VALIDATION OF AN INSTRUMENTAL MEASURING PROCEDURE ACCORDING TO THE EUROPEAN PHARMACOPOEIA CHAPTER 2.2.2

Validation of an instrumental measuring procedure to determine the color gradation of transparent liquids with LICO 690 according to the European Pharmacopoeia chapter 2.2.2

1 Basis and Purpose

This validation is based on the instrumental and objective color evaluation of optically clear tinted liquids as specified in the European Pharmacopoeia, chapter 2.2.2. "Degree of coloration of liquids" (https://www.edqm.eu/en). Even today, many pharmaceutical products are still assessed by visual methods to detect color changes during production, storage or ageing.

The visual comparison with color reference solutions by human eyes often involves problems if one product is judged by several persons. Human color perception is considerably influenced by surroundings, light and mental state of the person. Moreover, no method is defined to check color reference solutions for color fastness and constancy. Usually the user has to depend on the standards being replaced in regular intervals and stored orderly at any time.

This method was elaborated to develop a suitable measuring procedure on the basis of existing national and international regulations and standards that should enable objective instrumental color comparisons to be made.

2 Procedure

As early as in 1931, the fundamentals of instrumental colorimetry were standardized on an international level and an international color identification system was defined, called CIE-color system (CIE = Commission Internationale d'Eclairage). This CIE-color system is the basis of colorimetry and laid down in many international standards e.g. ISO 11664[1], ASTM E 308 or USP chapter <1061> "Color -Instrumental measurement"

Instrumental colorimetry is based on these definitions:

- Characteristics of the illuminating light source (ISO 11664 part 2)
- Spectral sensitivity of the human eye (ISO 11664 part 1)
- Color system for representation in numerical terms (ISO 11664 part 3,4)
- Pure transmittance of the sample (EN 1557[2] and DIN 5036[3]).

EN 1557 defines color measurement of transparent liquids following ISO 11664. The basic procedure is the photometric measurement of the pure transmittances of a transparent, optically clear, tinted liquid in the visible spectrum between 380nm and 720nm. The pure spectral transmittance ($\tau(\lambda)$) of a solution is the ratio of the directed spectral radiation flux $(\Phi(\lambda))_{ex}$ emerging from the solution to the incident spectral radiation flux $(\Phi(\lambda))_{in}$ (DIN 1349[4]). The standard tristimulus values X, Y and Z are calculated for standard illuminant C and the 2°-standard observer as provided by ISO 11664.

The standard tristimulus values X, Y and Z are calculated from the standard illuminant S(λ), the standard spectral function x(λ), y(λ) and z(λ) and the measured transmittances τ(λ) using equations (1) to (3) (cp. ISO 11664, spectral analysis). Factor k (equation (4)) serves to standardize the tristimulus value Yn for perfect white (τ(λ)=1).

$$X = k * \int_{\lambda=380}^{720} S(\lambda) * \bar{x}(\lambda) * \tau(\lambda)\, d\lambda \quad \textbf{(1)}$$

$$Y = k * \int_{\lambda=380}^{720} S(\lambda) * \bar{y}(\lambda) * \tau(\lambda)\, d\lambda \quad \textbf{(2)}$$

$$Z = k * \int_{\lambda=380}^{720} S(\lambda) * \bar{z}(\lambda) * \tau(\lambda)\, d\lambda \quad \textbf{(3)}$$

The sample's color values are described by the color's position within the CIE-L*a*b* color space (ISO 11664).

The CIE-L*a*b* color space is a color system adapted to the subjective human color perception.

$$k = \frac{100}{\int_{\lambda=380}^{720} S(\lambda) * \bar{y}(\lambda) * d\lambda} \quad \textbf{(4)}$$

The L*-axis is a measure of a color's lightness, the a*-axis represents the red-green share and the b*-axis the yellow-blue share. On principle, the L*-values are positive and lie between 0 for absolute black hues and 100 for perfect white. The a*-values of red hues are positive, those of green hues negative. Accordingly, the b*-values of yellow hues are positive and those of blue ones negative. Spectrum loci encompassing the L*-axis are identical in their chroma C*, but differ in hue. The spectrum loci on a radius vector starting from the L*-axis are identical in hue h but of increasing chroma. The angle between a radius vector and the positive a*-axis is denoted by hue h_{ab}, given in angular degrees between 0° and 360° and counted in mathematically positive sense.

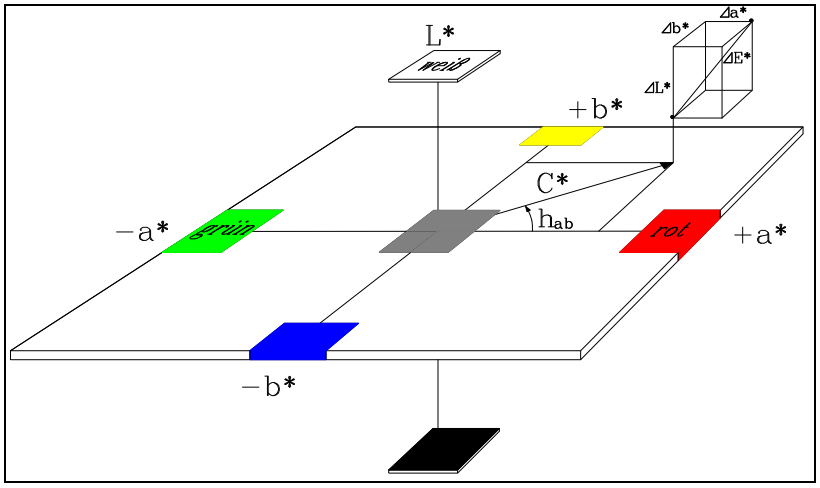


Fig. 1: CIE-Lab-system (ISO 11664)

