PHARMACOPOEIAL DISCUSSION GROUP

SIGN-OFF DOCUMENT
Q-07 COLOUR (instrumental method)

Revision 1

It is understood that sign-off covers the technical content of the draft and each party will adapt it as necessary to conform to the usual presentation of the pharmacopoeia in question; such adaptation includes stipulation of the particular pharmacopoeia's reference materials and general chapters.

Harmonised provisions:

Provision	EP (1)	JP	USP
Principle	+	+	+
Spectrophotometric method	+	+	+
Determination of coloration	+	+	+

Legend
+: will adopt and implement
–: will not stipulate

(1) In EP this text will be part of chapter 2.2.2 which will cover the visual and the instrumental methods.

Non-harmonised provisions:

N/A

Local requirement

EP will include a sentence at the beginning of chapter 2.2.2: "Report the result with the method used (method I, method II or method III)".

JP will include the italicised sentence: "The relationship between the distribution coefficients and the tristimulus values (X, Y and Z) is given by the following equations, expressed in terms of integrals. *According to the definition in JIS Z 8120: 2001, the lower limit of the wavelength of visible radiation is generally taken between 360 nm and 400 nm and the upper limit between 760 nm and 830 nm*:"

European Pharmacopoeia

Signature	Name	Date
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Japanese Pharmacopoeia

Signature	Name	Date
H. Okuda	Haruhiro Okuda for Fumi Yamamoto	June 19, 2019

United States Pharmacopeia

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Q-07 INSTRUMENTAL MEASUREMENT OF COLORATION OF LIQUIDS

PRINCIPLE

The observed colour of an object depends primarily on its light absorbing characteristics. However a variety of conditions such as light-source differences, spectral energy of the illuminant, visual sensitivity of the observer, size differences, background differences and directional differences affect the perception of colour. Hue, lightness or brightness and saturation are three attributes of the colour. Instrumental measurement under defined conditions allows numerical expression of a colour. The base of any instrumental measurement of colour is that the human eye has been shown to detect colour via three types of receptors.

Instrumental methods for measurement of colour provide more objective data than the subjective viewing of colours by a small number of individuals. With adequate maintenance and calibration, instrumental methods can provide accurate, precise and consistent measurements of colour that do not drift with time. Through extensive colour matching experiments with human subjects having normal colour vision, distribution coefficients (weighting factors) have been measured for each wavelength within the wavelength range of the visible spectrum, giving the relative amount of stimulation of each receptor type caused by the light of that wavelength.

The International Commission on Illumination (CIE)[1] has developed models taking into account the light source and the angle at which the observer is looking at the target (field of view). In a visual test for colour of solution, there are requirements that lead to the use of a 2° angle and diffuse daylight. The mean sensitivity of the human eye is represented by the distribution coefficients $\bar{x}_\lambda$, $\bar{y}_\lambda$ and $\bar{z}_\lambda$ (Fig. 1).

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[1] International Commission on Illumination publication CIE 15:2004 Colorimetry 3rd Edition.

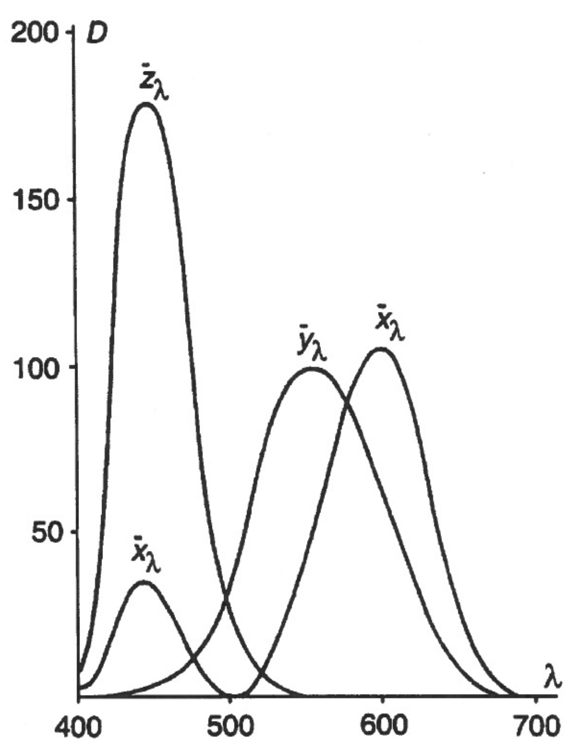

Fig 1. *Mean sensitivity of the human eye represented by distribution coefficients, CIE 2° standard observer* (D = distribution coefficient; λ = wavelength in nm)

For any colour, the amount of stimulation of each receptor type is defined by the set of tristimulus values (*XYZ*).

The relationship between the distribution coefficients and the tristimulus values (X, Y and Z) is given by the following equations, expressed in terms of integrals:

$$X = k\int_0^\infty f_\lambda \bar{x}_\lambda S_\lambda d\lambda$$

$$Y = k\int_0^\infty f_\lambda \bar{y}_\lambda S_\lambda d\lambda$$

$$Z = k\int_0^\infty f_\lambda \bar{z}_\lambda S_\lambda d\lambda$$

$$k = 100 / \int_0^\infty \bar{y}_\lambda S_\lambda d\lambda$$

k = normalising constant characterising the stimulation of one receptor type and the used illumination;

S_λ = relative spectral power distribution of the illuminant;

$\bar{x}_\lambda$, $\bar{y}_\lambda$ and $\bar{z}_\lambda$ = colour matching distribution coefficients for CIE 2° Standard Observer;

f_λ = spectral transmittance T_λ of the material;

λ = wavelength in nanometres.

