Accuracy

3.2 Checking the 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 attenuation caused by the filter.

3.2.1 Potassium dichromate liquid filter for checking the photometric accuracy in accordance to USP <857> (and Ph. Eur.)

PRODUCT DESCRIPTION

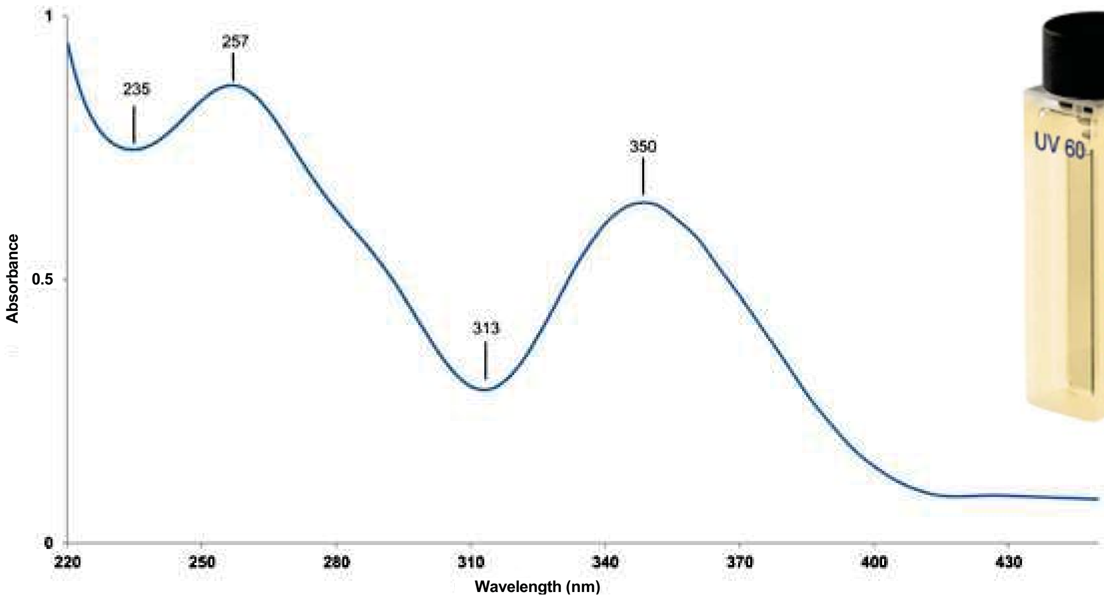
Potassium dichromate in perchloric acid is very suitable for checking the photometric accuracy of spectrophotometers. In the UV range, the potassium dichromate spectrum has characteristic maxima at 257 nm and 350 nm and characteristic minima at 235 nm and 313 nm. The spectrum reaches a plateau at 430 nm, which is used to determine photometric accuracy in the visible range. Hellma Analytics purchases the reference material for this filter directly from NIST (SRMR 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.

APPLICATION

The potassium dichromate liquid filters are used to check the photometric accuracy (absorbance) as well as the linearity of the absorbance in the UV range, according to the specifications of the United States Pharmacopeia (USP<857>). In sealed quartz cuvettes, the potassium dichromate liquid filters can still be used for Ph. Eur. verification.

NOTE

Until the end of 2019, the Ph. Eur. still recommended the potassium dichromate solution for checking the photometric accuracy. With the publication of EP 10 in January 2020, the manual production of potassium dichromate solutions is no longer provided because potassium dichromate is listed in Annex XIV of the REACH regulation. Instead, the manufacturing process of a nicotinic acid solution is described. In principle, however, traceable potassium dichromate solutions in sealed quartz cuvettes are still permitted without restrictions for checking the photometric accuracy and linearity according to Ph. Eur.



Typical spectrum of a potassium dichromate solution with 60 mg/L

ARTICLE NO.	667020, 667040, 667060, 667080, 6670100, 6670120, 6670140, 6670160, 6670180, 6670200, 667600, 667014 (REFERENCE FILTER)
APPLICATION	Checking the photometric accuracy in the UV range (235 nm to 350 nm) and Vis range (wavelength 430 nm)
CONTENT	UV20, 20 mg/L potassium dichromate in $HClO_4$, (0.1 – 0.3 Abs), UV40, 40 mg/L potassium dichromate in $HClO_4$, (0.2 – 0.6 Abs) UV60, 60 mg/L potassium dichromate in $HClO_4$, (0.3 – 0.9 Abs) UV80, 80 mg/L potassium dichromate in $HClO_4$, (0.4 – 1.2 Abs) UV0100, 100 mg/L potassium dichromate in $HClO_4$ (0.5 – 1.5 Abs) UV0120, 120 mg/L potassium dichromate in $HClO_4$ (0.6 – 1.8 Abs) UV0140, 140 mg/L potassium dichromate in $HClO_4$ (0.7 – 2.0 Abs) UV0160, 160 mg/L potassium dichromate in $HClO_4$ (0.8 – 2.3 Abs) UV0180, 180 mg/L potassium dichromate in $HClO_4$ (0.9 – 2.6 Abs) UV0200, 200 mg/L potassium dichromate in $HClO_4$ (1.0 – 3.0 Abs) UV600, 600 mg/L potassium dichromate in $HClO_4$ (1.0 Abs) UV14, perchloric acid ($HClO_4$), (reference filter)
STANDARD MEASUREMENT	**UV20 – UV0200 wavelengths:** 235; 257; 313; 350 nm (UV range) **UV600 Wavelengths:** 430 nm (Vis range) **Slit width:** 2 nm
POSSIBLE MEASUREMENTS	**Wavelengths:** fixed **Slit width:** 1 nm and 2 nm possible

Photometric Accuracy

3.2.2 Nicotinic acid (niacin) liquid filter for checking photometric accuracy according to Ph. Eur. and <USP>

PRODUCT DESCRIPTION

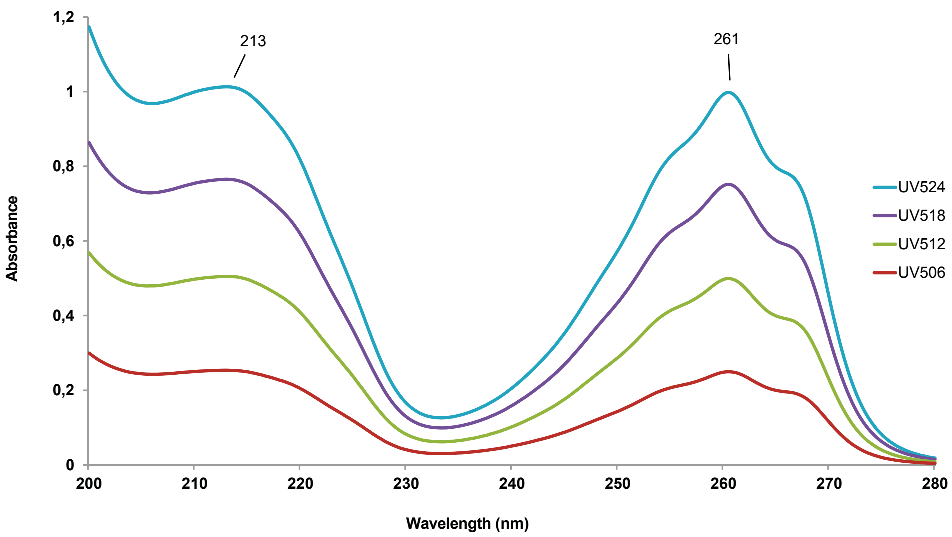
Niacin (nicotinic acid) in hydrochloric acid is highly suitable for checking the photometric accuracy of spectrometers. The niacin spectrum shows in the UV range two characteristic peaks at 213 nm and 261 nm. The niacin filter solutions are filled and immediately fused under controlled conditions to become permanently airtight.

APPLICATION

The niacin liquid filters are used to check the photometric accuracy (absorbance) as well as the linearity of the absorbance in the UV range, according to the specifications of the European Pharmacopoeia and the United States Pharmacopeia (USP <857>).

NOTE

The individually measured absorbance values are free from any systematic errors. The measured data of the reference filter UV599 (hydrochloric acid measured against air) appears separately on the calibration certificate. To check absorbance linearity, perform the measurement with niacin filters of different concentrations. List the measured absorbance values for each filter and each wavelength in a diagram against the values on the calibration certificate.



Typical spectrum of a niacin filter measured at a slit width of 1 nm

ARTICLE NO.	667506, 667512, 667518, 667524, 667536, 667548, 667599 (REFERENCE FILTER)
APPLICATION	Checking the photometric accuracy in the UV range (213 nm and 261 nm)
CONTENT	UV506, 6 mg/L niacin in HCl (approx. 0.25 Abs) UV512, 12 mg/L niacin in HCl (approx. 0.50 Abs) UV518, 18 mg/L niacin in HCl (approx. 0.75 Abs) UV524, 24 mg/L niacin in HCl (approx. 1.0 Abs) UV536, 36 mg/L niacin in HCl (approx. 1.5 Abs) UV548, 48 mg/L niacin in HCl (approx. 2.0 Abs) UV599, hydrochloric acid (HCl) (reference filter)
STANDARD MEASUREMENT	**UV506 - UV548 Wavelengths:** 213 nm and 261 nm **Slit width:** 1 nm
POSSIBLE MEASUREMENTS	**Wavelengths:** fixed **Slit width:** 1 nm and 2 nm possible

Stray light

Measurement in accordance with
Ph. Eur. and USP <857> procedure B

3.3 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.