The L*a*b*-values are calculated from the standard tristimulus values X, Y and Z by equations (5) to (9). They also depend on the employed standard illuminant C and 2°-standard observer.

$$L^* = 116 * \sqrt[3]{\frac{Y}{Y_n}} - 16 \qquad (5)$$

$$a^* = 500 * \left(\sqrt[3]{\frac{X}{X_n}} - \sqrt[3]{\frac{Y}{Y_n}} \right) \qquad (6)$$

$$b^* = 200 * \left(\sqrt[3]{\frac{Y}{Y_n}} - \sqrt[3]{\frac{Z}{Z_n}} \right) \qquad (7)$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \qquad (8)$$

$$h_{ab} = \arctan\frac{b^*}{a^*} \qquad (9)$$

A color difference between reference and sample is indicated by three color differences ΔL*, Δa* and Δb*. ΔL* indicates the lightness difference L_P* - L_B*, Δa* the color difference on the red-green axis expressed as a_P* - a_B* and Δb* that on the yellow-blue axis referred to b_P* - b_B*.

The three-dimensional color distance between two spectrum loci is given as color difference ΔE* and calculated by equation (10).

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad (10)$$

3 Validation of the Analyzing Method

The chemicals - i. e. ferric chloride, potassium iodide, cobaltous sulphate-5-hydrate chloride, cupric and soluble starch - required to prepare the parent solutions red, yellow and blue were in PhD resp. Ph.Eur.-quality supplied by Behr Labor-Technik on July 15th, 1993.

On August 2nd, 1993, the Pharm. Eur. stock solutions were prepared at Dr. Lange DIN Laboratories, Willstätter Str. 11, 40549 Düsseldorf with the a.m. chemicals. After titration they were used to prepare the colour reference solutions for the 5 colour scales Yellow (Y), Greenish-Yellow (GY), Brownish-Yellow (BY), Brown (B) and Red (R). These solutions were then diluted to the colour reference solutions Y1 to Y7, GY1 to GY7, BY1 to BY7, B1 to B9 and R1 to R7.

To determine the CIE-L*a*b*-values of the 37 colour reference solutions the following instruments and cuvettes were used:

3 Dr. Lange colorimeters LICO 200, type LMG099
Instrument numbers 670105, 670123, 6760133

Dr. Lange cuvettes
LYY621 (round glass cuvettes, average path length 11.3mm)
LYY214 (PS-plastic cuvettes, path length 10mm)
LZM130 (PMMA-plastic cuvettes, path length 50mm)

Usual lab accessories like transfer pipettes and glassware.

Before starting the measurement series, the instruments were checked for their good function and accuracy with test filter set LZM134, serial no. 0046.

All measurements are performed at room temperature 22°C +/- 1°C. The mean was taken of the CIE-L*a*b*-values of the 37 color reference solutions measured with these instruments and the results are rounded to one decimal place.

The applicability of this method and the comparability of the reading results of LICO 690 have been tested during the development phase of the instrument when hundreds of measurements were taken for the determination and definition of the CIE-Lab values of the 50 CRS standards prepared according to the Chinese Pharmacopoeia (PPRC). The following instruments have been used: Hach Lange colorimeters LICO 690, type LMG187, Instruments serial numbers 1450640, 1450648, 1450651.

4 Instrument Validation

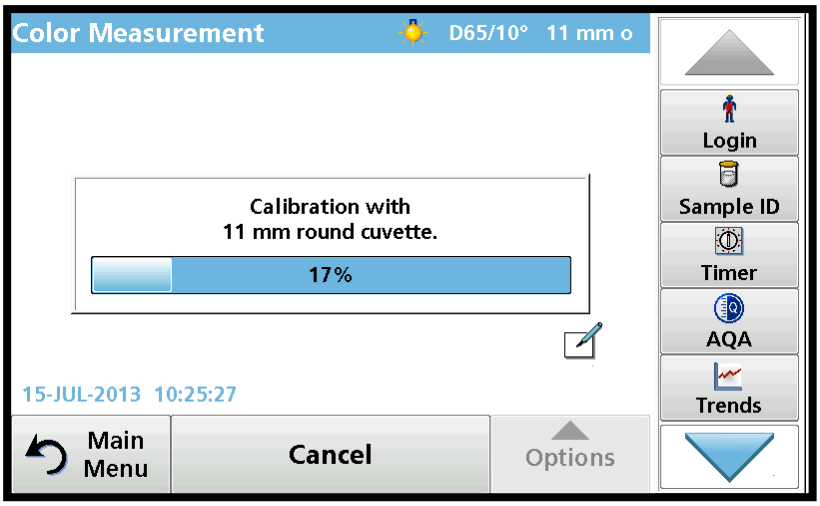
4.1 Instrument Description

LICO 690 from Hach Lange GmbH, type LMG 187, is a single-beam spectrophotometer with reference beam path. A halogen lamp is used to illuminate the sample. A concave grid of 1200 lines/mm splits the light beam into its spectral components. The cuvette slot accepts commercial standard square cuvettes with path lengths between 10mm and 50mm or round cuvettes with an average path length of 11.3mm. The transmittances are measured in the wavelength range between 380nm and 720nm in steps of 10nm.

