Performance report fluidlab R-300

Spectrometer

Introduction

Spectrophotometers are an important and widely used measuring instrument for evaluating a variety of chemical, biological and medical tests. In order to guarantee the measuring accuracy of spectrophotometers, reference materials have been established that can determine both the photometric and the spectral accuracy of the measuring system. These reference materials are glass vessels in cuvette form, both neutral glass filters as well as vessels which are filled with various liquids. Using the certified reference filters from Hellma Analytics, every fluidlab R-300 is calibrated during production and checked in a final quality control. For better traceability, these values are saved in our database for each device.
Permissible limit values for these tests are defined in US Pharmacopeia 857 (USP). Pharmaceutical companies in the United States are required by law to use only devices that meet this guideline. European companies also work in accordance with this guideline, as they can only operate in the US market if they comply with it. With regard to the limit values for tests, the European Pharmacopoeia 2.2.25 (EP) is identical in most respects to the US version, but contains further limit values, e.g. concerning the linearity of the measurement, which for the sake of completeness shall also be examined in the course of this analysis.
At anvajo we work in accordance with this guideline, as the standards for UV/VIS spectrometers that are described in it are among the most demanding worldwide and set the benchmark in both the pharmaceutical and the biotechnological sectors. These stringent quality standards make it possible to use the anvajo fluidlab for analyses in regulated markets such as in-vitro diagnostics and food or water analysis.

Material and methods

Materials

For the photometric measurements with the fluidlab R-300, the F201, F202, F203 and F4 filters from Hellma Analytics (Müllheim, Germany) were used.
In addition, various dye concentrations of methylene blue (Sigma, Darmstadt, Germany) in standard PMMA cuvettes (10x10 mm) were used to determine reproducibility and for a comparison of linearity between various devices

Photometric parameters

For this pupose neutral glass filters with various absorption characteristics were used. The check is performed on discrete certified wavelengths over a wide extinction range from 0.3 to 2.1

Wavelength parameters

For this purpose, a holmium/didymium liquid filter was used. Certified absorption maxima of this material can be checked onthe basis of discrete wavelengths over theentire VIS range.

	USP limit	EP limit
Accuracy		
VIS region	0.01 < 1 A 1 % > 1 A	0.01 < 1 A 1 % > 1 A
Precision		
VIS region	0.005	n/a
Coefficient of determination R^2		
VIS region	n/a	0.999

	USP limit	EP limit
Accuracy		
VIS region	±2 nm	±3 nm
Precision		
VIS Region	±0,5 nm	±0,5 nm

The measurement protocol is specified by US Pharmacopeia 857 and was performed with an anvajo fluidlab R-300. A blank measurement (I_{blank}) with an empty metal frame was performed for the photometric accuracy measurement. The sample measurement (I_{sample}) of the reference filter was then performed in the same frame with clamped neutral glass, which has specific absorption characteristics.
The two measurements were correlated using the mathematical equation:
$E = -\log10(I_{blank}/I_{sample})$. Finally, the measured values were transferred manually from the display into an Excel file. The calculated extinction values (E) were compared, without any further signal improvements, with the datasheet supplied by Hellma Analytics.

Photometric check

Accuracy

One of the most important tests for the quality control of spectrometers is the photometric accuracy check, as this has a direct influence on the quality of the measurement results. To ensure that scientists and laboratory technicians can rely on the measurement results, all reference measurements over the entire wavelength range must be within the extinction tolerance of 0.01 or 1% specified by the pharmacopoeia.
As can be seen from Table 1, the anvajo fluidlab R-300 meets the required level of accuracy. With the reference filters from Hellma Analytics, this quality control can also be performed independently by any laboratory in which the fluidlab R-300 is used. In general, spectrometers are checked in this manner every one to two years.

Wavelength [nm]	Target F201	Actual F201	Difference	Soll F202	Ist F202	Difference	Soll F203	Ist F203	Difference	Soll F4	Ist F4	Difference
440.0	0.3304	0.325	0.0054	1.6321	1.625	0.0071	2.177	2.157	0.0200	1.0674	1.057	0.0104
465.0	0.2946	0.291	0.0036	1.5121	1.504	0.0081	2.0212	2.011	0.0102	0.9897	0.984	0.0057
546.1	0.3049	0.304	0.0009	1.5066	1.499	0.0076	1.9823	1.971	0.0113	1.0108	1.002	0.0088
590.0	0.3354	0.335	0.0004	1.5409	1.53	0.0109	2.0305	2.014	0.0165	1.0694	1.058	0.0114
635.0	0.3386	0.342	-0.0034	1.4512	1.44	0.0112	1.9193	1.902	0.0173	1.0291	1.018	0.0111

Table 1: Overview of reference and measured values. In accordance with US Pharmacopeia 857, the difference between thetwo values should be less than 0.02.

Reproductability

The reproducibility of measurements is an important factor for assessing the effect of mechanical tolerances in the device (especially in the sample shaft), the stability over time of the spectrometric measurement and the accuracy of the recording of the measured values. In this regard, the US Pharmacopeia specifies a standard deviation of 0.005 or 0.5% of the extinction value across at least 6 measurements in each case. These values could be easily achieved with all the reference glass filters. Table 2 shows the values for n = 10 repeated measurements.