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

3.3.1 Checking for stray light – measurement in accordance with Ph. Eur. and USP <857> Procedure B

PRODUCT DESCRIPTION

In the European Pharmacopeia (Chapter 2.2.25), checking for stray light is described as follows:

Stray light should be determined with suitable filters or solutions at an appropriate wavelength. The acceptance criterion according to Ph. Eur. depends on the filter used:

Filter / Solution	Absorbance at wavelength
Potassium chloride (12 g/L)	≥ 2.0 Abs at 198 nm
Sodium iodide (10 g/L)	≥ 3.0 Abs at 220 nm
Potassium iodide (10 g/L)	≥ 3.0 Abs at 250 nm
Sodium nitrite (50 g/L)	≥ 3.0 Abs at 340 and 370 nm

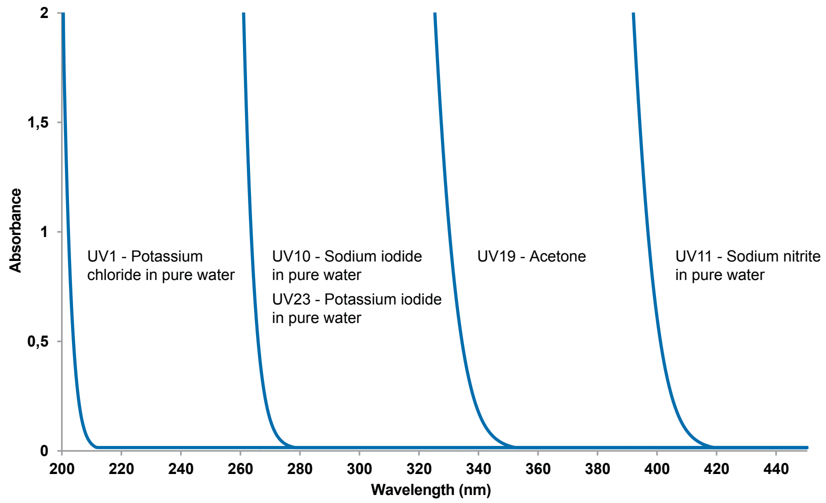
The USP <857> procedure B acceptance criteria are given as follows:

Filter / Solution	Absorbance at wavelength
Potassium chloride (12 g/L)	≥ 2.0 Abs at 198 nm
Sodium iodide (10 g/L)	≥ 2.0 Abs at 220 nm
Potassium iodide (10 g/L)	≥ 2.0 Abs at 220 nm
Acetone (high purity)	≥ 2.0 Abs at 300 nm
Sodium nitrite (50 g/L)	≥ 2.0 Abs at 340 nm

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.

When checking for stray light, both Ph. Eur. and USP <857> Procedure B require the respective stray light filter used to be measured against the reference filter filled with ultrapure water, with the exception of acetone, which is measured against air. The measured absorbance value must correspond to the respective acceptance criterion at the specified wavelength.

Stray light



Typical spectrum of the stray light filters

ARTICLE NO.	667001, 667010, 667011, 667019*, 667023, 667012 (REFERENCE FILTER FOR ZU 667001, 667010, 667011 AND 667023)
APPLICATION	Checking for stray light in the UV range, measurement according to Ph. Eur. and USP <857> Procedure B (at wavelengths from 198 nm to 370 nm, depending on the filter selected)
CONTENT	UV1, potassium chloride in pure water, ≥ 2 Abs at 198 nm UV10, sodium iodide in pure water, ≥ 3 Abs at 220 nm UV23, potassium iodide in pure water, ≥ 2,0 Abs at 220 nm and ≥ 3,0 Abs at 250nm UV19, acetone* (high purity), ≥ 2 Abs at 300 nm and 320 nm UV11, sodium nitrite in pure water, ≥ 3 Abs at 340 and 370 nm UV12, pure water (reference filter for UV1, UV10, UV11 and UV23)
STANDARD MEASUREMENT	**UV1:** Cut-off 200 nm **UV10:** Cut-off 259 nm **UV23:** Cut-off 259 nm **UV19:** Cut-off 325 nm **UV11:** Cut-off 388 nm **UV12:** Reference filter for UV1, UV10, UV11, UV23, measured against air at: 198, 200, 300, 400 nm **Slit width:** 2 nm
POSSIBLE MEASUREMENTS	**Wavelengths:** fixed **Possible slit width:** 1 nm and 2 nm possible

* measured against air

Measurement according USP <857> procedure A

3.3.2 Checking for stray light – measurement according to USP <857> Procedure A

PRODUCT DESCRIPTION

In USP <857> (update Dec. 2022), two possible procedures are described for checking for stray light:

Procedure A

Here the stray light filter with a path length of 10 mm is measured against the reference filter (filled with the same solution) with a path length of 5 mm. The stray light value can now be calculated from the absorbance maximum obtained, using the following formula: $s_\lambda = 0.25 \times 10^{-2\Delta A}$

The following acceptance criteria apply:

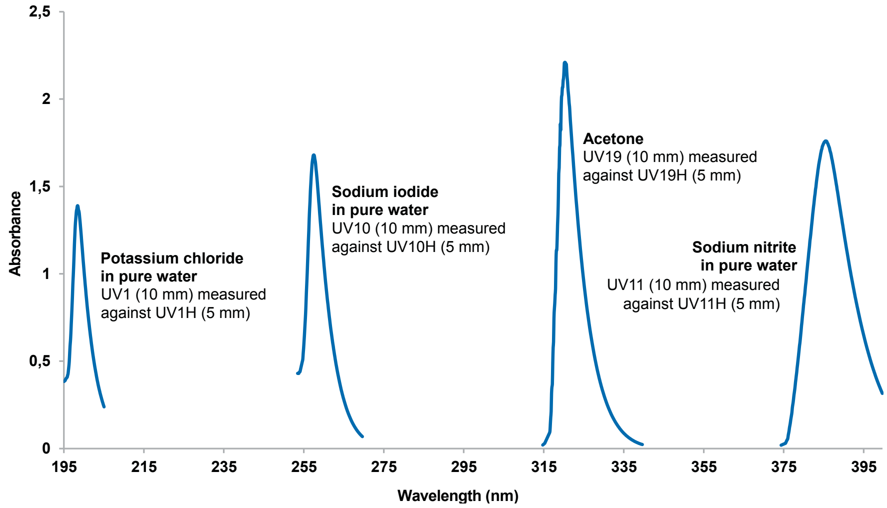
$\Delta A \geq 0.7$ Abs and $s_\lambda \leq 0.01$

ΔA = observed maximum absorbance

s_λ = stray light value in transmission (T)

Note

The measurement indicated on the calibration certificate for the wavelength at the peak maximum refers only to the measurement taken with the UV-Vis-NIR spectrophotometer shown on the calibration certificate. This wavelength is instrument-dependent due to the different optical components installed and the resulting differences in performance, and so it is not applicable to other UV-Vis-NIR spectrophotometers. The indicated measurement of the wavelength at the peak maximum is not suitable for checking the wavelength scale.



Typical spectrum of the stray light filters according to USP <857>, procedure A. The reference filter with 5 mm light path is marked with "H" for half.

Stray light

NOTE

Experience from everyday use shows that the values obtained using this stray light measurement method are extremely instrument-dependent, i.e. the wavelength of the peak position varies depending on the type of instrument and its performance. The important thing for the user to know is that, when using this testing method, the maximum absorbance measured in the testing range is the deciding factor and should be ≥ 0.7 Abs.

The Hellma Analytics USP stray light filter sets each consist of a 10 mm stray light filter and a 5 mm reference filter, both filled with the same solution. These filter sets meet the criteria of USP <857> and are therefore ideal for qualifying the stray light level of spectrophotometers in accordance with the requirements of the USP. The procedure for determining the stray light value is the same for all stray light filter sets; the difference lies in the Cut-off range for each set.

For improved handling of the reference filters with 5 mm light path, our USP stray light reference filters have the same external dimensions as a cuvette with 10 mm path length. The reference filters with 5 mm path length are marked with the letter H. There is no need for an additional spacer.

ARTICLE NO.	**667001/667001H, 667010/667010H, 667011/667011H, 667019/667019H** Letter H indicates the reference filter with 5 mm path length
APPLICATION	Checking for stray light in the UV range, measurement acc. to USP <857> procedure A (at wavelength 198 nm to 385 nm, depending on the selected filter)
CONTENT	UV1/UV1H, potassium chloride in pure water, acc. to USP <857> Procedure A UV10/UV10H, sodium iodide in pure water, acc. to USP <857> Procedure A UV11/UV11H, sodium nitrite in pure water, acc. to USP <857> Procedure A UV19/UV19H, pure acetone, acc. to USP <857> Procedure A
STANDARD MEASUREMENT	UV1/UV1H: Cut-off ca. 198 nm* UV10/UV10H: Cut-off ca. 258 nm* UV11/UV11H: Cut-off ca. 385 nm* UV19/UV19H: Cut-off ca. 322 nm* **Slit width: 2 nm**

* depending on the device

Spectral Resolution

3.4 Checking the spectral resolution

APPLICATION

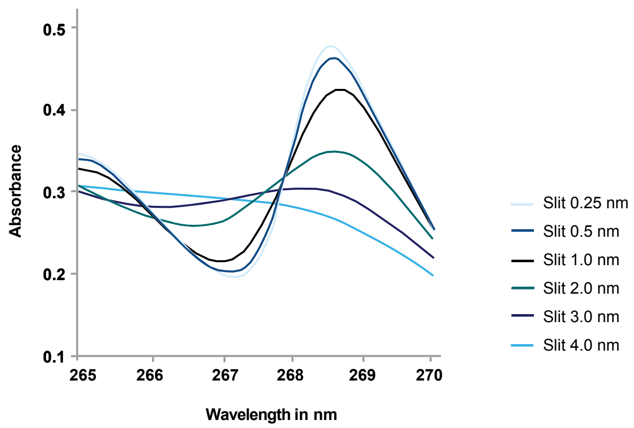
Regularly checking the spectral resolution of spectrophotometers ensures, for example, that neighboring peaks are resolved and not superimposed on the peaks of bordering wavelengths. This also prevents absorbance errors.

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 Pharmacopeia, and also the USP <857>.

NOTE

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. 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 peak maximum. If the spectrophotometer's spectral resolution is impaired, two different peaks will be shown as a combined peak, leading to inaccurate measurement.