4.2 Software Description and Validation

The LICO 690 method of determining color in accordance with the European Pharmacopoeia (Ph.Eur.) corresponds to the specifications in *Chapter 2.2.2* of the pharmacopoeia ”Degree of coloration of liquids“, in which a total of 37 color reference solutions (CRS) are defined for the hues yellow (Y1-Y7), greenish-yellow (GY1-GY7), brownish-yellow (BY1-BY7), brown (B1-B9) and red (R1-R7). Each color reference solution is unambiguously defined in the CIE-Lab color space according chapter 6.1 in terms of its brightness, hue and saturation.

After the instrument is switched on it must be calibrated before any color measurements can be carried out.



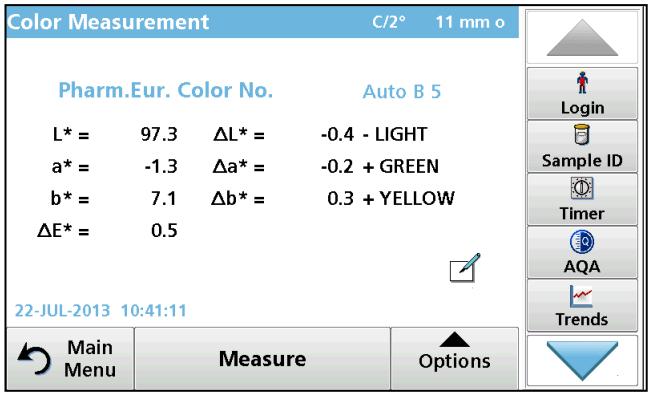
Introduce distilled water into a clean cuvette (the material and path length of the cuvette must correspond to the measurement requirements) and insert the cuvette into the appropriate cell compartment. Recommended cuvettes are listed in chapter 4.5.

A calibration remains valid until the instrument is turned off or recalibrated.

LICO 690 features a calibration memory for all three path lengths employed (10 mm and 50 mm rectangular cuvettes, and 11 mm round cuvettes).

Fill the sample liquid into a cuvette, clean the outside of the cuvette and insert it into the appropriate cuvette compartment. When the measurement has been carried out, the color value of the sample will be displayed in the most recently set color system. Press on the measurement result several times until **Pharm.Eur. Color No.** is displayed above the measurement result.

The display shows the L*, a* and b* values of the sample on the left, then the color difference values ΔL*, Δa* and Δb* to the closest reference solution displayed above, and at the bottom line the total color difference ΔE* between the color of the sample and the reference solution (e.g. reference solution B5). The selected correlation mode is indicated at the top right (e.g. “Auto”). Underneath is a qualitative description of the colour difference (lightness, green, yellow).



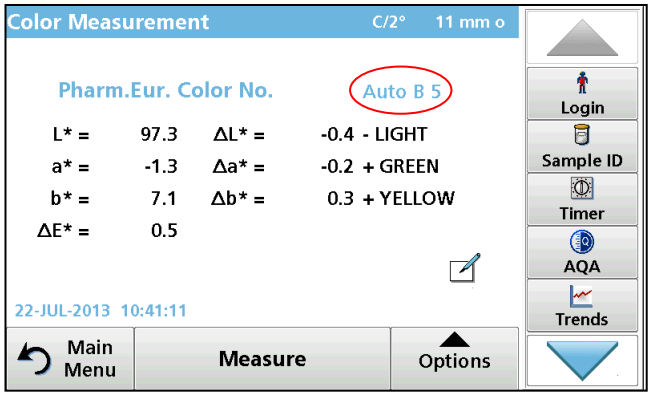
ΔE* Overall spatial color distance between the sample and the Ph.Eur.-reference solution.

ΔL* Gives the lightness difference between the sample and the Ph.Eur.-reference solution. A positive value means that the sample is lighter than the indicated reference solution.

Δa* Gives the red-greed-difference between the sample and the Ph.Eur.-reference solution. A positive value means that the sample is redder or less green than the selected reference solution.

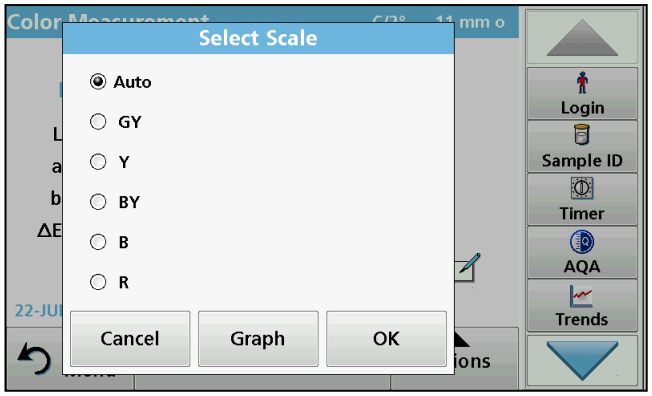
Δb* Gives the yellow-blue-difference between the sample and the Ph.Eur.-reference solution. A positive value means that the sample is yellower than the selected reference solution.

To change the correlation mode, touch the *Scale* area. Select in the following window the comparison mode “Auto” (comparison of the sample within all 5 color scales) or specify a single scale (GY, Y, BY, B or R) for the evaluation.



Basically, there are two ways of correlating the color of a sample with the Ph.Eur. system:

1. **Auto**: The color of the solution is compared to all the 37 color reference solutions. The identifier of the reference solution whose color is closest to that of the sample (i.e. the reference solution with the smallest color difference ΔE* to the sample) is displayed.



2. **Specified scale**: A scale is specified (e.g. B). The identifier of the reference solution whose color is closest to that of the sample within the specified scale (i.e. the reference solution with the smallest color difference ΔE* to the sample within the specified scale) is displayed.