Wavelength [nm]	s(F201)	s(F202)	s(F203)	s(F4)
440.0	0.0017	0.0008	0.0014	0.0017
465.0	0.0018	0.0010	0.0014	0.0020
546.1	0.0016	0.0010	0.0014	0.0023
590.0	0.0017	0.0009	0.0010	0.0015
635.0	0.0011	0.0016	0.0016	0.0007

Table 2: Reproducibility measurements with reference glass filters. The standard deviation s of all measurement series isbelow the required limit value of 0.005 and thus complies with the US Pharmacopeia (n = 10).

In Table 3, the reproducibility is examined again in a further step with a dye sample. For this examination, a methylene blue sample in a cuvette was inserted into the device 20 times in a row and the measured values were recorded. The standard deviation of less than 1% is very good, but slightly higher than with the reference glass filters. This is primarily on account of the poorer optical and inhomogeneous surface of the cuvettes and the processes used with this sample, which are a significant factor in comparison to the reference glass filters.

N = 20	Methylene blue (33±5%)	Methylene blue (66±5%)
1	1.5	1.905
2	1.494	1.916
3	1.508	1.947
4	1.494	1.904
5	1.508	1.91
6	1.51	1.944
7	1.512	1.947
8	1.518	1.928
9	1.526	1.941
10	1.508	1.968
11	1.512	1.927
12	1.504	1.952
13	1.506	1.919
14	1.504	1.907
15	1.502	1.909
16	1.52	1.925
17	1.517	1.936
18	1.504	1.914
19	1.514	1.93
20	1.512	1.903
Mittelwert	1.509	1.927
Std	0.0054	0.0097
Std %	0.54 %	0.97 %

Table 3: Reproducibility measurements with a methylene blue sample which was inserted into and removed from the device20 times at short intervals.

Linearity

The linearity can be derived from the accuracy measurements with the reference filters. If measurements display a high level of accuracy, it may be inferred that the linearity is also very high, as can be seen from Figure 1. The coefficient of determination of the linear fit across the measured values is R2 = 1 and is above the limit value of R2 = 0.999 specified in the European Pharmacopoeia. There is no target value for linearity in the US Pharmacopeia. The linearity thus attains the theoretical maximum over the extinction range from 0.3 to 2.15. As a result, the linear range reflects the range of values of the spectrometer, which must display an extinction value of between 0 and 2.0. The linear range of most biochemical and chemical assays also lays between these limit values.

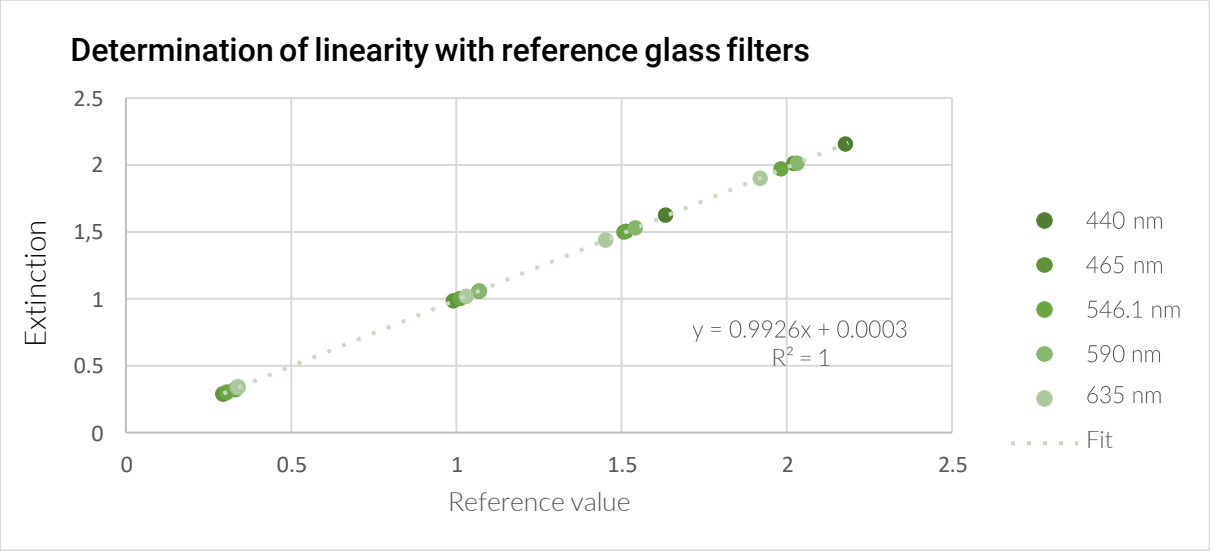

Figure 1: Comparison of the reference values from Hellma Analytics and the measured values of the fluidlab R-300. With a coefficient of determination of R2=1, the linearity value is excellent. With a value of 0.0003, the intersection with the y-axis is below the measuring accuracy of the system

The linearity measurements of a dilution series are plotted in the graph below (Figure 2). For this purpose, a dilution series with trypan blue (a commonly used dye for staining cells) was measured both with the fluidlab R-300 and with one of the most frequently used spectrometers worldwide. It is clear from the curve progressions that the anvajo fluidlab R-300 is significantly more linear than the competitor device over an extinction range of 1 to approximately 2.4 (R2 = 0.998 and R2 = 0.962). The more linear the measurement results of a device are over a wide range of values, the more precisely can assays be evaluated in high concentration ranges.