Typical Spectrum of toluene liquid filter measured with different slit widths

ARTICLE NO.	667006, 667009
APPLICATION	Testing the resolution according to Ph. Eur. and USP <857>
CONTENT	UV6, toluene in hexane UV9, hexane (reference filter)
STANDARD MEASUREMENT	**Wavelengths:** scan from 265 to 270 nm **Slit width:** 0.5; 1.0; 1.5; 2.0; 3.0 nm with Hellma Analytics Calibration Certificate (no DAkkS* Calibration Certificate)
POSSIBLE MEASUREMENTS	**Wavelengths:** fixed **Possible slit width:** from 0.5 to 3 nm on request

* Accredited calibration laboratory by DAkkS according to DIN EN ISO/IEC 17025.The accreditation is valid only for the scope listed in the annex of the accreditation certificate D-K-18752-01-00

Sets

Measurement acc. to Ph. Eur.

3.5 Liquid Filter Sets

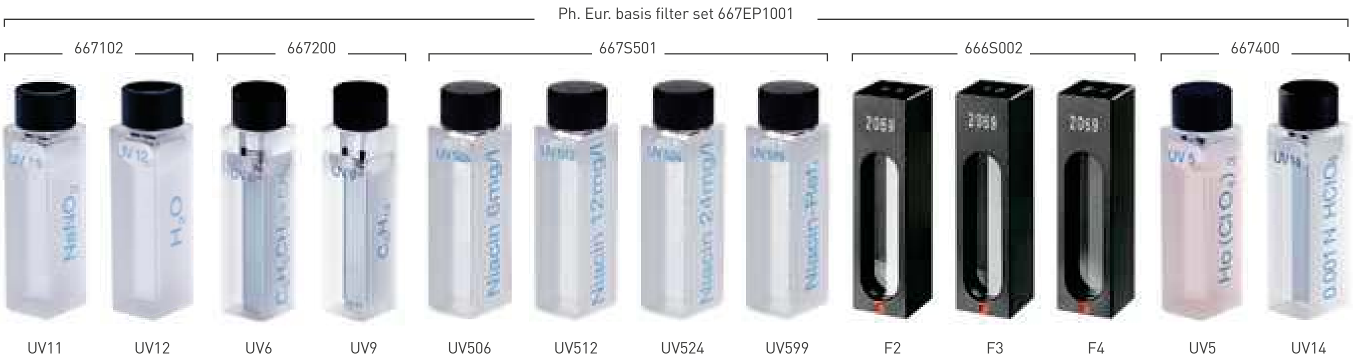
3.5.1 Basis Filter Set for Ph. Eur. Conformity

Basis filter set Art. no. 667EP1001 was compiled according to the specifications of the European Pharmacopeia and contains all filters for complete checking of the spectrophotometer in the wavelength range: 240 – 640 nm and in the absorbance range: **0.25 – 1.0 Abs.**

- ✔ **Checking of wavelength accuracy (UV5 /UV14)**
- ✔ **Checking of photometric accuracy and linearity: in the UV range (UV506, UV512, UV524, UV599) and Vis range (F2, F3, F4)**
- ✔ **Checking for stray light (UV11 and UV12)**
- ✔ **Checking spectral resolution (UV6 and UV9)**

All liquid filters consist of reference materials that are inserted into precision Hellma cuvettes made of high performance quartz glass. 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 calibration certificates* and Hellma Analytics calibration certificates provided.

* Accredited calibration laboratory by DAkkS according to DIN EN ISO/IEC 17025. The accreditation is valid only for the scope listed in the annex of the accreditation certificate D-K-18752-01-00.



PARAMETER	ARTICLE NO.	CONTENT	WAVELENGTH nm
EP Basis set UV/Vis (for wavelength range: 240 – 640 nm and absorbance range: 0.25 – 1.0 Abs)			
Photometric accuracy and linearity in UV-range	667S501	Filter set S501 consists of: UV506, 6 mg/L niacin in HCl, (0.25 Abs) UV512, 12 mg/L niacin in HCl, (0.5 Abs) UV524, 24 mg/L niacin in HCl, (1.0 Abs) UV599, hydrochloric acid (HCl), reference filter	213, 261
Photometric accuracy and linearity in the Vis-range	666S002	Glass Filter set S002 consists of: F2 (0.25 Abs), F3 (0.5 Abs), F4 (1.0 Abs)	440, 465, 546.1, 590, 635
Wavelength accuracy	667400	Filter set, UV5, holmium oxide in $HClO_4$ and UV14, perchloric acid, reference filter	241, 287, 361, 451, 485, 536, 640
Stray light	667102	Filter set, UV11, sodium nitrite in H_2O and UV12, pure water (H_2O), reference filter Absorbance ≥ 3.0 Abs at 340 nm and 370 nm	Cut-off 388
Spectral resolution	667200	Filter set, UV6, toluene in hexane and UV9, hexane, reference filter	Scan: 265 – 270 Slit widths: 0.5, 1.0, 1.5, 2.0, 3.0
TOTAL	**667EP1001**	**EP BASIS SET UV/VIS**	

Measurement acc. to USP <857>
Revision December 2022

3.5.2 Basis Filter Set for USP <857> Conformity

The new USP basis filter set – Art. no. 667857V4 – was compiled on the basis of the USP specifications updated in December 2022. The set contains all filters for a complete check of the spectrophotometer in the wavelength range from 240 nm to 640 nm and in the absorbance range from **0.2 – 2.0 Abs.**

This basis set can be supplemented accordingly depending on the user's intended range of application. We will be happy to advise you.

- ✔ **Checking of wavelength accuracy (UV5)**
- ✔ **Checking of photometric accuracy in the UV range (UV20, UV0140) and Vis range (F2, F203)**
- ✔ **Checking for stray light (UV19)**
- ✔ **Checking spectral resolution (UV6 and UV9)**

USP basis filter set 667857V4

667200

UV5 UV20 UV0140 UV14 F2 F203 UV19 UV6 UV9

PARAMETER	ARTICLE NO.	CONTENT	WAVELENGTH nm
USP Basis set UV/Vis (for wavelength range: 240 – 640 nm and absorbance range: 0.2 – 2.0 Abs)			
Photometric accuracy in UV-range	667020 6670140 667014	Potassium dichromate filters: UV20, 20 mg/L in $HClO_4$ (0.1 – 0.3 Abs) UV0140, 140 mg/L in $HClO_4$ (0.7 – 2.0 Abs) UV14, $HClO_4$ (reference filter)	235, 257, 313, 350
Photometric accuracy in the Vis-range	666F2-39 666F203-36	Neutral density glass filters: F2 (0.25 Abs), F203 (2.0 Abs)	440, 465, 546.1, 590, 635
Wavelength accuracy	667005USP	UV5, holmium oxide in perchloric acid	241, 250, 278, 287, 333, 345, 361, 386, 416, 451, 468, 485, 536, 640
Stray light	667019	UV19, acetone* (high purity), ≥ 2 Abs at 300 nm and 320 nm	Cut-off 325
Spectral resolution	667200	Filter set, UV6, toluene in hexane and UV9, hexane, reference filter	Scan: 265 – 270 Slit widths: 0.5, 1.0, 1.5, 2.0, 3.0
TOTAL	**667857V4**	**USP <857> BASIS SET UV/VIS**	

* measurement against air

4. REFERENCE PLATES

Photometric Accuracy

4.1 Checking the photometric accuracy

APPLICATION

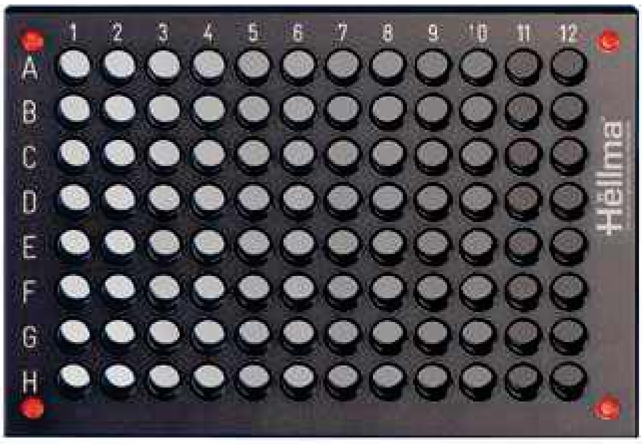
The Hellma Analytics reference plate 666R013 can be used to check the photometric accuracy of microplate readers.

PRODUCT DESCRIPTION

The reference plate dimensions are equivalent to a 96-well microplate with a diameter of 6.6 mm per window (H 13.0 mm x D 127 mm x L 85.5 mm). At each of the five neutral density glass filters (columns 3 – 12) in the reference plate the absorbance value for 16 windows can be measured. The other 16 windows do not contain glass (column 1 + 2) and serve as references.

NOTE

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 calibration certificate in a diagram.



ARTICLE NO.	666R013
APPLICATION	Reference plate for microplate readers for testing the photometric accuracy
CONTENT	Neutral density glass filter (0.25 Abs); column 3 + 4 Neutral density glass filter (0.5 Abs); column 5 + 6 Neutral density glass filter (1.0 Abs); column 7 + 8 Neutral density glass filter (1.5 Abs); column 9 + 10 Neutral density glass filter (2.5 Abs); column 11 + 12 column 1 + 2 without glass (reference)
STANDARD MEASUREMENT	**Photometric accuracy measured at wavelengths:** 405, 450, 490, 650 nm, at 8 points in a column **Slit width:** 1 nm
POSSIBLE MEASUREMENTS	**Wavelengths:** all possible from 405 to 800 nm **Slit width:** all up to 2 nm

Photometric Accuracy and Wavelength Accuracy

4.2 Checking photometric and wavelength accuracy

APPLICATION

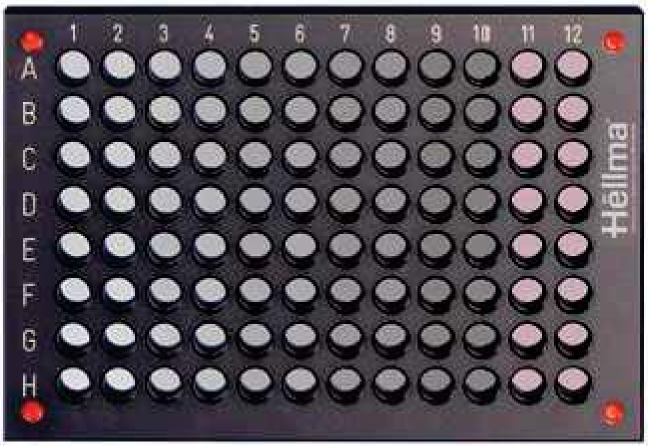
The Hellma Analytics reference plate 666R113 can be used to check the photometric and wavelength accuracy of microplate readers.