If a scale is specified (e.g. scale B), one of the following five signs is used to define the correlation between the measurement results and the scale more precisely:

= equal: The color of the sample is equal to the color of the reference solution. A sample is considered as "equal (=)" or “the same as” if the color difference ΔE* to the nearest three-dimensional CRS is less than the given " ΔE* equal" - limit of this CRS (see nominal values of the CRS, page 14).

< less: The color number of the sample is less than that of the reference solution

> greater: The color number of the sample is greater than that of the reference solution

<> between: The color number of the sample lies between those of two reference solutions

-> next: The sample colour lies outside the tolerance ranges of the selected colour scale. In this case, the system gives the Ph.Eur.-solution whose colorimetric values are the closest to that of the measured sample. For instance, "-> B5" means that the location of the sample colour is outside scale B, but reference solution B5 is the one with the smallest colour distance ΔE* to the sample.

The limits of the descriptive terms "equal, less” etc. is stipulated for each color reference solution (CRS). These limits are three-dimensional spheres around each CRS. For better understanding, fig. 5 shows these limits as two-dimensional ellipses, although not true to scale.

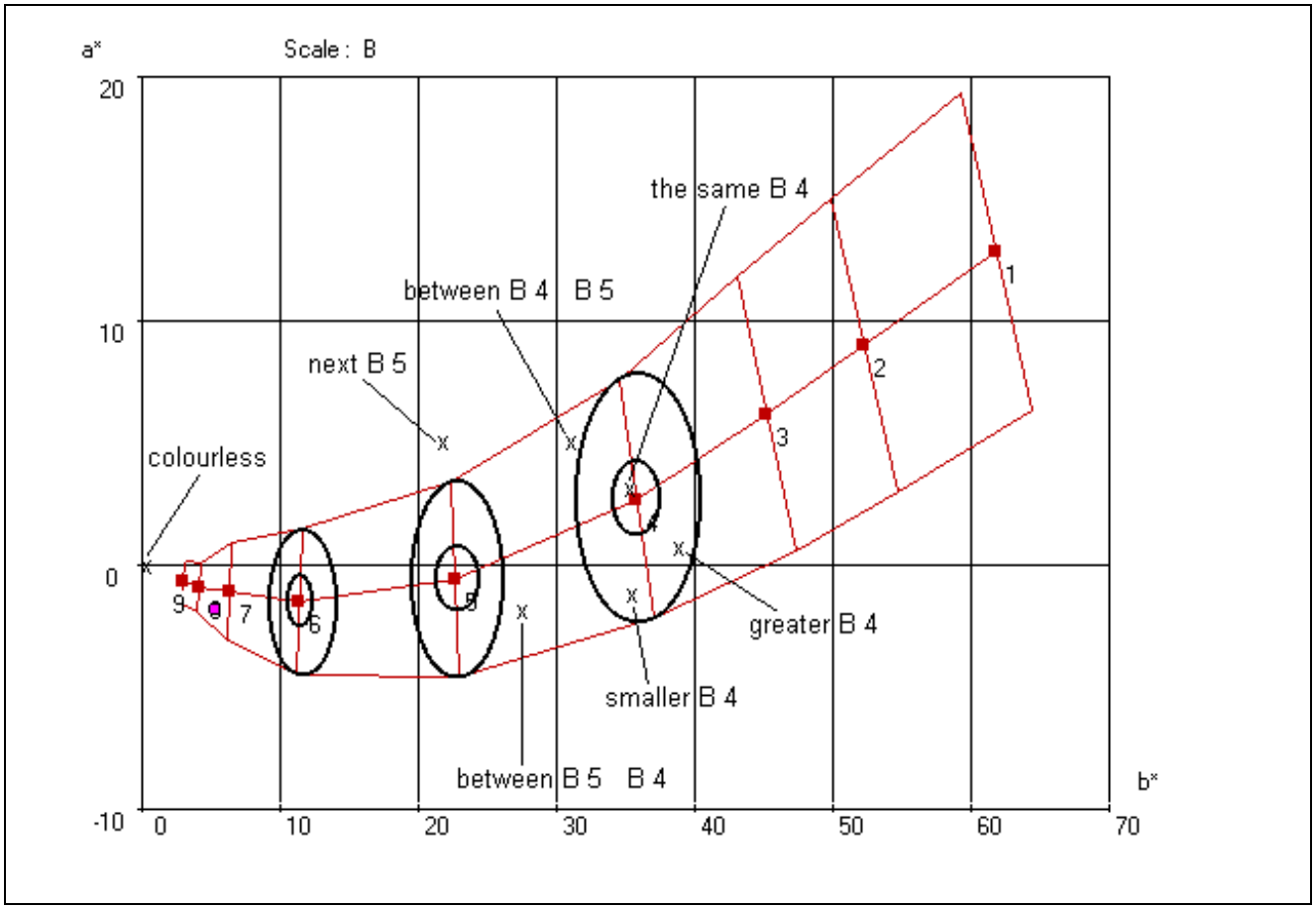
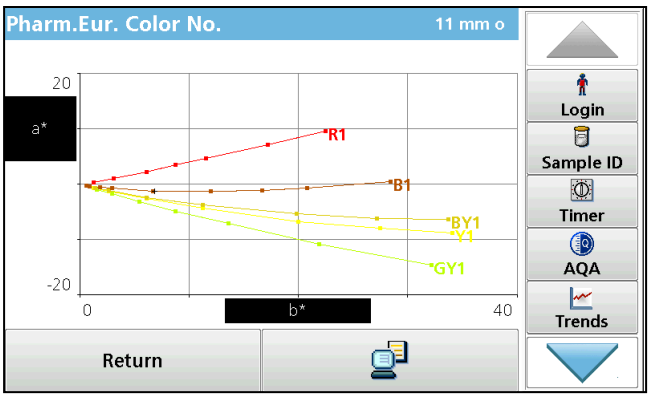
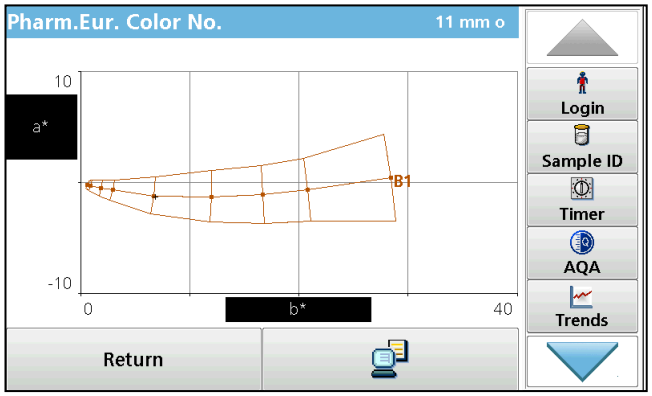