In practical calculations of tristimulus values, the integration is approximated by a summation, as follows:

$$X = k\sum_{\lambda} T_{\lambda}\bar{x}_{\lambda}S_{\lambda}\Delta\lambda$$

$$Y = k\sum_{\lambda} T_{\lambda}\bar{y}_{\lambda}S_{\lambda}\Delta\lambda$$

$$Z = k\sum_{\lambda} T_{\lambda}\bar{z}_{\lambda}S_{\lambda}\Delta\lambda$$

$$k = \frac{100}{\sum_{\lambda} S_{\lambda}\bar{y}_{\lambda}\Delta\lambda}$$

The tristimulus values can be used to calculate the CIE *Lab* colour space coordinates: *L*(lightness or brightness), a*(red-green)* and *b*(yellow-blue)*; these are defined by:

$$L^* = 116 f\left(Y/Y_n\right) - 16$$

$$a^* = 500\left[f\left(X/X_n\right) - f\left(Y/Y_n\right)\right]$$

$$b^* = 200\left[f\left(Y/Y_n\right) - f\left(Z/Z_n\right)\right]$$

Where

$f\left(X/X_n\right) = \left(X/X_n\right)^{1/3}$ if $X/X_n > (6/29)^3$, otherwise $f\left(X/X_n\right) = 841/108\left(X/X_n\right) + 4/29$;

$f\left(Y/Y_n\right) = \left(Y/Y_n\right)^{1/3}$ if $Y/Y_n > (6/29)^3$, otherwise, $f\left(Y/Y_n\right) = 841/108\left(Y/Y_n\right) + 4/29$;

$f\left(Z/Z_n\right) = \left(Z/Z_n\right)^{1/3}$ if $Z/Z_n > (6/29)^3$, otherwise, $f\left(Z/Z_n\right) = 841/108\left(Z/Z_n\right) + 4/29$.

X_n, Y_n and Z_n, are the tristimulus values of *purified water*.

In the spectrophotometric method, transmittance values are obtained at discrete wavelengths throughout the visible spectrum. These values are then used to calculate the tristimulus values through the use of weighting factors $\bar{x}_{\lambda}$, $\bar{y}_{\lambda}$ and $\bar{z}_{\lambda}$ for a 2° Standard Observer and CIE standard illuminant C (see the International Commission on Illumination publication, CIE).

SPECTROPHOTOMETRIC METHOD

Operate a suitable spectrophotometer according to the instructions of the manufacturer and determine the transmittance *T* at least from 400 nm to 700 nm, at intervals of not greater than 10 nm. Express the result as a percentage. Calculate the tristimulus values *X*, *Y*, and *Z* and the colour co-ordinates *L**, *a** and *b**.

DETERMINATION OF COLORATION

Calibrate the instrument as per the instrument manufacturer's recommendation. System performance tests are done prior to each measurement or at regular intervals, depending on the use of the apparatus. To this purpose, use certified reference materials [2] within the measurement range.

Operate the apparatus according to the manufacturer's instructions and test the sample solution and reference solution(s) under the same conditions (e.g. path length of the cuvette, temperature)

For transmittance measurements *purified water* is used as standard and assigned a transmittance of 100.0 per cent at all wavelengths throughout the visible spectrum. Then the weighing factors $\bar{x}_\lambda$, $\bar{y}_\lambda$ and $\bar{z}_\lambda$ for CIE standard illuminant C are used to calculate the tristimulus values corresponding to colour co-ordinates $L^* = 100$, $a^* = 0$ and $b^* = 0$

Reference measurements can be made using the colour co-ordinates of *purified water* or freshly prepared pharmacopoeial reference solutions, or using the respective colour co-ordinates stored in the instrument manufacturer's database, provided the latter have been obtained under the same testing conditions.

If the test solution is turbid or hazy, it is filtered or centrifuged. If the test solution is not filtered or centrifuged, any haziness or turbidity is reported with the results. Air bubbles are to be avoided and removed.

The instrumental method is used to compare two solutions in respect to their colour or colour difference, or a deviation from a defined colour. Calculate the colour difference between the *test solution t* and a *reference r* as ΔE^*_{tr} using the following equation:

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

where ΔL^*, Δa^* and Δb^* are the differences in colour co-ordinates.

Instead of the colour coordinates CIE *Lab,* the colour coordinates CIE *LCh* may be used.

[2] Certified filters or certified reference solutions recommended by the instrument's manufacturer.

Assessment of location within the $L^*a^*b^*$ colour space

Instruments may provide information on the actual location of the test solution within the $L^*a^*b^*$ colour space. Using appropriate algorithms, correspondence to pharmacopoeial reference solutions (such as "test solution equals reference solution XY", "test solution close to reference solution XY" or "test solution between reference solutions XY and XZ") can be obtained.