PRODUCT DESCRIPTION

The reference plate dimensions are equivalent to a 96-well microplate with a diameter of 6.6 mm per window (H 13.0 mm x D 127 mm x L 85.5 mm). At each of the four neutral density glass filters used (columns 3 – 10) the absorbance value for 16 windows can be measured. The holmium glass filter (column 11+12) is used to test the wavelength accuracy in 16 windows while a further 16 windows (columns 1+2) do not contain glass and serve as references.

NOTE

The positions of the holmium peaks may vary slightly depending on the glass batches. For this reason, Hellma Analytics measures each holmium glass filter individually.



ARTICLE NO.	666R113
APPLICATION	Reference plate for microplate readers for testing the photometric and wavelength accuracy
CONTENT	Neutral density glass filter (0.5 Abs), column 3+4 Neutral density glass filter (1.0 Abs), column 5+6 Neutral density glass filter (1.5 Abs), column 7+8 Neutral density glass filter (2.0 Abs), column 9+10 Holmium glass filter, column 11+12 column 1 + 2 without glass (reference)
STANDARD MEASUREMENT	**Photometric accuracy measured at 8 points in column at wavelengths:** 405; 450; 490; 650 nm; **Wavelength accuracy measured at:** 279; 361; 453; 536; 638 nm **Slit width:** 1 nm
POSSIBLE MEASUREMENTS	**Photometric accuracy:** **Wavelengths:** additional wavelengths: all possible from 405 to 800 nm **Slit widths:** all up to 2 nm **Wavelength accuracy:** **Wavelengths:** 279; 287; 361; 418; 445; 453; 460; 536; 638 nm **Slit width:** 1-2 nm (0,1-0,9 nm only on request)

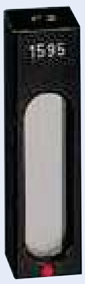
5. RECALIBRATION

Continuously assured quality: Recalibration intervals for reference materials

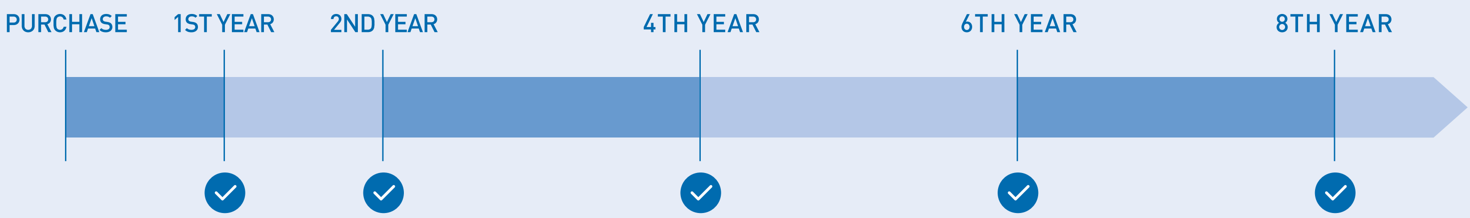
As is the case for all measuring devices, the **reference materials used to verify spectrophotometers** must also be **checked and recalibrated at regular intervals** (see for example ISO 9001 "Control of Monitoring and Measuring Equipment"). This allows you to ensure that you consistently fulfill your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

Important parameters for recalibration

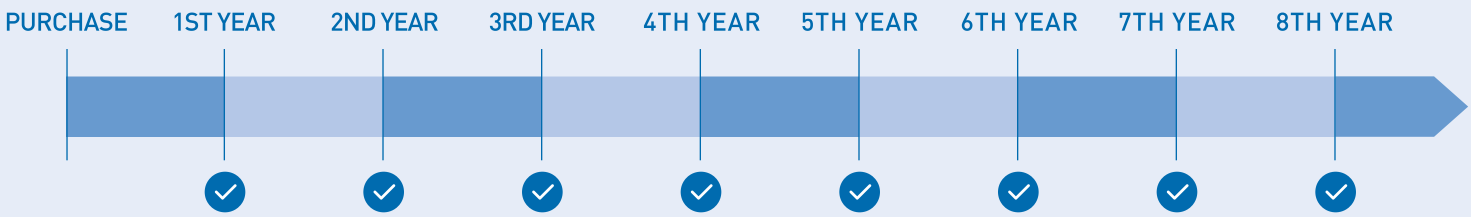
The **length of intervals** between the recalibration of reference materials depends on how frequently materials are used, the wear associated with this, accuracy requirements, and the requirements of a company's internal auditing. In general, a recalibration interval of **12 months** is recommended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



GLASS FILTER RECALIBRATION EVERY 24 MONTHS



LIQUID FILTER RECALIBRATION OF 12 MONTHS IS RECOMMENDED



Fast and reliable – recalibration service

In our calibration laboratory, accredited by DAkkS according to DIN EN ISO/IEC 17025, your reference materials are cleaned and measured with a high-performance spectrophotometer according to your requirements. The accreditation is valid only for the scope listed in the annex of the accreditation certificate D-K-18752-01-00. If necessary, filters are repaired, or are exchanged following a consultation. You will receive your filters with a new DAkkS calibration certificate or Hellma Analytics calibration certificate. **Filters are usually recalibrated within five working days** of their arrival at the calibration laboratory.

Recalibration of reference materials from other manufacturers. We also recalibrate reference materials for UV/Vis spectroscopy from other suppliers. If you require a quotation first, please send your inquiry via email to your **local Hellma partner.**

Returning your reference materials for recalibration

Efficient processing of the reference materials you send us ensures that you will be able to use your reference materials again within just a few days. We need your support to make this possible. Please include all information needed to process the reference materials:

- **Article no.***
- **Serial no.***
- **Wavelength(s) to be measured***
- **Slit width(s) to be measured***
- **Documentation of measurement data prior to cleaning** Yes / No**
- **Quotation no.** (if you have already received a quotation from us)
- **Billing address**
- **Delivery address** (if different from billing address)
- **Special requests, e. g. additional wavelengths etc.**

* This information is not necessary if you enclose a copy of the current calibration certificate.

** Documentation of measurement data prior to cleaning
If you require documentation of measurement data prior to cleaning, please note this on your order. Depending on your quality management requirements, you have two options:
1. Documentation of measurement data prior to cleaning with DAkkS certificate.
2. Documentation of measurement data prior to cleaning with simple measurement report.

Please enclose a copy of your order or send this via email to your **local Hellma partner.**

If you send your reference materials with only a delivery note, **it is essential that you indicate your order number.** Please include this on the delivery note, otherwise we will be unable to process your order.

PLEASE NOTE:

Liquid filters can only be sent when the external temperature is above 4 °C, as the liquid can freeze, which will destroy the reference materials.

6. PRODUCT OVERVIEW

UV/Vis Reference Materials

GLASS FILTERS WITH CALIBRATION CERTIFICATE

TYPE	MATERIAL	WAVELENGTH nm	ARTICLE NO.
Glass filters for checking wavelength accuracy			
666-F1	Holmium glass filter F1	279; 361; 453; 536; 638	666F1-339
666-F7W	Didymium glass filter F7W	329; 472; 512; 681; 875	666F7W-323
Glass filters checking the photometric accuracy			
666-F390	Glass filter F390; 0.04 Abs	440; 465; 546.1; 590; 635	666F390-25
666-F2	Neutral density glass filter F2; 0.25 Abs	440; 465; 546.1; 590; 635	666F2-39
666-F201	Neutral density glass filter F201; 0.3 Abs	440; 465; 546.1; 590; 635	666F201-39
666-F3	Neutral density glass filter F3; 0.5 Abs	440; 465; 546.1; 590; 635	666F3-38
666-F204	Neutral density glass filter F204; 0.7 Abs	440; 465; 546.1; 590; 635	666F204-37
666-F4	Neutral density glass filter F4; 1.0 Abs	440; 465; 546.1; 590; 635	666F4-37
666-F202	Neutral density glass filter F202; 1.5 Abs	440; 465; 546.1; 590; 635	666F202-36
666-F203	Neutral density glass filter F203; 2.0 Abs	440; 465; 546.1; 590; 635	666F203-36
666-F301	Neutral density glass filter F301; 2.5 Abs	440; 465; 546.1; 590; 635	666F301-361
666-F303	Neutral density glass filter F303; 3.0 Abs	440; 465; 546.1; 590; 635	666F303-361
Empty frame			
666-F0	Empty frame (without glass), reference filter		666F0-71
Glass filter sets for checking photometric accuracy, photometric linearity and wavelength accuracy			
666-S000	Complete Glass filter set: F0, F1, F2, F3, F4 (Abs: 0.25; 0.5; 1.0)	A: 440; 465; 546.1; 590; 635 W: 279; 361; 453; 536; 638	666S000
666-S002	Glass filter set: F2, F3, F4 (Abs: 0.25; 0.5; 1.0)	A: 440; 465; 546.1; 590; 635	666S002
666-S004	Glass filter set: F0, F201, F202, F203 (Abs: 0.3; 1.5; 2.0)	A: 440; 465; 546.1; 590; 635	666S004
666-S005	Glass filter set: F0, F1, F3, F4 (Abs: 0.5; 1.0)	A: 440; 465; 546.1; 590; 635 W: 279; 361; 453; 536; 638	666S005
666-S006	Glass filter set: F0, F2, F3, F4 (Abs: 0.25; 0.5; 1.0)	A: 440; 465; 546.1; 590; 635	666S006
666-S010	Glass filter set: F1, F390, F2, F3, F4 (Abs: 0.04; 0.25; 0.5; 1.0)	A: 440; 465; 546.1; 590, 635 W: 279; 361: 453; 536; 638	666S010
666-S200	Glass filter set: F2, F4, F203 (Abs: 0.25; 1.0; 2.0)	A: 440; 465; 546.1; 590; 635	666S200
666-S300	Glass filter set: F390, F301, F303 (Abs: 0.04; 2.5; 3.0)	A: 440; 465; 546.1; 590; 635	666S300