Fig. 2: CIE-L*a*b*-graphic for "B"

A sample is considered as "equal (=)" or “the same as” if the color difference ΔE* to the nearest three-dimensional CRS is less than the given " ΔE* equal"-limit of this CRS (see nominal values of the CRS, p. 13). For a color difference greater than " ΔE* equal" but less than the first " ΔE*"-limit the sample is assessed with greater (higher hue value) or less (smaller hue value).





Note:

Depending on the selected correlation mode (“Auto” or specific scale) the Graph – button will show the CIE a*-b* locus of the sample solution either in all 5 scales or only within the selected scale.

Press button  to print this graph or **Return** to return to the reading result.

The definition of “colourless” is defined as follow:

colourless The measured sample has no perceivable colouration. The definition of the tolerance for colourless are:

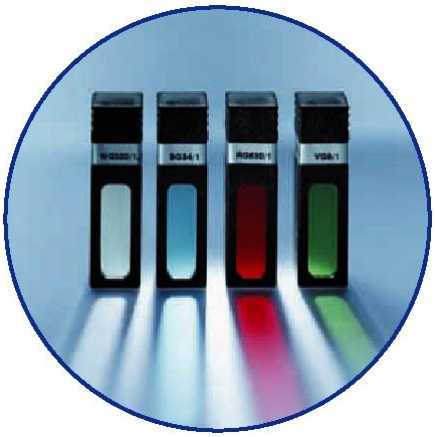
10mm square cuvette	11mm tube	50mm square cuvette
99,90 < L* < 100.40	99,80 < L* < 100.40	99,60 < L* < 100.60
-0,20 < a* < + 0,20	-0,30 < a* < + 0,30	-1,40 < a* < + 1,40
-0,20 < b* < + 0,30	-0,30 < b* < + 0,40	-1,00 < b* < + 1,40

For all color measurements, cuvettes of glass or plastic with path lengths of 10mm, 11mm or 50mm can be used. The required sample volume is only about 2ml for the 10mm square cuvette and the 11mm round cuvette and about 5ml for the 50mm square one. The determined color values can be given a sample name and be stored and printed. It is possible to display and print single transmittances T_{380} - T_{720} and the spectral transmission or absorption (extinction) curves.

Note: Please perform tests to check the usability of the cuvettes with respect to the required or expected accuracy, repeatability and chemical resistant. Recommended cuvettes are listed in chapter 4.5.

4.3 Instrument Test

The operation of the instrument and its measuring accuracy can be checked with a test filter set LZM 339.



The nominal values of these test filters are determined with an average instrument of LICO and remain valid for two years. The average instrument is tested with a test filter set measured at the Federal Institute of Materials Research and Testing BAM.

Alternatively the instrument test can be performed with certified test solution *addista color (order-no. LZM282)*. The *addista color* - set consists 6 solutions (3 Hazen/APHA and 3 Gardner standard color solution, each 50ml).

The instrument test can also performed with certified reference solution from Fluka[1], Buchs/CH (distributed by Sigma Aldrich).

Please perform all tests at room temperature 22°C +/- 1°C.

4.4 Instrument Operation

The operation is described in the producer's operating instructions accompanying the instrument.

4.5 Recommended cuvettes:

Cuvette type	10mm glass	11mm glass	50mm glass	50mm PMMA[2]
Dimensions inner pathlength (mm) outer (mm)	 10 x 10 12 x 12	 11,3 ∅ 13,2 ∅	 50 x 10 52 x 12	 50 x 5 52 x 12
Filling volume approx.	2 ml	2 ml	10 ml	5 ml
Max. temperature	90° C	150° C	90° C	80° C
Pieces/pack	1	560	1	50
Order no.	LYY 215	LYY 621	LZP 167	LZM 130

[1] http://www.sigmaaldrich.com order-no.: 90232 Colour Reference Solution B, BY, Y, GY, R acc. to Ph Eur, 10ml
[2] Polymethylmethacrylate

5 System Aptitude Test

The system (Instrument and Method) was tested for its aptitude and compliance with visual color assessment by using freshly prepared color reference solutions as well as in everyday practice with real samples in the pharmaceutical industry.

