Determination of the unsulfonated color concentration from D&C Yellow No. 10 by the derivative spectrophotometry

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Abstract. The method that we used is based on the measurement of the first derivative of the mixture of the two colorants at the wavelength for which one of them has the first derivative equal to zero.

The Code of Federal Regulations (21 CFR 74.1710) specifies for D&C Yellow No. 10 the maximum permitted levels of an unsulfonated subsidiary color and of diethyl ether-soluble matter other than that specified.

In the proposed method a color additive sample is dissolved in water and the unsulfonated subsidiary color are extracted from this solution with dichloromethane. The analysts in dichloromethane solution are determined by spectrophotometry.

The unsulfonated subsidiary colors determined are:
- D&C Yellow No. 11 [2-(2-Quinolyl)-1H-indene-1,3(2H)-dione] (Y11), from which D&C Yellow No. 10 is manufactured by sulfonating and
- 1,5-Naphthyridinequinophthalone (1,5-NQ).

Another compound soluble in water and dichloromethane (which I called S) is present in dichloromethane solution after extraction together with the other two colors and can affect the correct determination of the concentrations. The dichloromethane-soluble matter other than specified is a mixture consisting mostly of chlorinated derivatives of the unsulfonated subsidiary color. Because the S color is present both in aqueous and in dichloromethane solutions, the spectra of calibration solutions should be corrected. The applied correction does not affect the determination of the unsulfonated subsidiary colors concentrations.

D&C Yellow No. 11 and 1,5-NQ are used as standard for unsulfonated subsidiary colors.

1. Introduction

D&C Yellow # 10 (2-(2-Quinolyl)-1,3-indandione disulfonic acid disodium salt) is a U.S. Food and Drug Administration (FDA)-approved colorant commonly used to color dose forms such as tablets and capsules. The FDA states that D&C Yellow No. 10 may be safely used as a colorant. D&C Yellow No. 10 is approved for use in drugs and cosmetics but is not approved as a colorant for food [1], [2]. The structure of D&C Yellow # 10 is presented in Figure 1.

D&C Yellow No. 10 is obtained by sulfonating of D&C Yellow No. 11. D&C Yellow No. 11 is manufactured by condensing quinaldine with phthalic anhydride to give the unsulfonated dye.

The color additive D&C Yellow No. 10 is a mixture of disulfonates (principally), monosulfonates and trisulfonates of 2-(2-quinolyl)indan-1,3-dione consisting principally of the sodium salts of: 2-(2,3-dihydro-1,3-dioxo-1H-indene-2-yl)-6-quinolinesulfonic acid and 2-(2,3-dihydro-1,3-dioxo-1H-indene-
2-yl)-8-quinolinesulfonic acid with lesser amounts of the disodium salts of the disulfonic acids of 2-(2-quinolinyl)-1H-indene-1,3(2H)-dione.

![Structure of D&C Yellow # 10](image)

**Figure 1.** The structure of D&C Yellow # 10

In the manufacture of Y10 remain as impurities, especially Y11 unreacted and 1,5-Naphthyridinequinophthalone.

The Code of Federal Regulation (21 CFR 74.1710) specifies for D&C Yellow No. 10 minimum concentration 85% and the maximum permitted levels 4ppm for D&C Yellow No. 11. The maximum concentration of diethyl ether soluble matter other than that specified, must be 2 parts per million, using added 2-(2-quinolinyl)-1H-indene-1,3(2H)-dione for calibration [3].

The unsulfonated subsidiary colors determined are:
- D&C Yellow No. 11 [2-(2-Quinolinyl)-1H-indene-1,3(2H)-dione] (Y11) [4], from which D&C Yellow No. 10 is manufactured by sulfonating and
- 1,5-Naphthyridinequinophthalone (1,5-NQ).

Another compound soluble in water and dichloromethane (called S) is present in dichloromethane solution after extraction together with the other two colors and can affect the correct determination of the concentrations. The four compounds present in D & C Yellow No.10 are shown in Figure 2.

|          | Water soluble | D&C Yellow No. 10 | S     | 1,5-NQ | D&C Yellow No. 11 |
|----------|---------------|-------------------|-------|--------|-------------------|
|          |               | 85% Min.          | 2ppm Max. | 4ppm Max. |                    |
|          |               | Dichloromethane soluble |

**Figure 2.** The compounds water soluble and dichloromethane soluble

The dichloromethane-soluble matter other than specified is a mixture consisting mostly of chlorinated derivatives of the unsulfonated subsidiary color [5].

The D&C Yellow No. 11 is used as the standard for the unsulfonated subsidiary color as a surrogate standard for the other ether-soluble matter.

The structure of D&C Yellow # 11 and 1,5-Naphthyridinequinophthalone are presented in Figure 3. The UV-vis absorption spectra of the two sulfonated compounds that are present in the dichloromethane phase are shown in Figure 4. The absorption bands of the two compounds are very close, making it difficult to spectrophotometrically determine their concentrations.