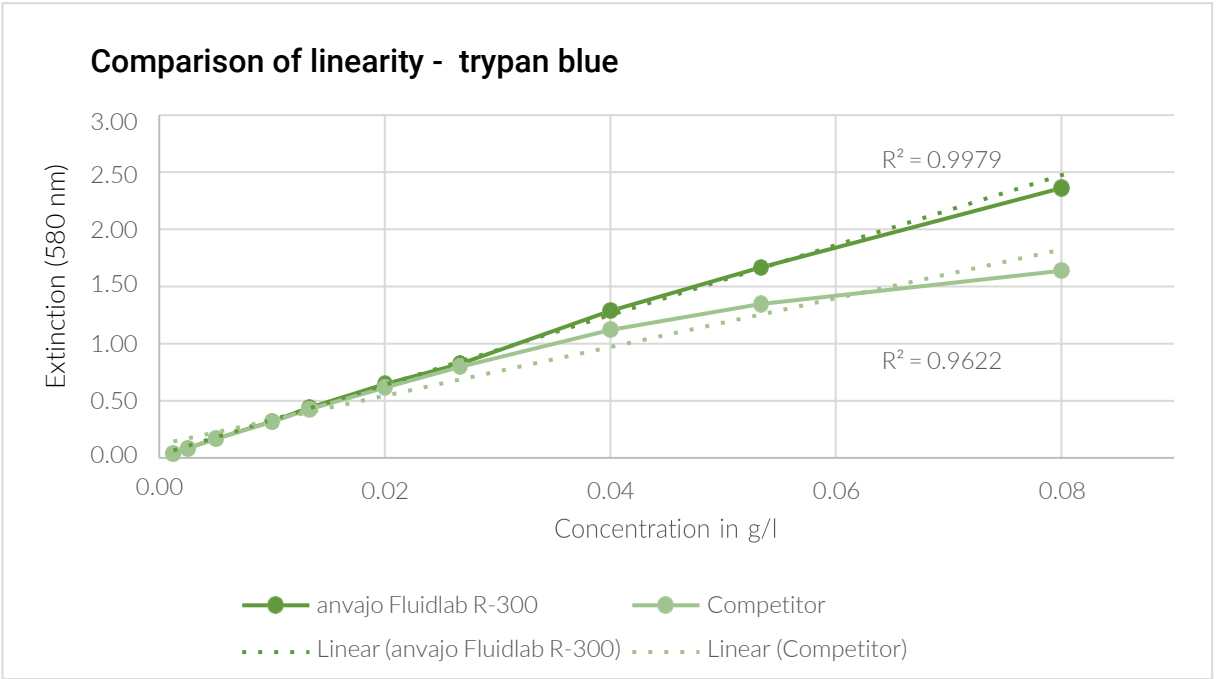

Figure 2: Comparison of the linearity of a trypan blue dilution series between the anvajo fluidlab R-300 and a widely used competitor device. The linearity of the fluidlab R-300 is higher than that of the competitor device over the range of values under consideration.

Device-to-device reproducibility

Every spectrometer that we produce at anvajo must meet the tolerance conditions described above in the section on 'Accuracy'. Device-to-device reproducibility with small deviations in the measurement results is an important factor for ensuring the comparability of measurements that have been performed with different devices. This is applicable to various application examples: in a production plant multiple devices are often placed at different stations for measurements, or different laboratories may wish to exchange their findings and results, so that the comparability of the results must also be guaranteed in this case. Table 4 shows the deviation from device to device, after a series of measurements was performed with 4 random devices from Production. The maximum deviation between the measuring points is 0.6%. The device-to-device deviation is therefore small and permits reliable comparison of measurement results between different devices.

Wavelength [nm]	F201	F202	F203	F4
440.0	0.26%	0.29%	0.59%	0.48%
465.0	0.33%	0.22%	0.49%	0.33%
546.1	0.40%	0.33%	0.46%	0.29%
590.0	0.31%	0.50%	0.59%	0.37%
635.0	0.43%	0.57%	0.65%	0.40%

Table 4: Device-to-device tolerance of 4 randomly selected fluidlab R-300s at different wavelengths and with different extinction values.

Summary

It has been possible to show that the anvajo fluidlab R-300 meets all the requirements of the US Pharmacopeia and the European Pharmacopoeia and can thus potentially be used in the production of medicines. This forms a very good and reliable basis for high-quality spectrometric measurement with the fluidlab and furthermore for the establishment of POC tests in the medical field or in the life sciences, with the laboratory-level quality that users expect.