The system is in use at:

ABBOTT, Illinois/USA
ABBOTT GmbH&CoKG, 67061 Ludwigshafen
A&M StabTest, 50126 Bergheim
A&M StabTest, 55129 Mainz
ASTA Medica. 60001 Frankfurt
ASTA Medica, 33790 Halle
Astellas Pharma Netherlands, NL-2353 Leiderdorp
ASTRA Chemicals GmbH, 22880 Wedel
AKZO Nobel, NL -5340 BH Oss
BASF, 67056 Ludwigshafen
BASF Geismar/USA
Bayer AG, 51368 Leverkusen
Biotest, 63276 Dreieich
Boehringer Ingelheim KG, 55218 Ingelheim
Boehringer Ingelheim KG, 88397 Biberach
Boehringer Mannheim GmbH, 68298 Mannheim
B.Braun Melsungen AG, 34209 Melsungen
B.Braun Medical, Malaysia
BYK-Gulden, 78403 Konstanz
CKW Pharma Extrakt GmbH, 48529 Nordhorn
Diosynth B.V., NL-Apeldoorn
Diosynth B.V., NL-Oss
Dolorgiet, 53754 St. Augustin
Dressin, 95302 Kulmbach
DSM Andeno B.V., NL-Maarssen
EBW-Arzneimittel, A-Unterach
Eczacibasi-Baxter, 80670 Istanbul, Turkey
Fluka-Chemie AG, CH-9470 Buchs
Guildford Europe (I.S.P.), GB-Guildford
Helopharm, 1000 Berlin
Heumann Pharma GmbH, 90537 Feucht
Hoffmann-La Roche, 79630 Grenzach-Wyhlen
Hoffmann-La Roche AG, CH-4002 Basel
Hoffmann-La Roche AG, F-Paris
Inspec Finechemical GmbH, 69221 Dossenheim
Intervet International B.V., NL-Boxmeer
Jena*pharm* GmbH & Co. KG, 07745 Jena
Johnson&Johnson USA
Dr. Kade GmbH, 12277 Berlin
Knoll Farmaceutici Spa, I-20060 Liscate MI
KVP Pharma+Veterinär, 24106 Kiel
Novartis Pharma GmbH, 79664 Wehr
Labo Serobiologique, Frankreich
Lonza AG, CH-3930 Visp
Merck KGaA, 64293 Darmstadt
Pentapharm AG, CH-4144 Aesch
PharmaForce, Inc., Columbus, Ohio
Rhône-Poulenc Rorer, 50792 Köln
Rhône-Poulenc Rorer, F-Vitry
Rhône-Poulenc, F-Melle
Rhône-Poulenc , F-St. Fons
Rhône-Poulenc Rorer, F-Anthony
Roche, F-Paris
Sandoz, 8500 Nürnberg
Sandoz, F-Orléans
Schering GmbH & Co. KG, 07745 Jena
Schering AG, 13342 Berlin
Schering AG, 59179 Bergkamen
Schwarz Pharma, 40789 Monheim
Smith. K. Beetcham, Worthing/GB
Solvay Pharma, Frankreich
Thomae GmbH, 88397 Biberach a.d. Riss
Weleda AG, 73525 Schwäbisch Gmünd
Zeneca Pharma, Macclesfield/GB

6 Appendix

6.1 Nominal Value of the color reference solutions (CRS)

The next table shows CIE-L*a*b*- nominal values of the color reference solutions used by LICO 690 instrument. It indicates the stored CIE-Lab-values and ΔE*-limits for every CRS under consideration of the cuvette path length.

