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UV-1900i Plus UV-Vis Spectrometer, LabSolutions™ DBCS Multi Data Report

Application News

Analysis of the Degree of Coloration of Liquids Compliant to European Pharmacopeia

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User Benefits

- Semi-Automated analysis with LabSolutions UV-Vis and Multi Data Report.
- Fully Part 11 CFR compliant through use of audit trail and electronic signatures for raw data and report.
- Highly customizable to fit any corporate identity, SOP or other applicable documents.

■ Introduction

Chapter 2.2.2. Degree of Coloration of Liquids of the European Pharmacopeia (short EP in the following text) specifies how to determine the coloration of a liquid sample, which is an important criterion in pharmaceutical quality control.[1] By definition, a liquid is colorless if it has the appearance of water or the solvent used in its preparation.

Several reference solutions are specified in this chapter, which are created from mixing yellow, red and blue primary solutions. In the past, each of these solutions was compared visually to a sample as shown in Fig. 1.

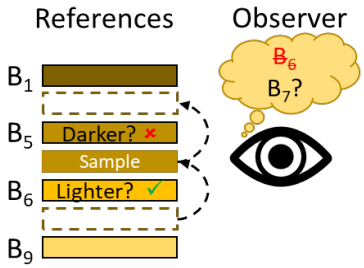

Fig.1 Schematic of visual color comparison

Since EP 10.3, using a spectrophotometer and software-based analysis is allowed as alternative to this traditional method. In this application, a semi-automated approach is shown that uses LabSolutions UV-Vis Color analysis software and the LabSolutions Multi Data Report to assign the correct color number to a sample. This solution significantly reduces the number of manual steps and allows full traceability of the data via audit trail and electronic signatures for raw data, templates and finished reports.

■ Sample Preparation

Commercially available primary solutions (Carl Roth ROTI Calipure Reag. Ph. Eur., Primary solution) and hydrochloric acid (10 g/L HCl in water) were mixed according to the recipes in table 2.2.2.-1. of EP 11.5 to create the brown (B), brownish-yellow (BY), yellow (Y), greenish yellow (GY) and red (R) stock solutions.

Each stock solution was diluted with hydrochloric acid (10 g/L HCl in water) to create the dilution series of B_1 to B_9, BY_1 to BY_7, Y_1 to Y_7, GY_1 to GY_7 and R_1 to R_7 following the recipes specified in tables 2.2.2-2. to 2.2.2-6. in EP10.

Disposable 10 mm cells (Brand plastic square cells, 10 mm pathlength, 340 – 900 nm transparency range) were filled with the reference solutions as shown in Fig. 2, for use in the UV-1900i Plus UV-Vis Spectrophotometer.

Reference solutions B_1, B_4, BY_1, BY_3, GY_1, GY_3, R_1, R_3 and Y_3 were remeasured in different cells (Hellma, 10 mm, Quartz), to create slightly different spectra and simulate "unknown" samples for testing the analysis procedure.