W: Wavelength for wavelength accuracy; A: Wavelength for absorbance (photometric accuracy)

UV/Vis Reference Materials

LIQUID FILTERS WITH CALIBRATION CERTIFICATE

TYPE	MATERIAL	WAVELENGTH nm	ARTICLE NO.
Potassium dichromate liquid filters for checking photometric accuracy			
667-UV20	20 mg/L Potassium dichromate in $HClO_4$ (0.1–0.3 Abs)	235; 257; 313; 350	667020
667-UV40	40 mg/L Potassium dichromate in $HClO_4$ (0.2–0.6 Abs)	235; 257; 313; 350	667040
667-UV60	60 mg/L Potassium dichromate in $HClO_4$ (0.3–0.9 Abs)	235; 257; 313; 350	667060
667-UV80	80 mg/L Potassium dichromate in $HClO_4$ (0.4–1.2 Abs)	235; 257; 313; 350	667080
667-UV0100	100 mg/L Potassium dichromate in $HClO_4$ (0.5–1.5 Abs)	235; 257; 313; 350	6670100
667-UV0120	120 mg/L Potassium dichromate in $HClO_4$ (0.6–1.8 Abs)	235; 257; 313; 350	6670120
667-UV0140	140 mg/L Potassium dichromate in $HClO_4$ (0.7–2.0 Abs)	235; 257; 313; 350	6670140
667-UV0160	160 mg/L Potassium dichromate in $HClO_4$ (0.8–2.3 Abs)	235; 257; 313; 350	6670160
667-UV0180	180 mg/L Potassium dichromate in $HClO_4$ (0.9–2.6 Abs)	235; 257; 313; 350	6670180
667-UV0200	200 mg/L Potassium dichromate in $HClO_4$ (1.0–3.0 Abs)	235; 257; 313; 350	6670200
667-UV600	600 mg/L Potassium dichromate in $HClO_4$ (1.0 Abs)	430	667600
667-UV14	Perchloric acid	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
Potassium dichromate liquid filter set for checking the photometric linearity			
667-UV307	Filter set: UV20, UV40, UV60, UV80, UV0100, UV14	235; 257; 313; 350	667307
Nicotinic acid (Niacin) liquid filters for checking the photometric accuracy			
667-UV506	6 mg/L Niacin in HCl (0.25 Abs)	213; 261	667506
667-UV512	12 mg/L Niacin in HCl (0.5 Abs)	213; 261	667512
667-UV518	18 mg/L Niacin in HCl (0.75 Abs)	213; 261	667518
667-UV524	24 mg/L Niacin in HCl (1.0 Abs)	213; 261	667524
667-UV536	36 mg/L Niacin in HCl (1.5 Abs)	213; 261	667536
667-UV548	48 mg/L Niacin in HCl (2.0 Abs)	213; 261	667548
667-UV599	Hydrochloric acid (HCl) (reference filter)	213; 261	667599
Nicotinic acid liquid filter sets for checking the photometric linearity			
667-UV350	Filter set: UV506, UV512, UV518, UV524, UV599	213; 261	667350
667-UVS501	Filter set: UV506, UV512, UV524, UV599	213; 261	667S501

UV/Vis Reference Materials

LIQUID FILTERS WITH CALIBRATION CERTIFICATE

TYP	MATERIAL	WAVELENGTH nm	ARTICLE NO.
Liquid filters for checking the wavelength accuracy acc. to EP and / or USP			
667-UV5	Holmium oxide in perchloric acid acc. to Ph. Eur.	241; 287; 361; 451; 485; 536; 640	667005
667-UV5USP	Holmium oxide in perchloric acid acc. to USP <857>	241; 250; 278; 287; 333; 345; 361; 386; 416; 451; 468; 485; 536; 640	667005USP
667-UV25	Didymium in perchloric acid	329; 469; 575; 740; 864	667025
667-UV25EPUSP	UV25, didymium liquid filter for checking the wavelength accuracy (acc. to Ph. Eur. and USP <857>)	512; 732; 740; 794; 801; 864	667025EPUSP
667-UV35	Rare earth (cerium) liquid filter for checking the wavelength accuracy below 240 nm (acc. to Ph. Eur. and USP <857>)	201; 211; 222; 239; 252	667035
667-UV45	Holmium/Didymium in perchloric acid	241; 354; 444; 575; 641; 740; 864	667045
667-UV45EPUSP	UV45, HoDi liquid filter (holmium and didymium in perchloric acid) for checking the wavelength accuracy from 240 to 870 nm (acc. to Ph. Eur. and USP <857>	241; 287; 361; 451; 482; 512; 537; 641; 732; 740; 794; 801; 864	667045EPUSP
667-UV400	Liquid filter set: UV5, holmium and UV14, perchloric acid (reference filter) acc. to Ph. Eur.	241; 287; 361; 451; 485; 536; 640	667400
667-UV425	Liquid filter set: UV5, holmium and UV25, didymium for checking the wavelength accuracy from 240 to 870 nm acc. to USP <857>	UV5: 241; 250; 278; 287; 333; 345; 361; 386; 416; 451; 468; 485; 536; 640; UV25: 732; 740; 794; 801; 864	667425
Liquid filters for checking stray light			
667-UV1	Potassium chloride in pure water, 10 mm path length, ≥ 2 Abs at 198 nm	200 Cut-off	667001
667-UV1H*	Potassium chloride in pure water, reference filter 5 mm path length	200 Cut-off	667001H
667-UV10	Sodium iodide in pure water, 10 mm path length, ≥ 3 Abs at 220 nm	259 Cut-off	667010
667-UV10H*	Sodium iodide in pure water, reference filter 5 mm path length	259 Cut-off	667010H
667-UV23	Potassium iodide in pure water, 10 mm path length, ≥ 2 Abs at 220 nm and ≥ 3 Abs at 250 nm	259 Cut-off	667023
667-UV11	Sodium nitrite in pure water, 10 mm path length, ≥ 3 Abs at 340 nm and 370 nm	388 Cut-off	667011
667-UV11H*	Sodium nitrite in pure water, reference filter 5 mm path length	388 Cut-off	667011H
667-UV12	Pure water, reference filter 10 mm path length	198; 200; 300; 400	667012
667-UV19	Acetone (high purity), 10 mm path length, ≥ 2 Abs at 300 nm and 320 nm	325 Cut-off	667019
667-UV19H*	Acetone (high purity), reference filter 5 mm path length	325 Cut-off	667019H
Liquid filter sets for checking stray light acc. to Ph. Eur. and USP <857> procedure B			
667-UV100	Filter set includes: UV1 and UV12; ≥ 2 Abs at 198 nm	200 Cut-off	667100
667-UV101	Filter set includes: UV10 and UV12; ≥ 3 Abs at 220 nm	259 Cut-off	667101
667-UV107	Filter set includes: U23 and UV12; ≥ 2 Abs at 220nm and ≥ 3 Abs at 250 nm	259 Cut-off	667107
667-UV102	Filter set includes: UV11 and UV12; ≥ 3 Abs at 340 and 370 nm	388 Cut-off	667102
667-UV103	Filter set includes: UV1, UV10, UV11 and UV12	Cut-off: 200; 259; 388	667103
667-UV104	Filter set includes: UV10, UV11 and UV12	Cut-off: 259; 388	667104

* with Hellma Analytics calibration certificate

UV/Vis Reference Materials

LIQUID FILTERS WITH CALIBRATION CERTIFICATE

TYPE	MATERIAL	WAVELENGTH nm	ARTICLE NO.
Liquid filter sets for checking stray light acc. to USP <857> procedure A			
667-UV100H	Filter set includes: UV1 and U1H, PL 10 and 5 mm	198 Cut-off	667100H
667-UV101H	Filter set includes: UV10 and U10H, PL 10 and 5 mm	258 Cut-off	667101H
667-UV102H	Filter set includes: UV11 and U11H, PL 10 and 5 mm	385 Cut-off	667102H
667-UV119H	Filter set includes: UV19 and U19H, PL 10 and 5 mm	322 Cut-off	667119H
667-UV105H	Filter set includes: UV1/UV1H; UV10/UV10H; UV11/UV11H; UV19/UV19H; PL 10 mm and 5 mm	Cut-off: 198; 258; 322; 385	667105H
667-UV106H	Filter set includes: UV1/UV1H; UV10/UV10H; UV19/UV19H; PL 10 mm and 5 mm	Cut-off: 198; 258; 322	667106H
Liquid filters for checking the resolution			
667-UV6*	Toluene in hexane	Scan: 265–270	667006
667-UV9	Hexane (reference filter)	without calibration certificate	667009
667-UV200*	Filter set includes: UV6, UV9	Scan: 265–270 Slit width: 0.5; 1.0; 1.5; 2.0; 3.0	667200

PL: path length * with Hellma Analytics certificate

Basis Filter Set for USP <857> Conformity

USP BASIS SET UV/VIS (for wavelength range: 240 - 640 nm and absorbance range: 0.2 - 2.0)