	10mm square cuvette					11mm vial					50mm square cuvette				
	L*	a*	b*	ΔE*	=ΔE*	L*	a*	b*	ΔE*	=ΔE*	L*	a*	b*	ΔE*	=ΔE*
B1	88,3	0,0	26,2	4,0	2,0	87,0	0,5	28,5	4,0	2,0	58,9	12,8	61,3	7,0	3,5
B2	91,9	-0,9	19,1	3,5	1,8	91,0	-0,6	20,9	2,9	1,5	69,1	9,0	51,8	6,5	3,3
B3	93,9	-1,1	15,3	3,0	1,5	93,1	-1,0	16,8	2,6	1,3	75,1	6,2	44,8	6,0	3,0
B4	95,8	-1,2	10,9	2,4	1,2	95,3	-1,2	12,1	2,3	1,1	82,1	2,7	35,5	5,0	2,5
B5	97,9	-1,0	6,1	1,8	0,9	97,7	-1,1	6,8	1,8	1,0	90,3	-0,6	22,5	4,0	2,0
B6	99,2	-0,6	2,7	1,0	0,5	99,1	-0,6	3,0	1,0	0,5	96,0	-1,5	11,3	3,0	1,5
B7	99,5	-0,4	1,8	0,8	0,4	99,4	-0,4	2,0	0,8	0,4	98,0	-1,1	6,3	2,0	1,0
B8	99,8	-0,2	0,9	0,6	0,3	99,7	-0,2	1,0	0,6	0,3	98,7	-0,9	4,1	1,1	0,6
B9	99,9	-0,2	0,6	0,4	0,2	99,8	-0,2	0,7	0,4	0,2	99,1	-0,7	2,9	1,1	0,5
BY1	94,4	-6,3	31,2	3,2	1,6	93,7	-6,2	33,8	3,4	1,6	78,2	7,6	70,3	7,0	3,5
BY2	95,8	-5,9	24,9	3,0	1,4	95,3	-6,0	27,2	3,3	1,5	82,8	2,9	61,7	6,5	3,3
BY3	97,1	-5,0	18,1	2,8	1,2	96,8	-5,3	19,9	3,2	1,4	87,8	-1,9	50,3	6,0	3,0
BY4	98,5	-3,3	10,2	2,5	1,0	98,3	-3,6	11,3	2,7	1,0	93,5	-5,5	33,4	5,0	2,5
BY5	99,2	-2,0	5,5	1,6	0,8	99,2	-2,2	6,1	1,8	0,9	96,6	-5,3	20,4	4,0	2,0
BY6	99,7	-0,9	2,4	0,7	0,4	99,7	-1,0	2,7	0,7	0,3	98,6	-3,3	9,9	2,5	1,25
BY7	99,8	-0,5	1,3	0,6	0,3	99,8	-0,5	1,5	0,6	0,3	99,3	-1,9	5,4	2,0	1,0
Y1	96,6	-8,5	31,6	3,2	1,6	96,2	-8,6	34,2	3,4	1,6	86,3	2,8	72,5	6,5	3,3
Y2	97,5	-7,6	25,4	2,8	1,4	97,2	-7,9	27,6	3,3	1,5	89,3	-2,0	63,4	6,0	3,0
Y3	98,3	-6,2	18,3	2,6	1,3	98,1	-6,6	20,1	3,2	1,4	92,5	-6,1	51,1	5,5	2,8
Y4	99,1	-3,9	10,2	2,4	1,2	99,0	-4,2	11,3	2,7	1,0	96,1	-8,1	33,6	5,0	2,5
Y5	99,5	-2,2	5,4	1,6	0,8	99,5	-2,5	6,1	1,8	0,9	97,9	-6,7	20,6	4,0	2,0
Y6	99,7	-1,0	2,5	0,8	0,4	99,7	-1,1	2,8	0,7	0,3	99,2	-3,9	10,1	2,5	1,25
Y7	99,9	-0,5	1,2	0,6	0,3	99,9	-0,6	1,4	0,6	0,3	99,6	-2,3	5,5	1,5	0,75
GY1	98,9	-13,7	29,8	5,2	2,5	98,8	-14,5	32,2	5,5	2,5	95,8	-21,6	68,3	7,0	3,5
GY2	99,4	-9,9	20,0	4,1	2,0	99,3	-10,7	21,9	4,5	2,0	97,4	-19,9	53,1	6,5	3,3
GY3	99,5	-6,4	12,4	2,4	1,2	99,5	-7,0	13,7	2,6	1,3	98,5	-16,8	39,5	5,0	2,5
GY4	99,7	-4,3	8,0	1,7	0,8	99,7	-4,7	8,9	1,9	1,0	99,0	-13,3	28,5	4,0	2,0
GY5	99,8	-2,7	5,0	1,3	0,6	99,8	-3,0	5,6	1,5	0,7	99,3	-9,8	19,8	3,0	1,5
GY6	99,9	-1,5	2,6	0,6	0,3	99,9	-1,6	3,0	0,8	0,4	99,7	-6,1	11,7	2,0	1,0
GY7	99,9	-0,8	1,4	0,5	0,3	99,9	-0,9	1,6	0,8	0,4	99,8	-3,5	6,5	1,5	0,75
R1	91,4	8,5	20,3	2,8	1,4	90,4	9,8	22,5	4,1	2,0	71,3	35,7	65,4	8,0	4,0
R2	93,4	6,4	15,5	2,4	1,2	92,6	7,3	17,3	3,1	1,0	76,2	29,5	53,9	7,0	3,5
R3	95,5	4,2	10,5	1,9	0,9	95,0	4,8	11,7	2,0	0,8	82,1	21,3	39,7	6,0	3,0
R4	96,6	3,1	7,9	1,5	0,8	96,2	3,6	8,9	1,6	0,7	85,8	16,4	31,5	5,0	2,5
R5	97,7	2,0	5,4	1,3	0,7	97,4	2,3	6,1	1,5	0,6	90,0	11,0	22,4	4,0	2,0
R6	98,8	1,0	2,8	0,9	0,4	98,7	1,1	3,2	1,0	0,5	94,6	5,2	12,4	3,0	1,5
R7	99,5	0,4	1,2	0,7	0,4	99,5	0,4	1,4	0,8	0,5	97,8	2,0	5,4	2,0	1,0

6.2 Calculation of Standard Tristimulus Values

The example given on the next page shows the calculation of the standard tristimulus values of color reference solution BY 7 according to ISO 11664 determined under standard illuminant C and 2° standard observer.

The spectral transmittance $\tau(\lambda)\%$ of color reference solution BY 7 is referred to an optical path length of 50mm and distilled water as 100%.

The following abbreviations are used in the table:

λ = wavelength in mm
k = scaling factor of Yn = 100
$S_C(\lambda)$ = spectral energy distribution of standard illuminant C
$x(\lambda)$ = CIE color matching function x for 2° standard observer
$y(\lambda)$ = CIE color matching function y for 2° standard observer
$z(\lambda)$ = CIE color matching function z for 2° standard observer
$\Delta\lambda$ = step size in nm
$\tau(\lambda)\%$ = spectral transmittance of color reference solution BY 7

$$G_x(\lambda) = k * S_C(\lambda) * x(\lambda) * \tau(\lambda)\% * \Delta\lambda$$

$$G_y(\lambda) = k * S_C(\lambda) * y(\lambda) * \tau(\lambda)\% * \Delta\lambda$$

$$G_z(\lambda) = k * S_C(\lambda) * z(\lambda) * \tau(\lambda)\% * \Delta\lambda$$

Tristimulus value $X = \Sigma G_x(\lambda)$
Tristimulus value $Y = \Sigma G_y(\lambda)$
Tristimulus value $Z = \Sigma G_z(\lambda)$

L* = CIE-L*-value calculated from the tristimulus value Y according to ISO 11664
a* = CIE-a*-value calculated from the tristimulus values X,Y according to ISO 11664
b* = CIE-b*-value calculated from the tristimulus values Y,Z according to ISO 11664