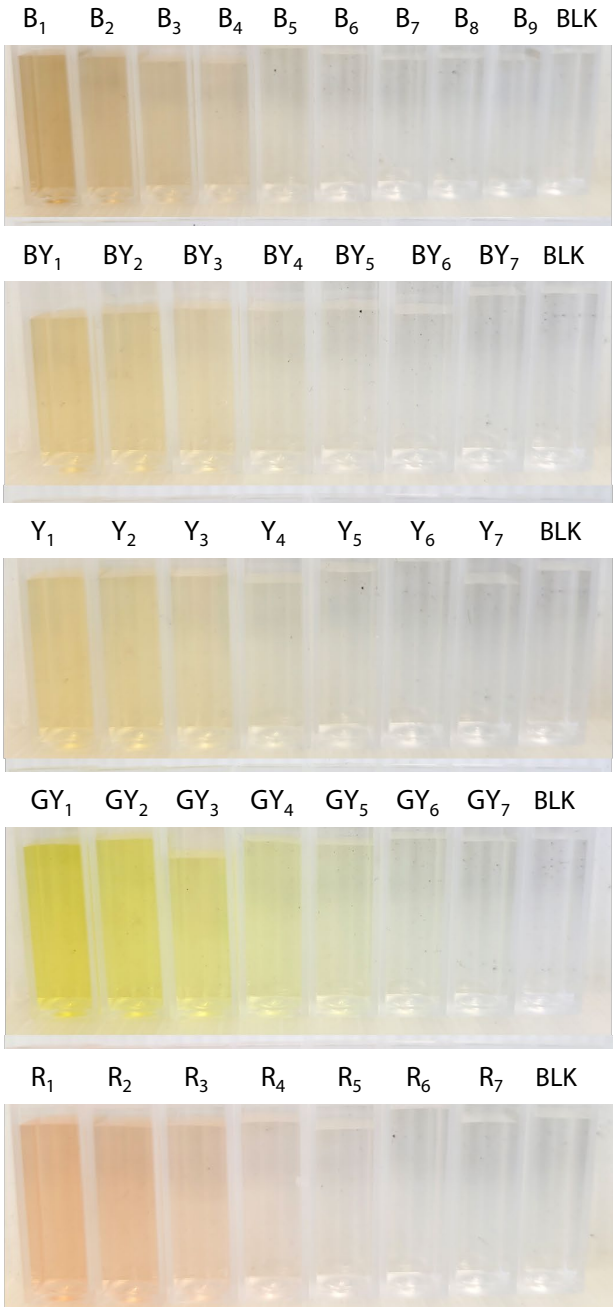

Fig. 2 from top to bottom: Series of reference solutions B, BY, Y, GY, and R.

■ Color Coordinates

The instrumental method to determine the degree of coloration is based on the CIE color model.[2,3] This model describes a three-dimensional color coordinate system: Lightness index L* ranges from black (0) to white (100), color coordinate a* ranges from green (a* < 0) to red (a* > 0) and color coordinate b* ranges from blue (b* < 0) to yellow (b* > 0), as shown in Fig. 3. Instead of the cartesian coordinates a* and b*, the polar coordinates C* (chroma, distance to the L-axis) and h° (hue, angle to the a* axis) can be used to describe a color in the CIE system.

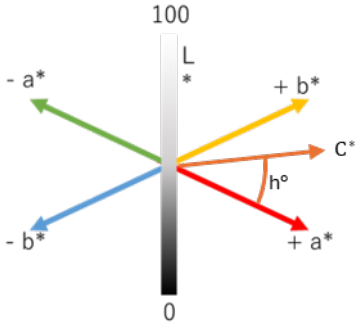

Fig. 3 Coordinate system of the CIELAB color model.

Since color stimulus is highly individual and depends on the lighting conditions (e.g. warm yellow light vs. cold white light), standard illuminant and observer are specified for the conversion from a UV-Vis transmittance spectrum to color coordinates by the equations given in ASTM E 308.

Fig. 4 shows the sensitivity to red, green and blue color stimulus of the 2° standard observer and the emissivity of standard illuminant C as specified in EP 11.5.

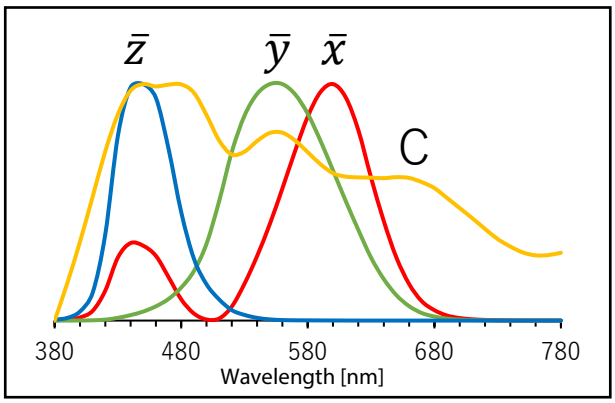

Fig.4 Tristimulus curves $\bar{x}$ (red line), $\bar{y}$ (green line) and $\bar{z}$ (blue line) and standard illuminant C (yellow line) from ASTM E 308, normalized values.

Illuminant C simulates the conditions of bright daylight, while the 2° standard observer simulates the color stimulus of an object observed at close range. Therefore, these parameters approximate the conditions specified for the visual comparison well.

■ Analysis Condition

The UV-1900i Plus (shown in Fig 5) was used, as it comes perfectly equipped for analysis compliant to European Pharmacopeia: It has a fixed slit for 1 nm spectral bandwidth, a dual-beam optical layout and comes with build-in EP validation functions, which can be set to be forced on each startup of the instrument to ensure EP compliant data acquisition.