For simultaneous determination of the concentrations from a mixture of two or more compounds, derivative spectrophotometry can be successfully used [6], [7]. Derivatization of the spectrum is especially useful when the absorption bands of the compounds in the mixture are very close and overlap. The overlapping signals may be thus separated and background may be eliminated by the presence of other compounds in the sample [8].
The structures of: a) D&C Yellow No. 11 \[2-(2-\text{Quinolinyl})-1H\text{-indene-1,3(2H)-dione}] and b) 1,5-Naphthyridinequinophthalone

The UV-vis spectra of D&C Yellow No.11 and 1,5-Naphthyridinequinophthalone from the dicloromethane extract of D&C Yellow No. 10 batches

The derivative spectrophotometry is based on the measurement of the first derivative of the mixture of the two colorants at the wavelength for which one of them has the first derivative equal to zero.

For each colorant on a specific concentration domain, absorbance shows a linear dependence on the concentration of the form:

\[ A = \varepsilon \cdot d \cdot c + n = m \cdot c + n \]  

(1)

where:

- \( A \) = measured absorbance of the color solutions at the wavelength \( \lambda \), corrected for the absorbance of the blank at the same wavelength;
- \( \varepsilon \) = absorptivity value of the color at the wavelength \( \lambda \);
- \( d \) = cell path length (cm);
- \( c \) = the concentration of color in solution (mg/L);
- \( m \) = the slope of calibration line;
- \( n \) = intercept of calibration line.

We denote with \( D^1 \) the first derivative of absorbance with respect to wavelength:
\[
D' = \frac{dA}{d\lambda} = \frac{d\varepsilon}{d\lambda} \cdot c \cdot d + \frac{dn}{d\lambda} = m^i \cdot c + n^i
\]  

(2)

The first order derivative depends linearly on the concentration. The slope \( m^i \) and the intercept \( n^i \) are dependent on the wavelength.

For a mixture of two dyes with the concentrations \( c_1 \) and \( c_2 \) respectively, the absorbance of the mixture is additive:

\[
A = A_1 + A_2 = \varepsilon_1 \cdot d \cdot c_1 + n_1 + \varepsilon_2 \cdot d \cdot c_2 + n_2 = m_1 \cdot c_1 + n_1 + m_2 \cdot c_2 + n_2
\]

(3)

where:

- \( A \) = the measured absorbance of the mixture solutions at \( \lambda \);
- \( A_1 \) = the measured absorbance of the first color solution at \( \lambda \);
- \( A_2 \) = the measured absorbance of the second color solutions at \( \lambda \);
- \( m_1 \) = the slope of the calibration line for the first color;
- \( m_2 \) = the slope of the calibration line for the second color;
- \( n_1 \) = the intercept of the calibration line for the first color;
- \( n_2 \) = the intercept of the calibration line for the second color.

It follows that in a solution containing a mixture of two dyes (Y11 and 1,5-NQ) the first derivative of the absorbance in relation to the wavelength, is additive.

\[
D' = D'_{Y11} + D'_{NQ} = m_{Y11}^i \cdot c_{Y11} + n_{Y11}^i + m_{YNQ}^i \cdot c_{YNQ} + n_{YNQ}^i
\]

(4)

The additivity is also valid for the higher order derivatives [9].

2. Experimental

2.1. Materials and instrumentation

All of the spectrophotometric measurements were made with a scanning UV-visible PerkinElmer Lambda25 spectrometer provided with quartz windows cells, 1 cm path length, 0.4 mL.

For all samples UV-VIZ spectra was recorded in 230÷500 nm, scan speed 120nm/min, data interval 1nm, split 1nm. Use the dichloromethane as blank.

The samples were weighed using microbalance, accurate to 0.01 mg and analytical balance, accurate to 0.1mg.

Micro-syringe 500 μL Gastight® and 50 μL Hamilton Co. Microliter® (Reno, Nevada, USA) was used to prepare the standard solutions and calibration solutions.

The solutions were prepared in silanized vials. The glassware surface deactivating reagent (Sylon CT, 5% dimethyldichlorosilane in toluene) was purchased from Supelco, (Bellefonte, PA, USA).

A hot plate magnetic stirrer with the speed controlled, IKA® Works Inc. (Wilmington, NC, USA) (model IKAMAG RT10 power) was used to stir the samples.

2.2. Reagents

- Water, HPLC grade, deionized with a Milli-Q water system from Millipore (Bedford, MA, USA).
- Dichloromethane, anhydrous (≥99.8%) obtained from Sigma-Aldrich (Milwaukee, WI, USA).
- Standards: D&C Yellow No.11 and 1,5-NQ, standards for the unsulfonated subsidiary color and for the other diethyl ether-soluble matter.