λ	k	$S_C(\lambda)$	x(λ)	y(λ)	z(λ)	Δλ	τ(λ)%	$G_x(\lambda)$	$G_y(\lambda)$	$G_z(\lambda)$
380	0,00009891	31,34	0,0014	0,0000	0,0065	10	3,25	0,0001	0,0000	0,0007
390	0,00009891	45,01	0,0042	0,0001	0,0201	10	12,50	0,0023	0,0001	0,0112
400	0,00009891	60,11	0,0143	0,0004	0,0679	10	29,88	0,0254	0,0007	0,1206
410	0,00009891	76,54	0,0435	0,0012	0,2074	10	50,87	0,1675	0,0046	0,7987
420	0,00009891	93,16	0,1344	0,0040	0,6456	10	69,03	0,8549	0,0254	4,1064
430	0,00009891	106,74	0,2839	0,0116	1,3856	10	81,85	2,4532	0,1002	11,9732
440	0,00009891	115,38	0,3483	0,0230	1,7471	10	89,54	3,5590	0,2350	17,8523
450	0,00009891	117,76	0,3362	0,0380	1,7721	10	93,34	3,6550	0,4131	19,2656
460	0,00009891	116,90	0,2908	0,0600	1,6692	10	95,28	3,2036	0,6610	18,3888
470	0,00009891	117,57	0,1954	0,0910	1,2876	10	96,32	2,1886	1,0193	14,4219
480	0,00009891	117,66	0,0956	0,1390	0,8130	10	96,74	1,0763	1,5649	9,1528
490	0,00009891	114,62	0,0320	0,2080	0,4652	10	96,74	0,3509	2,2812	5,1019
500	0,00009891	106,46	0,0049	0,3230	0,2720	10	96,61	0,0498	3,2858	2,7670
510	0,00009891	97,15	0,0093	0,5030	0,1582	10	96,45	0,0862	4,6617	1,4662
520	0,00009891	92,02	0,0633	0,7100	0,0782	10	96,61	0,5566	6,2430	0,6876
530	0,00009891	93,07	0,1655	0,8620	0,0422	10	97,01	1,4779	7,6977	0,3768
540	0,00009891	96,96	0,2904	0,9540	0,0203	10	97,66	2,7198	8,9348	0,1901
550	0,00009891	99,91	0,4334	0,9950	0,0087	10	98,34	4,2117	9,6692	0,0845
560	0,00009891	100,00	0,5945	0,9950	0,0039	10	98,76	5,8071	9,7192	0,0381
570	0,00009891	97,15	0,7621	0,9520	0,0021	10	99,19	7,2636	9,0735	0,0200
580	0,00009891	92,88	0,9163	0,8700	0,0017	10	99,33	8,3612	7,9387	0,0155
590	0,00009891	88,51	1,0263	0,7570	0,0011	10	99,47	8,9369	6,5919	0,0096
600	0,00009891	85,19	1,0622	0,6310	0,0008	10	99,31	8,8882	5,2801	0,0067
610	0,00009891	83,95	1,0026	0,5030	0,0003	10	99,33	8,2691	4,1486	0,0025
620	0,00009891	83,67	0,8544	0,3810	0,0002	10	99,33	7,0233	3,1319	0,0016
630	0,00009891	83,57	0,6424	0,2650	0,0000	10	99,02	5,2578	2,1689	0,0000
640	0,00009891	83,38	0,4479	0,1750	0,0000	10	99,02	3,6576	1,4291	0,0000
650	0,00009891	83,76	0,2835	0,1070	0,0000	10	98,90	2,3228	0,8767	0,0000
660	0,00009891	83,48	0,1649	0,0610	0,0000	10	98,76	1,3447	0,4974	0,0000
670	0,00009891	81,96	0,0874	0,0320	0,0000	10	98,47	0,6977	0,2554	0,0000
680	0,00009891	79,77	0,0468	0,0170	0,0000	10	98,37	0,3632	0,1319	0,0000
690	0,00009891	76,16	0,0227	0,0082	0,0000	10	97,97	0,1675	0,0605	0,0000
700	0,00009891	72,46	0,0114	0,0041	0,0000	10	97,83	0,0799	0,0287	0,0000
710	0,00009891	68,76	0,0058	0,0021	0,0000	10	97,54	0,0385	0,0139	0,0000
720	0,00009891	64,86	0,0029	0,0010	0,0000	10	97,43	0,0181	0,0063	0,0000
								X= 95,1362	Y= 98,1504	Z= 106,8603

After this, the CIE-L*a*b*-values are calculated from the tristimulus values XYZ according to ISO 11664 part 4.

$$L^* = 116 * \sqrt[3]{\frac{98.15}{100.00}} - 16 = 99.28$$

$$a^* = 500 * \left(\sqrt[3]{\frac{95.14}{98.03}} - \sqrt[3]{\frac{98.15}{100.00}} \right) = -1.87$$

$$b^* = 200 * \left(\sqrt[3]{\frac{98.15}{100.00}} - \sqrt[3]{\frac{106.86}{118.11}} \right) = 5.32$$

6.3 Standards

[1] ISO 11664 Colorimetry Part 1 to 5 (replacement for DIN 5033)

[2] ISO 11664-4 CIE 1976 L*a*b* Color space (replacement for DIN 6174 - Colorimetric Determination of Color Distances for Pigment Colors According to the CIELAB-Formula)

[3] EN 1557 Colorimetric Characterization of Optically Clear Tinted Liquids

[4] DIN 5036 Assessment and Measurement of Luminous Characteristics of Materials

[5] DIN 1349-1 Transmission of optical radiation; optical clear (nonscattering) media

6.4 Literature

(some in German language only)

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