Fig. 5 UV-1900i Plus UV-Vis spectrophotometer

The system can be used in standalone mode via integrated touchscreen with a simple user management and security functions. Here, it was connected to LabSolutions UV-Vis DB for CFR Part 11 compliant data integrity and automated color analysis. In a high-throughput scenario it can be connected to an Autosampler with flow cell for automated analysis of up to 360 samples.

The measurement parameters are shown in Table 1.

Table 1 Analysis Conditions of UV-1900i Plus

System	: UV-1900i Plus
Wavelength Range	: 800 to 380 nm
Data Interval	: 1 nm
Scan Speed	: Medium
Value Type	: Transmittance T%
Slit Width	: 1 nm (fixed)
Light Source Switch	: 340 nm

The EP specifies the following workflow for determining the degree of coloration of a liquid from UV-Vis spectrum data:

1. Measure water R as blank (100 T% by definition).
2. Measure each reference solution and sample.
3. Calculate the CIE values L*, a* and b* or L*, C* and h°
4. Calculate the LAB or LCh color difference for each combination of sample and reference:
   a) $\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$
   b) $\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta C^*)^2 + (\Delta h^\circ)^2}$
5. Assign to each sample the color number of the reference, which had the smallest color difference ΔE

The lightness index L* and color coordinates a* and b* were calculated with LabSolutions UV-Vis Color software. The calculation parameters are shown in Table 2. The evaluation template was set up so that each measured spectrum was automatically added after the measurement.

Table 2 Detail settings for the calculation of L*, a* and b*

Color System	: CIELAB
Evaluation value	: L*, a*, b*, C* and h°
Standard	: ASTM E308-22
Colorimetric Illuminant	: C
Observation Field of view	: 2°
Number of decimal	: 3

For the calculation of the color difference and automated assignment of the correct color number, a multi data report template was prepared. This tool collects multiple raw data files in a single report. The EP Degree of Coloration report template uses a combination of VLOOKUP() and HLOOKUP() to assign the color number of the best fitting reference to each sample. This final step can be quite tedious without automated data analysis, especially if many samples are measured in a short time. For each sample, 37 values of ΔE must be calculated to find the correct degree of coloration.

■ Multi Data Report Template

The multi data report (short MDR) is a module of LabSolutions to combine data from multiple raw data files into a single report. The report editor allows to prepare equations as typical for spreadsheet software, but the input values are read from the data files just as this template is used to print a report. No manual changes are possible in the final report and data integrity is preserved.

For this application, a report template was prepared that collects the color coordinates of all unknown samples into one table and the color coordinates of all reference solutions into a second table as shown in Fig. 6

EP10 Degree of Coloration

Sample Table

File Name	Sample Name	Option	L*	a*	b*	Color	ΔE
EP10 - 0-66-2 - UV1900_Quartz_B1.vspd	Sample 1	B1	89.304	-0.510	24.143	B1	0.99
EP10 - 0-67-2 - UV1900_Quartz_B4.vspd	Sample 2	B4	96.276	-1.066	9.572	B4	0.31
EP10 - 0-68-2 - UV1900_Quartz_BY1.vspd	Sample 3	BY1	94.899	-6.548	29.929	BY1	1.15
EP10 - 0-69-2 - UV1900_Quartz_BY3.vspd	Sample 4	BY3	97.411	-4.850	16.603	BY3	0.63
EP10 - 0-70-2 - UV1900_Quartz_GY1.vspd	Sample 5	GY1	96.317	-22.271	72.690	GY1	2.04

Standard Table

File Name	Sample Name	Option	L*	a*	b*	Color	ΔE
EPColor_UV1900_GY_001.vspd	GY1		96.247	-22.147	70.659	GY1	0
EPColor_UV1900_GY_002.vspd	GY2		97.262	-21.142	59.637	GY2	0
EPColor_UV1900_GY_003.vspd	GY3		98.161	-18.576	45.796	GY3	0
EPColor_UV1900_GY_004.vspd	GY4		99.068	-12.941	27.554	GY4	0
EPColor_UV1900_GY_005.vspd	GY5		99.495	-8.017	15.696	GY5	0

Fig. 6 Print preview of the multi data report for degree of coloration

Contrary to normal spreadsheet software, the table is populated using repeat rows and the sheet is protected when the final report is generated. The repeat row function adds new rows for each raw data file when the final report is generated. It is not necessary to know the number of expected samples when the template is created.
Filters can be set when specifying the repeat row. Therefore, the multi data report can be structured independently from the structure of each raw data file. For example, Fig. 7 shows the filter conditions for reading only values from reference solutions. Any other data is ignored for populating this specific repeat row.