PARAMETER	ARTICLE NO.	CONTENT	WAVELENGTH nm
Photometric accuracy UV range	667020 6670140 667014	UV20: 20 mg/L Potassium dichromate in $HClO_4$ (0.1–0.3 Abs) UV0140: 140 mg/L Potassium dichromate in $HClO_4$ (0.7–2.0 Abs) UV14: Perchloric acid (reference filter)	235; 257; 313; 350
Photometric accuracy Vis range	666F2-39 666F203-36	Neutral density glass filters: F2 (0.25 Abs), F203 (2.0 Abs)	440; 465; 546.1; 590; 635
Wavelength accuracy	667005USP	UV5USP: Holmium oxide in perchloric acid	241; 250; 278; 287; 333; 345; 361; 386; 416; 451; 468; 485; 536; 640
Stray light	667019	UV19: Acetone measured against air, ≥ 2 Abs at 300 nm and 320 nm	325 Cut-off
Spectral resolution	667200	Filter set UV6 toluene in hexane and UV9 hexane (reference filter)	Scan: 265–270, Slit width: 0.5; 1.0; 1.5; 2.0; 3.0
TOTAL	**667857V4**	**USP <857> BASIS SET UV/VIS**	

Basis Filter Sets for Ph. Eur. Conformity

EP BASIS SET UV/VIS (for wavelength range: 240 - 640 nm and absorbance range: 0.25 - 1.0)

PARAMETER	ARTICLE NO.	CONTENT	WAVELENGTH nm
Photometric accuracy and linearity for UV range	667S501	Filter set S501 includes: UV506: Niacin filter 6 mg/L (0.25 Abs) UV512: Niacin filter 12 mg/L (0.5 Abs) UV524: Niacin filter 24 mg/L (1.0 Abs) UV599: Hydrochloric acid (reference filter)	213; 261
Photometric accuracy and linearity for Vis range	666S002	Glass filter set S002 includes: F2 (0.25 Abs), F3 (0.5 Abs), F4 (1.0 Abs)	440; 465; 546.1; 590; 635
Wavelength accuracy	667400	Filter set, UV5 holmium oxide in perchloric acid and UV14 perchloric acid (reference filter)	241; 287; 361; 451; 485; 536; 640
Stray light	667102	Filter set, UV11 sodium nitrite in pure water and UV12 pure water (reference filter) ≥ 3 Abs at 340 and 370 nm	388 Cut-off
Spectral resolution	667200	Filter set, UV6 toluene in hexane and UV9 hexane (reference filter)	Scan: 265–270 Slit width: 0.5; 1.0; 1.5; 2.0; 3.0
TOTAL	667EP1001	EP BASIS SET UV/VIS	

EP-BASIS SET UV (for wavelength range: 240 - 400 nm and absorbance range: 0.25 - 1.0)

PARAMETER	ARTICLE NO.	CONTENT	WAVELENGTH nm
Photometric accuracy and linearity for UV range	667S501	Filter set S501 includes: UV506: Niacin filter 6 mg/L (0.25 Abs) UV512: Niacin filter 12 mg/L (0.5 Abs) UV524: Niacin filter 24 mg/L (1.0 Abs) UV599: Hydrochloric acid (reference filter)	213; 261
Wavelength accuracy	667400	Filter set, UV5 holmium oxide in perchloric acid and UV14 perchloric acid (reference filter)	241; 287; 361; 451; 485; 536; 640
Stray light	667107	Filter set, UV23 potassium dichromate in pure water and UV12 pure water (reference filter) ≥ 2 Abs at 220 nm and ≥ 3 Abs at 250 nm	259 Cut-off
Spectral resolution	667200	Filter set, UV6 toluene in hexane and UV9 hexane (reference filter)	Scan: 265–270 Slit width: 0.5; 1.0; 1.5; 2.0; 3.0
TOTAL	667EP1002	EP BASIS SET UV	

UV/Vis Reference Materials

REFERENCE PLATES WITH CALIBRATION CERTIFICATE

TYPE	MATERIAL	WAVELENGTH nm	ARTICLE NO.
Checking photometric accuracy			
666-R013	Neutral density glass filters NG 11 (0.25), NG 5 (0.5), NG 4 (1.0), NG 3 (1.5), (2.5)	A: 405; 450; 490; 650	666R013
Checking photometric and wavelength accuracy			
666-R113	Neutral density glass filters NG 5 (0.5), NG 4 (1.0), NG 3 (1.5), (2.0) Holmium glass filter	A: 405; 450; 490; 650 W: 279; 361; 453; 536; 638	666R113

A: Wavelength for absorbance (photometric accuracy) W: Wavelength for wavelength accuracy

7. FAQ

How does the recalibration of my filters work?

Hints for the return and recalibration of your reference materials can be found on page 35.

Why do holmium 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. The filter can be easily wiped down using alcohol and a soft cloth. The filter should generally be stored in a dry place.

How long can a reference material 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 recalibrated so that any signs of deterioration can be recognized at an early stage.

How often should filters be recalibrated?

Reference materials should be recalibrated 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 recalibration intervals, we recommend having all reference materials recalibrated at least every 12 months during their first two years of use, and then selecting a suitable recalibration interval based on the values measured (please see chapter 5).

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 and PTB 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.

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.

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.

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.

* Accredited calibration laboratory by DAkkS according to DIN EN ISO/IEC 17025. The accreditation is valid only for the scope listed in the annex of the accreditation certificate D-K-18752-01-00.

Why is the potassium dichromate filter no longer recommended in Ph.Eur. for checking photometric accuracy?

In the Ph. Eur. 9 valid until the end of 2019, the potassium dichromate solution with 60 mg/L was still recommended for checking the photometric accuracy. The Ph. Eur. now no longer recommends the manual preparation of potassium dichromate solutions, as potassium dichromate is listed in Annex XIV of REACH. Instead, Ph. Eur. describes the preparation of a nicotinic acid solution to check photometric accuracy in the UV range at 213 and 261 nm. The use of potassium dichromate filters in sealed quartz cuvettes for checking photometric accuracy in the UV range is still permitted without restriction according to the current Ph. Eur.

Why are these peaks measured for calibrating holmium 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.

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 densitiy glass filter (highly simplified): The calibration certificate states the following measurement values and measurement uncertainties:

SERIAL NO.	3524	Optical Density (Abs) +MU(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 (x_b) – refer to the operating instructions for more details – and define a value for the measuring error due to environmental influences at your company (x_u) (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 them statistically. 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 9 of this user manual.

8. GLOSSARY

Abbreviations:

ΔA	observed maximum absorbance
Abs	Absorbance
ASTM	American Society for Testing and Materials
DAkkS	Deutsche Akkreditierungsstelle (National accreditation body for the Federal Republic of Germany)
EP	European Pharmacopoeia
Ph. Eur.	European Pharmacopoeia
FAQ	Frequently Asked Questions
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
I	Intensity of light beam
I_0	Output intensity of the light beam
k	Coverage factor for measurement uncertainty
λmax	Peak Maximum at defined wavelength
λmin	Peak Minimum at defined wavelength
MU	Measurement uncertainty
NG	Neutral density glass
NIR	Near infrared
NIST	National Institute of Standards and Technology
QM-System	Quality management system
PL	Path length
PTB	Physikalisch-Technische Bundesanstalt (Germany's national metrology institute)
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
s_λ	Stray light value, in transmittance (T)
SR	Spectral range
SRM®	Standard Reference Material (registered trademark of NIST)
T	Transmission
USP	United States Pharmacopeia
UV	Ultra violet (wavelength range approx. 180 – 400 nm)
Vis	Visible (visible wavelength range) approx. 400 – 800nm

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 I_0 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 400 nm to 800 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 I_0 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: $T = I/I_0$ or $\%T = (I/I_0) * 100$.

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 180 nm to 400 nm.

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

9. LITERATURE REFERENCE

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

JCGM 100:2008: 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.)

EA-4/02 M: 2022
Evaluation of the Uncertainty of Measurements in Calibration

United States Pharmacopeia (USP)

10. GENERAL USAGE GUIDELINES

Glass Filters

10.1 General usage guidelines for glass filters

Glass filters are made of glass doped with metal ions/rare earth metals, which are assembled stress-free into 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 / TRANSPORT

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

Storage: relative humidity not condensing at temperatures of 4 - 40 °C.
Transport: relative humidity not condensing at temperatures of 4 - 40 °C, short-term transients are permissible.

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.

INFLUENCE OF TEMPERATURE ON MEASUREMENTS

Temperature has a very small influence on certified measurement values, and temperatures between 20°C and 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.

10.2 Calibration with glass filters (wavelength accuracy and photometric accuracy)

10.2.1 Preparations

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.

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.

VIDEO TUTORIAL

Preparation and execution of measurements with glass filters

Glass Filters

10.2.2 Steps for checking wavelength accuracy with holmium glass filter or didymium glass filter

1. First, carry out the "Preparations" according to chapter 10.2.1
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 – if possible – a slow 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 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 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.
   Note: USP <857> requires at least 6 replicate measurements to be made and the mean value to be taken. This is used as the basis for calculating the deviations.
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 scanning 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 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.

Watch a video of the individual steps here.

10.2.3 Steps for checking photometric accuracy with a neutral density glass filter

1. First, carry out the "Preparations" according to chapter 10.2.1
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. If this is not possible, please ensure that the integration time selected is not too short.
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 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)
   Note: USP <857> requires at least 6 replicate measurements to be made and the mean value to be taken. This is used as the basis for calculating the deviations
8. Compare your measurement values with the certified ones.

Watch a video of the individual steps here.

EXPERTS TIP:

Factors in measurement uncertainty

"Measurement uncertainty arises in particular from the device-specific measurement deviation of the spectrophotometer used and from the measurement uncertainties listed on the calibration certificate."

Carola Senger,
Chemical Laboratory Assistant

Glass Filters

10.2.4 Procedure for checking photometric linearity with at least 3 neutral density glass filters

1. First carry out the "Preparations" according to chapter 10.2.1.
2. Set the wavelength selection program on your spectrophotometer. Observe the instructions in the associated operating manual. Use the wavelengths specified in the calibration certificate. Enter the number of neutral density filters to be measured in your wavelength selection program.
3. Set the measurement parameters specified on the supplied calibration certificate on your spectrophotometer. If this is not possible, please ensure that the integration time selected is not too short.
4. Perform a zero point adjustment (autozero).
5. The measurement is performed against air, i.e. for double-beam spectrophotometers, the reference cell holder remains empty, for single-beam spectrophotometers, a reference measurement is performed against the empty cell holder.
6. Insert the first neutral density 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. Proceed similarly with the other neutral glass filters to be measured. Perform several measurements and average your measured values to avoid errors.
8. Compare your measurement values with the certified ones.

