PHARMACOPOEIAL DISCUSSION GROUP

SIGN-OFF DOCUMENT
CODE:Q-07
NAME: COLOUR (instrumental method)

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.
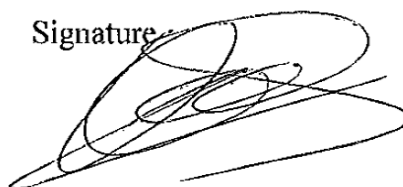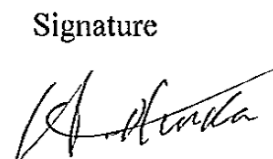
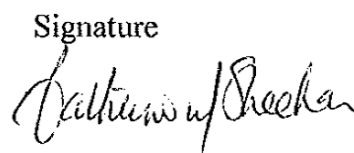## Non-harmonised provisions:

N/A

Local requirement

N/A

European Pharmacopoeia

Signature _[signature]_   Name C. Violle   Date 26 Oct 2016

P.O.

Japanese Pharmacopoeia

Signature _[signature]_   Name Haruhiro Okuda
for Masanobu Yamada   Date Oct 26, 2016

United States Pharmacopeia

Signature _[signature]_   Name CATHERINE SHEEHAN   Date Oct 26, 2016

1

2   Q-07 INSTRUMENTAL MEASUREMENT OF COLORATION OF LIQUIDS

3

4   PRINCIPLE

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

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

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

26

---

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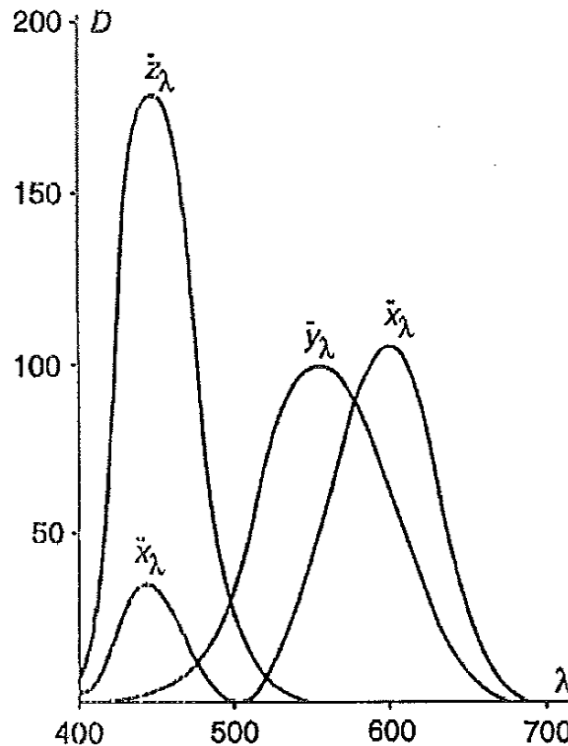
1

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;

1   λ = wavelength in nanometres.

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

4
$$X = k\sum_{\lambda} T_{\lambda}\bar{x}_{\lambda}S_{\lambda}\Delta\lambda$$

5
$$Y = k\sum_{\lambda} T_{\lambda}\bar{y}_{\lambda}S_{\lambda}\Delta\lambda$$

6
$$Z = k\sum_{\lambda} T_{\lambda}\bar{z}_{\lambda}S_{\lambda}\Delta\lambda$$

7
$$k = \frac{100}{\sum_{\lambda} S_{\lambda}\bar{y}_{\lambda}\Delta\lambda}$$

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

10
$$L^* = 116 f\left(\frac{Y}{Y_n}\right) - 16$$

11
$$a^* = 500\left[f\left(\frac{X}{X_n}\right) - f\left(\frac{Y}{Y_n}\right)\right]$$

12
$$b^* = 200\left[f\left(\frac{Y}{Y_n}\right) - f\left(\frac{Z}{Z_n}\right)\right]$$

13   Where

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

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

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

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

18

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

1   **SPECTROPHOTOMETRIC METHOD**

2   Operate a suitable spectrophotometer according to the instructions of the manufacturer and
3   determine the transmittance T at least from 400 nm to 700 nm, at intervals of not greater than
4   10 nm. Express the result as a percentage. Calculate the tristimulus values X, Y, and Z and the
5   colour co-ordinates L^*, a^* and b^*.

6

7   **DETERMINATION OF COLORATION**

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

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

15   For transmittance measurements *purified water* is used as standard and assigned a
16   transmittance of 100.0 per cent at all wavelengths throughout the visible spectrum. Then the
17   tristimulus values for CIE standard illuminant C are: 98.03, 100.00, and 118.11, respectively,
18   corresponding to colour co-ordinates $L^* = 100$, $a^* = 0$ and $b^* = 0$

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

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

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

29
$$\Delta E^*_{tr} = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2}$$

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

31   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.

1   **Compliance with a pharmacopoeial requirement**

2   For existing monographs which currently include specifications using the visual method, use
3   the following procedure to adapt to instrumental determination. First calculate the value ΔE^*
4   of the test solution versus the reference solutions, as shown in the equation for determination
5   of ΔE^*_{tr}. Determine the nearest neighbour as the reference solution that corresponds to the
6   smallest ΔE^* versus the test solution. The test solution meets the requirements of the
7   monograph if its nearest neighbour is a reference solution whose colour and intensity would
8   pass the monograph requirements.

9   If the nearest neighbour is a reference solution at the colourless end of the reference scale, i.e.
10   in the area between 'colourless' and the faintest reference solution of a scale, determine the
11   ΔE^* of the test solution versus purified water. The test solution meets the monograph
12   requirement if the ΔE^* of the test solution versus purified water is not greater than the ΔE^* of
13   the reference solution versus purified water.

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

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