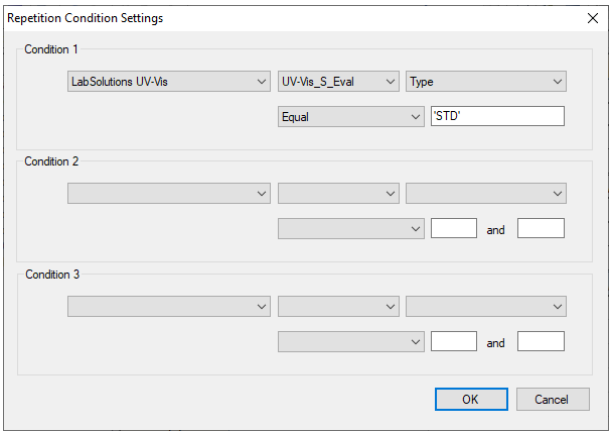

Fig. 7 Repetition conditions of the standard table in the MDR template

Up to three filter conditions can be set for each repeat row to further specify the fine structure of the report template. For example: By adding a filter for sample name, the standard table can be grouped by dilution series (B, R, etc.) instead of listing all reference values in arbitrary order.

The calculation of ΔE for each combination of sample and standard is done in hidden cells of the sheet, as shown in Fig. 8.

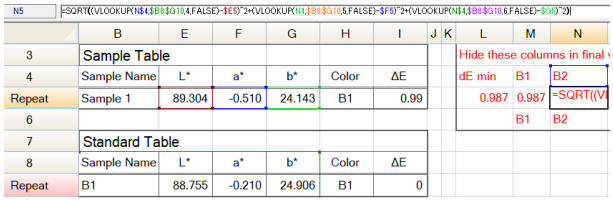

Fig. 8 Color difference calculation in the MDR template, part 1

In this application, one column is dedicated to each reference solution. VLOOKUP() is used to read the corresponding color coordinates from the standard table and calculate the color difference between each reference solution and the sample which is shown in the same row. For example: The color coordinates of Sample 1 are shown in row 5. The color difference between Sample 1 and reference B_1 is calculated in cell M5. The color coordinates from reference solution B_2 are found via VLOOKUP() to calculate the color difference between Sample 1 and reference B_2 in cell N5.

In the sample table, HLOOKUP() is used to find the name of the reference solution, which has the lowest color difference to the sample in the same row. The exact formula for Sample 1 in in row 5 is shown in Fig. 9, the range of HLOOKUP() is adjusted with the OFFSET() function as the table grows dynamically with each repeat row when measured data is added to the report.

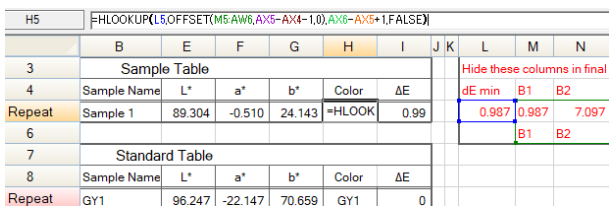

Fig. 9 Color difference calculation in the MDR template, part 2

In this example, reference B_1 had the lowest color difference of all combinations between Sample 1 and any reference solution and this value is $\Delta E = 0.987$.

■ Measurement Results – Standards

The spectra of each stock solution are shown in Fig. 10. Only the spectrum of solution B_1 has distinct peaks.

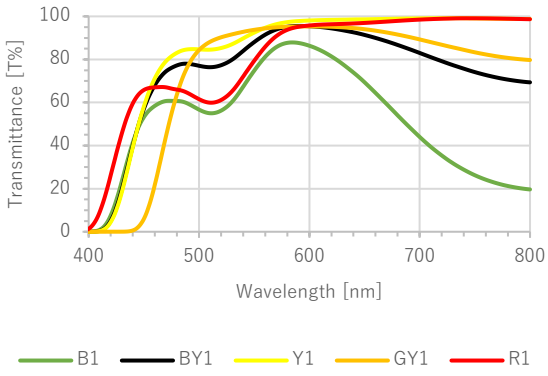

Fig. 10 Spectra of reference solutions B_1, BY_1, Y_1, GY_1 and R_1

The progression of color coordinates a* and b* for each series of reference solutions is shown in Fig. 10. The shade in the background of each image reflects the color impression at the average L* value of each series.