10.2.5 Calibration with glass filters – interpreting measurement results

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 with 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 9 of this user manual.

NOTE

Helpful tool for checking the photometric linearity

On our website you will find a helpful downloadable Excel tool with which you can conveniently check the linearity of your spectrophotometer. Based on your input, the correlation coefficient is calculated automatically and the values are displayed graphically. If the measured values deviate too much from the linear trend line ($R^2 < 0.999$), the R^2 field is automatically colored red.

www.hellma.com/en/hellma/downloads
Select format → Tools/Presentations → Download the tool

Liquid Filters

10.3 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.com/en/hellma/downloads
Select format → Safety Information CRM

FOR INFORMATION

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. Grease on the fingers may cause a greasy film on the polished surfaces, which may affect the measurement results. The filters are fragile and should be handled with the utmost care. It is very important that the pressure of the cuvette holder on the liquid filter is not set too high. If the pressure on the glass is too great, it can lead to cracks in the glass wall and the filter becomes unusable.

STORAGE / TRANSPORT

After use, we strongly recommend storing the filters at room temperature, in their storage box, in a dry, dust-free and light-protected area.

Storage: relative humidity non-condensing at temperatures of 15 - 30 °C.
Transport: relative humidity not condensing at temperatures of 4 - 40 °C.

Caution during the winter months! Liquid filters must not be allowed to freeze under any circumstances, as they may then break. When shipping, therefore, ensure adequate packaging!

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.

INFLUENCE OF TEMPERATURE ON MEASUREMENTS

Temperature has a very small influence on certified measurement values, and temperatures of between 20 °C and 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.

10.4 Calibration with liquid filters (wavelength accuracy and photometric accuracy)

10.4.1 Preparations

1. Warm up the spectrophotometer until the correct operating temperature has been reached and remains constant (e.g. for one hour). Please also note the instructions of your instrument manufacturer.

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 and vibrations (e.g. movement of fiber-optic cables) may also impair the accuracy of measurement results.

Measuring with deviating slit width:

"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 peaks lying close together from being resolved."

Thomas Brenn,
Product Manager

Liquid Filters

10.4.2 Steps for checking wavelength accuracy with holmium, didymium, rare earth or HoDi liquid filter

1. First carry out the "Preparations" according to chapter 10.4.1.

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. If this is not possible, select the slowest scanning speed and a small data interval.

4. If possible, carry out a baseline correction.

5. **A. Measurement against air:** In the case of double-beam spectrometers, the reference cuvette holder remains empty; in the case of single-beam spectrometers, a reference measurement is carried out against the empty cuvette holder.
   B. Measurement against reference: Observe the general handling instructions for liquid filters. The reference filter should always be inserted in the cuvette holder in the same orientation, e.g. always with the Hellma lettering facing the light source. If the measurement is carried out against a reference filter, the following procedure applies: With double-beam spectrometers: Carefully insert the reference filter into the reference cuvette holder. For single-beam spectrometers: Carefully insert the reference filter into the cuvette holder and first carry out a reference measurement.

6. Insert the wavelength 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. If peaks are not found, correct the threshold in your scan program.
   Note: USP<857> requires at least 6 replicate measurements to be made and the mean value to be taken. This is used as the basis for calculating the deviations.

9. Compare your measurement values with the certified ones. Suitable for this purpose is, for example, a control chart (see page 59).

MEASUREMENT PARAMETERS FOR CHECKING WAVELENGTH ACCURACY

Ensure that you have selected the correct measurement parameters before scanning 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 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.

10.4.3 Steps for checking photometric accuracy with potassium dichromate or niacin liquid filter

1. First carry out the "Preparations" according to chapter 10.4.1.

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. If this is not possible, please ensure that the integration time is not too short.

4. If possible, carry out a baseline correction.

5. The measurements are usually carried out against a reference filter filled with perchloric acid or hydrochloric acid. Observe the general handling instructions for liquid filters. The filters should always be placed in the same orientation in the cuvette holder, e.g. always with the Hellma lettering facing the light source.

6. **Measurement in a single-beam spectrophotometer:** Carefully insert the supplied perchloric acid or hydrochloric acid reference filter into the cuvette holder. Start the measurement. Then measure the certified reference material, which contains potassium dichromate dissolved in perchloric acid or niacin in hydrochloric acid. Then subtract the values of the reference measurement from the values of the measurement of the certified reference material.

7. **Measurement in a double-beam spectrophotometer:** Put the certified reference material with dissolved potassium dichromate in perchloric acid or niacin in hydrochloric acid, carefully into the sample holder and the perchloric acid or hydrochloric acid reference filter in the reference sample holder.

8. Start the program for measuring absorbance values at the wavelengths indicated on the calibration certificate. Take several measurements and average your measured values to avoid errors.
   Note: USP <857> requires at least 6 replicate measurements to be made and the mean value to be taken. This is used as the basis for calculating the deviations.

9. Compare your measurement values with the certified ones. Suitable for this purpose is, for example, a control chart (see page 59).

Liquid Filters

10.4.4 Control of photometric linearity with optical reference materials

The Ph. Eur. requires, among other things, the control of photometric linearity. To check the linearity at least 3 absorbance filters with different concentrations are required.

Procedure for checking photometric linearity in the UV range with potassium dichromate or niacin liquid filters

1. First carry out the "Preparations" according to Chapter 10.4.1.
2. Set the wavelength selection program on your spectrophotometer. Observe the instructions in the corresponding operating manual from your instrument manufacturer. Use the wavelengths specified in the calibration certificate. Enter the number of filters to be measured in your wavelength selection program.
3. If possible, set the measurement parameters specified on the supplied calibration certificate on your spectrophotometer. If this is not possible, please ensure that the integration time is not selected too short.
4. Perform a baseline correction (autozero).
5. The measurements are usually performed against a reference filter filled with perchloric acid or hydrochloric acid. Observe the general handling instructions for liquid filters. The filters should always be inserted in the cell holders in the same orientation, e.g. always with the Hellma lettering facing the light source.
6. **Measurement in the single-beam spectrophotometer:** Carefully insert the supplied perchloric acid or hydrochloric acid reference filter into the cuvette holder. Start the measurement. Then measure the first reference material containing potassium dichromate dissolved in perchloric acid or niacin in hydrochloric acid. Then subtract the value of the reference measurement from the value of the measurement with the respective reference material.
7. **Measurement in the double-beam spectrophotometer:** Carefully place the first reference material containing potassium dichromate dissolved in perchloric acid or niacin in hydrochloric acid into the sample holder and the perchloric acid or hydrochloric acid reference filter into the reference sample holder.
8. Start the program for measuring the absorbance values at the wavelengths specified on the calibration certificate. Proceed similarly with the other potassium dichromate or niacin filters to be measured. Perform several measurements and average your measured values to avoid errors.
9. Compare the measured values obtained with the values from the calibration certificate; a target value control chart, for example, is very suitable for this purpose.

NOTE ON LINEARITY:
The correlation coefficient is a measure of the degree of linear correlation. If it is 1, all measured values are on a straight line and thus perfectly linear to each other. The coefficient of determination R^2 is the square of the correlation coefficient; this is a measure of quality for describing a linear relationship. The European Pharmacopoeia (Ph. Eur.) requires that R^2 must be ≥ 0.999.

NOTE

Helpful tool for checking photometric linearity.

In this Excel tool provided for download, you can conveniently check the linearity of your spectrophotometer. Based on your entries, the correlation coefficient is automatically calculated and the values are displayed graphically. If the measured values deviate too much from the linear trend line ($R^2 < 0.999$), the R^2 field is automatically colored red.

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Select format → Tools/Presentations → Download the tool

Liquid Filters

10.4.5 Calibration with liquid filters – interpreting the measurement results (wavelength accuracy and photometric accuracy)

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 or the niacin 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.

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 9 of this user manual.

Documentation with target value chart

"Documenting the measurement results on the target value chart gives a valuable overview and helps to quickly identify trends and deviations."

Birgit Kehl,
Compliance Manager Calibration Laboratory

Control chart for certified reference materials

In order to achieve exact measurement results, it is important to test the spectrophotometer at regular intervals and to document the measurement results achieved. The results can be documented using control charts, for example, which also display the measurement values graphically. One example of a type of control chart is the target value chart used below.

Here, the value given on the Hellma Analytics calibration certificate is set as the target value. As the exclusion limit, you should use the measurement uncertainty you have determined (measurement uncertainty from the calibration certificate plus own measurement uncertainty), i.e. all measured values must lie within the margin of measurement uncertainty in order to avoid an out-of-control situation.