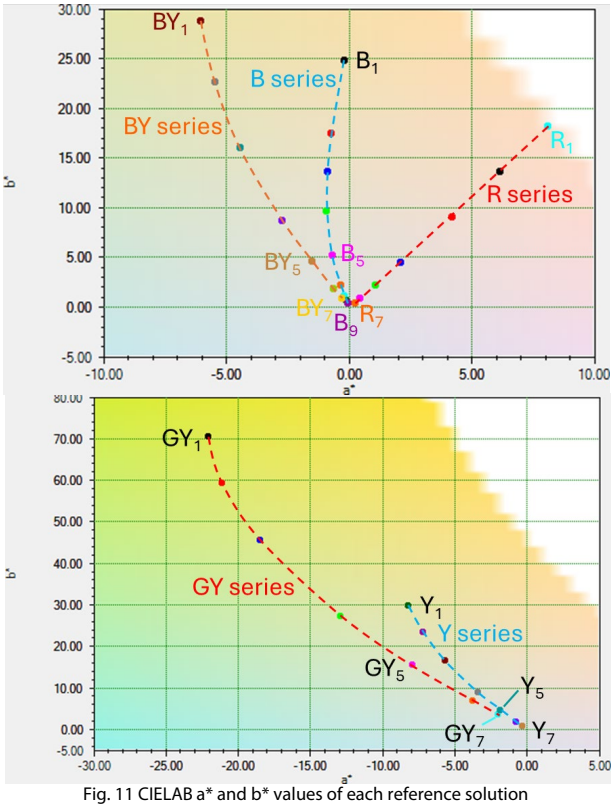

Fig. 11 CIELAB a* and b* values of each reference solution

As visible from Fig. 11, the color coordinates of all dilution series converge towards the same point of the nearly colorless blank with L* = 100 and a* = b* = C* = 0. The hue is nearly constant for each dilution series. Al expected from lightly colored samples, even the stock solutions have very high L* values close to 100. The color coordinates L*, a*, b*, chroma and hue of the highest and lowest concentration of each reference solution are shown in Table 3.

Table 3 CIE color coordinates of each highest and lowest reference

Name	L*	a*	b*	C*	h°
B1	88.76	-0.21	24.91	24.91	90.48
B9	99.84	-0.05	0.50	0.5	96.07
BY1	94.89	-6.09	28.88	29.51	101.91
BY7	99.78	-0.33	1.00	1.05	108.09
Y1	96.83	-8.28	30.05	31.17	105.4
Y7	99.89	-0.39	1.03	1.1	110.61
GY1	96.25	-22.15	70.66	74.05	107.4
GY7	99.86	-2.04	3.71	4.23	118.77
R1	92.06	8.05	18.22	19.92	66.17
R7	99.79	0.21	0.48	0.52	66.55

■ Measurement Results – Test Samples

As explained before, some reference solutions were remeasured in different cells (Quartz instead of plastic). As the absorbance of quartz and plastic are slightly different, the calculated color coordinates are different too.

These spectra were used as "unknown" samples to test, if the multi data report would show the expected color coordinate difference and EP degree of coloration analysis.

The results are shown in Table 4.

Table 4 EP Degree of Coloration results of the test samples

Sample Name	L*	a*	b*	C*	h°	EP Color	ΔE_{ab}	ΔE_{Ch}
B1 Quartz	89.30	-0.51	24.14	24.15	91.21	B1	0.99	1.18
B4 Quartz	96.28	-1.07	9.57	9.63	96.36	B4	0.31	0.81
BY1 Quartz	94.90	-6.55	29.93	30.64	102.34	BY1	1.15	1.2
BY3 Quartz	97.41	-4.85	16.60	17.30	106.28	BY3	0.63	0.96
Y3 Quartz	98.48	-5.95	17.16	18.16	109.12	Y3	0.51	0.58
GY1 Quartz	96.32	-22.27	72.69	76.03	107.03	GY1	2.04	2.01
GY3 Quartz	98.21	-18.90	47.06	50.71	111.89	GY3	1.3	1.31
R1 Quartz	92.18	7.76	18.80	20.34	67.58	R1	0.65	1.47
R3 Quartz	96.09	3.91	8.88	9.71	66.24	R3	0.42	0.79

Since the spectra are slightly different depending on the cell material, the color difference ΔE is not zero for any combination of reference solution (plastic) and test solution (quartz), but the expected EP Degree of Coloration is found for each test solution.

■ Further Customization of the Template

In this example, the sample name of each reference solution was used as search criterium for the VLOOKUP() and HLOOKUP() functions. It is possible to use any other sample information (Sample ID, Option or Comment) instead.

The multi data report template can be further customized to contain any information required by existing processes and fit the corporate identity. Fig 12 shows an example, where the target color number of each sample was typed into the Option field at the time of measurement and the IF() function and conditional formatting were added to the report template for a pass/fail judgement of each sample.

Sample Table							
Sample Name	Target Color	L*	a*	b*	Color	ΔE	Judgement
Sample 1	GY1	96.317	-22.271	72.690	GY1	2.04	PASS
Sample 2	R3	96.089	3.910	8.883	R3	0.42	PASS
Sample 3	BY2	4.968	-1.009	-0.843	B1	87.66	FAIL
Sample 4	BY4	96.276	-1.066	9.572	B4	0.31	FAIL

Fig. 12 Screenshot from a multi data report with pass/fail judgement

In this example, the expected EP Degree of Coloration values are assigned to the first two samples. Sample 3 is much too dark (low L* value) and fails with a very high color difference to the closest EP degree of coloration. Sample 4 has a color intensity in the expected range, but a different tint than expected. Instead of the expected degree of coloration BY4, it has degree of coloration B4.

■ EP Degree of Coloration Package

- ❑ Main Unit
  - UV-1900i Plus UV-Vis spectrophotometer with fixed 1 nm spectral bandwidth and automated validation for EP compliant measurements
- ❑ Consumables
  - Disposable cells with 10 mm pathlength and 15 mm center height
  - Pre-mixed primary solutions or ready to use EP Degree of Coloration reference solutions
- ❑ Software
  - LabSolutions DB UV-Vis for full data integrity
  - Color option for calculation of CIE color values
  - LabSolutions DB Multi Data Report for CFR Part 11 compliant analysis and reporting
  - Template for EP Degree of Coloration analysis

■ Conclusion

The UV-Vis spectra of color reference solutions as specified in chapter 2.2.2. of the European Pharmacopeia 11.5 were measured. The CIELAB L*, a* and b* color coordinates were calculated with LabSolutions UV-Vis Color software. The lightness index L* of all solutions is close to 100 and the lowest concentrated solutions of all dilution series are difficult to distinguish by then naked eye, as the color coordinates a* and b* converge to the blank values.

A LabSolutions Multi Data Report template was created to automatically calculate the color difference between unknown samples and all reference solutions and find the reference solution with the lowest color difference to the unknown samples.

With this combination of LabSolutions UV-Vis Color software and Multi Data Report, an automated analysis of the degree of coloration is achieved.

All data processing from measuring the first UV-Vis spectrum to signing the final report is done in LabSolutions. Automated EP validation checks (e.g. with the Shimadzu UV Performance Validation software or the UV-1900i Plus startup validation) ensure a reliable instrument performance.

The comparison of the color numbers calculated for the same reference solution measured in quartz or plastic cells shows the importance of using the same cell material for reference and sample measurement. Because of the slightly different absorbance of the cell windows, the color coordinates are slightly different even if the same solution is measured.

■ References

[1] European Directorate for the Quality of Medicines & HealthCare (EDQM). European Pharmacopoeia, 11.5 ed., 2.2.2. Degree of coloration of liquids. Strasbourg: EDQM, 2021.

[2] ASTM Standard E 308, 2018, "Standard Practice for Computing the Colors of Objects by Using the CIE System", ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, 2018, doi: 10.1520/E0308-18, www.astm.org

[3] Colorimetry, 4th Edition, CIE technical report 15:2018, 2018, doi: 10.25039/TR.015.2018

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