To help you in the analysis, you can download an example control chart template from our website
www.hellma.com/en/hellma/downloads
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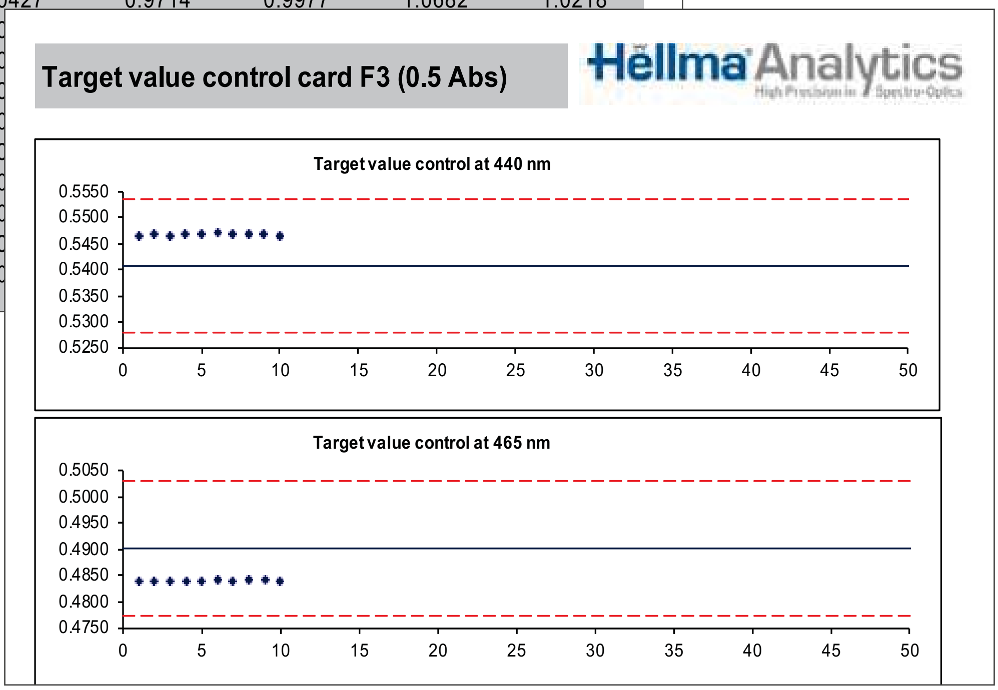
Measurement Neutral Density Glass Filter: F4 (1.0 Abs)

Measurement device: xxxx

Values from Hellma calibration certificate:

Target value	1.0514	0.9811	1.0067	1.0629	1.0206
upper cutoff	1.0682	0.9945	1.0201	1.0797	1.0340
lower cutoff	1.0346	0.9677	0.9933	1.0461	1.0072

Your values:	Wavelengths (from calibration certificate)				
Datum	**440 nm**	**465 nm**	**546.1 nm**	**590 nm**	**635 nm**
19.04.16	1.0427	0.9714	0.9977	1.0682	1.0218
09.05.16	1.0				
25.06.16	1.0				
09.08.16	1.0				
13.09.16	1.0				
26.10.16	1.0				
28.11.16	1.0				
20.12.16	1.0				
24.01.17	1.0				
22.02.17	1.0				



Liquid Filters

Measurement in accordance with Ph. Eur. and USP <857> Procedure B

10.5 Calibration with liquid filters (stray light and spectral resolution)

10.5.1 Steps for checking the stray light level in accordance to Ph. Eur. and USP <857> Procedure B and interpretation

1. First carry out the "Preparations" according to chapter 10.4.1.

2. Set the wavelength selection program on your spectrophotometer. To do this, follow the instructions in the corresponding operating manual from your instrument manufacturer. According to the solution used, use the wavelength specified by the respective pharmacopoeia for this solution (see table item 8; e.g. potassium chloride: 198 nm).

3. If possible, select the slit width specified on the supplied calibration certificate on your spectrophotometer.

4. Perform a zero point adjustment (autozero).

5. The measurement is usually performed against a reference filter filled with water (for acetone measurement against air). Observe the general handling instructions for liquid filters. The filters should always be inserted in the cuvette holder in the same orientation, e.g. always with the Hellma lettering facing the light source.

6. **Measurement in the single-beam spectrophotometer:** Carefully insert the supplied reference filter into the cuvette holder. Start the measurement. Then measure the optical reference material. Then subtract the value of the reference measurement from the value of the measurement with the optical reference material.

7. **Measurement in the double-beam spectrophotometer:** Carefully place the optical reference material in the sample holder and the reference filter in the reference sample holder. Start the measurement.

8. The acceptance criterion depends on the filter used and the respective pharmacopeia (EP/USP) see attached table:

Filter / Solution	**Requirements European Pharmacopoeia** absorbance at wavelength	**Requirements USP <857> Procedure B** absorbance at wavelength
Potassium chloride (12 g/L)	≥ 2.0 Abs at 198 nm	≥ 2.0 Abs at 198 nm
Sodium iodide (10 g/L)	≥ 3.0 Abs at 220 nm	≥ 2.0 Abs at 220 nm
Potassium iodide (10 g/L)	≥ 3.0 Abs at 250 nm	≥ 2.0 Abs at 220 nm
Acetone (pure)		≥ 2.0 Abs at 300 nm
Sodium nitrite (50 g/L)	≥ 3.0 Abs at 340 and 370 nm	≥ 2.0 Abs at 340 nm

9. The light level (remaining transmittance value) measured at the given 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 the slope to high transmissions is steep.

NOTE:

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.

FOR INFORMATION

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 less than 0.02 Abs, you should contact the service engineer.

Liquid Filters

Measurement in accordance with USP <857> Procedure A

10.5.2 Procedure for checking the stray light level according to USP <857> and interpretation

1. First carry out the "Preparations" according to chapter 10.4.1.
2. Run the scan program on your spectrophotometer, observing the guidelines in the user manual. Select the limits of the scanning range so that all values listed on the filter's calibration certificate are recorded.
3. If possible, 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 in each case with 10 mm path length (for Potassium chloride (UV1), for example, start at 220 nm) and scan to approx. 20 nm below the cut-off wavelength.
5. If possible, carry out a baseline correction.
6. The measurement is carried out against a reference filter with 5 mm path length, filled with the same solution. Follow the general usage guidelines for liquid filters. The filters should always be positioned in the cuvette holders in the same way, e.g. always with the Hellma lettering facing the light source.
7. **Measurements in a single-beam spectrophotometer:** Carefully insert the reference filter provided, with 5 mm path length, into the cuvette holder. Start the measurement. Next, measure the filter with 10 mm path length, filled with the same solution. Then subtract the values of the reference measurement from the values of the measurement of the optical reference material.
8. **Measurements in a double-beam spectrophotometer:** Carefully insert the filter with 10 mm path length into the sample holder, and the reference filter with 5 mm path length, filled with the same solution, into the reference sample holder.
9. Start the measurement. Scan a range of 20 nm around the cut-off.
10. Record the measured maximum absorbance value ΔA.
11. Check whether the recorded absorbance value is ≥ 0.7 Abs.
12. Next, use the following formula to calculate the stray light level:
    $s_{\lambda} = 0.25 \times 10^{-2\Delta A}$
    s_{λ} stray light value, in transmittance (T).
    ΔA observed maximum absorbance.
13. Check whether $S_{\lambda} \leq 0.01$.

Measurement in accordance with USP <857>, procedure A

667-UV105H liquid filter set for checking stray light acc. to USP <857>, procedure A

MEASUREMENT PARAMETERS FOR CHECKING STRAY LIGHT LEVEL

To enable a realistic estimate of the stray light level, choose a filter set with a cut-off wavelength as close above the required wavelength as possible. The stray light test is then carried out with the appropriate filter set, consisting of a stray light filter with 10 mm path length and the associated reference filter with 5 mm path length, both filters being filled with the same solution. The stray light level s_λ calculated using the formula corresponds to the stray light level of the device at the measurement wavelength. Since this value as well as the location of the peak maximum differ depending on the properties of the measuring system, the filter set can only be certified with regard to its suitability for use as a stray light filter in accordance with USP <857> procedure A.

INTERPRETING MEASUREMENT RESULTS WHEN CHECKING FOR STRAY LIGHT

If the stray light level you have observed does not correspond to the default value criteria of USP <857> procedure A, i.e. the absorbance at the peak maximum is less than 0.7 Abs and the stray light value calculated for the wavelength of the peak maximum s_λ is greater than 0.01, then first check whether your result was caused by extraneous light. If you can rule out extraneous light, please contact one of your device manufacturer's service technicians.

Liquid Filters

10.5.3 Steps for checking spectral resolution and interpretation

1. First carry out the "Preparations" according to chapter 10.4.1.

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 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 measured values, as stated on the calibration certificate provided.

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 λmax = 269 nm by the minimum peak measured at λmin = 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.

NOTE:
The USP <857> requires that the spectral band width of the spectrophotometer used should be 2 nm or less, this means that the calculated ratio between maximum and minimum is not less than 1.3.

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

SLIT WIDTH	ABSORBANCE RATIO (EXAMPLE)
0.5	2.2
1.0	2.0
2.0	1.4
3.0	1.1

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

FOR INFORMATION

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.

* Accredited calibration laboratory by DAkkS according to DIN EN ISO/IEC 17025. The accreditation is valid only for the scope listed in the annex of the accreditation certificate D-K-18752-01-00.

Reference plates

10.6 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 into black anodized precision frames made of aluminum. They are designed to fit into all **microplate 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 / TRANSPORT

It is recommended to store the reference plate after use in the supplied packaging in a dry, dust-free, light-protected place.

Storage: relative humidity non-condensing at temperatures of 4 - 40 °C.

Transport: relative humidity non-condensing at temperatures of 4 - 40 °C.

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 and 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.

10.7 Calibration with reference plates

10.7.1 Preparations

1. Warm up the microplate 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 columns 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 microplates 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).

Reference plates

10.7.2 Steps for checking photometric accuracy with reference plates

1. First carry out the "Preparations" according to chapter 10.7.1.
2. Run the wavelength selection program on your microplate reader, observing the guidelines in the user manual. Select the wavelengths stated on the calibration certificate.
3. Set your microplate 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 a slit width as small as possible. We recommend taking several measurements and then using the mean value to avoid errors during evaluation.

Composition of the measurement uncertainty

"The total measurement uncertainty consists of the device-specific measurement deviation of the instrument used and the calibration certificate listed measurement uncertainties combined."

Thomas Brenn,
Product Manager

10.7.3 Steps for checking wavelength accuracy with reference plates

1. First carry out the "Preparations" according to chapter 10.7.1.
2. Run the scan program on your microplate reader, observing the guidelines in the user manual. Select a scanning range that covers all of the peaks listed on the reference plate calibration certificate.
3. Set your microplate reader to the measurement parameters quoted on the calibration certificate provided. Select a slow 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 glass filters are inserted (usually column 11 + 12).
7. Detect 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.

MEASUREMENT PARAMETERS FOR CHECKING WAVELENGTH ACCURACY

Ensure that you have selected the correct measurement parameters before scanning the absorbance curve to detect 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 microplate 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.

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 microplate 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 9 of this user manual.

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