2.3. Glassware treatment

All the glassware used for analysis of Y11 and 1,5-NQ in the color mixture Y10 was silanized prior to use, as described previously [10]. The volumetric flasks and the vials used were filled with 5%
solution of dimethyldichlorosilane (DMDS) in toluene (Silon-CT). After 12 - 16 h they were rinsed once with toluene and once with methanol followed by oven drying at 150°C for 1 h.

2.4. Preparation of solutions

2.4.1. The stock-assay standard solutions. The stock solutions of Y11 (80 mg/L) and 1,5-NQ (40 mg/L) were prepared by dissolving each of them in methanol chloride in 25 mL volumetric flask. The stock solutions are kept in the refrigerator. They are weighed each time and kept constant mass by adding CH$_2$Cl$_2$ (to compensate for the solvent evaporated in time).

2.4.2. The calibration solutions. Calibration is performed in the absence of D&C Yellow No. 10. The D&C Yellow No. 10 (Y10) which does not contain D&C Yellow No. 11 and 1,5-NQ are used for preparing the calibration solutions.

Using an analytical balance, weigh 1 g (±0.001g) of D&C Yellow No. 10 (Y10) into six tarred 20 mL vials.

Add 10 mL water to the sample. Add a magnetic stirrer in vial and heat the mixture on a hot plate magnetic stirrer at 40°C until the sample dissolves, then allow the solution to cool to room temperature.

Add with a micro-syringe 12.5 µL, 25 µL, 50 µL, 75 µL, 100 µL, 125 µL from stock-assay standard solution of Y11 (80 mg/L). Are obtained thus mixtures of Y10 and Y11 dyes in which Y11 has the concentrations: 1ppm, 2ppm, 4ppm, 6ppm, 8ppm and 10ppm.

Add with a micro-syringe 50 µL, 100 µL, 150 µL, 200 µL, 250 µL from stock-assay standard solution of 1,5-NQ (40 mg/L). Are obtained thus mixtures of Y10 and 1,5-NQ dyes in which 1,5-NQ has the concentrations: 2ppm, 4ppm, 6ppm, 8ppm and 10ppm.

Add 3 mL dichloromethane in each vial, stopper and shake to mix, during 30 min at 1000 rpm on the hot plate magnetic stirrer, at room temperature, for extraction of unsulfonated subsidiary colors from aqueous solution.

Only the non-aqueous phase is transferred to other silanized glass vials with Pasteur pipette.

2.4.3. The reference solutions. Using an analytical balance, weigh 1 g (±0.001g) of D&C Yellow No. 10 (Y10) into six tarred 20 mL vials.

Add 10 mL water to the sample. Add a magnetic stirrer in vial and heat the mixture on a hot plate magnetic stirrer at 40°C until the sample dissolves, then allow the solution to cool to room temperature.

Add 3 mL dichloromethane in each vial, stopper and shake to mix, during 30 min at 1000 rpm on the hot plate magnetic stirrer, at room temperature, for extraction of unsulfonated subsidiary colors from aqueous solution.

Only the non-aqueous phase is transferred to other silanized glass vials with Pasteur pipette.

The reference solution is prepared from a color that does not contain the compounds: Y11 and 1,5-NQ. Unfortunately, the reference solution will also contain S-soluble compound in dichloromethane.

3. Result and discussion

3.1. The absorption spectra

The spectrum of the mixture of D&C Yellow No. 10 and S (water-soluble) is shown in Figure 5. It was measured using distilled water as a reference.

The spectrum of reference solution, corresponding to the color that is present in D&C Yellow No. 10 (soluble in both water and CH$_2$Cl$_2$), is different from that of the unsulfonated colors: Y11 and 1,5-NQ. Also it is different from that of D&C Yellow No. 10 due to the presence of S compound.

The spectrum of S compound (present in D&C Yellow No. 10) soluble both in water and dichloromethane is shown in Figure 6. Dichloromethane was used as the blank.
The spectrum of S compound is the average obtained from three spectra corresponding to three reference solutions.

**Figure 5.** The spectrum of water soluble compound mixture from D&C Yellow No. 10

**Figure 6.** The spectrum of S compound, soluble both in water and dichloromethane

Because the S compound is present both in aqueous and in dichloromethane solutions, the spectra of calibration solutions should be corrected. The spectrum of the calibration solution containing Y11 color (c=4ppm) without correction and with the corresponding correction of S compound is presented in Figure 7.

We can use two methods to obtain the spectra of the corrected calibration solutions.
- Use the reference solution as blank. The spectra are obtained directly from calibration solutions.
- Use the dichloromethane as blank. The uncorrected spectra of calibration solutions are obtained. These are mathematically corrected by subtracting the spectrum of reference solution from spectrum of the calibration solutions.

**Figure 7.** The spectra of the calibration solution containing D&C Yellow No.11 (c=4ppm)

The reference solution is prepared in both cases from a color that does not contain the compounds: Y11 and 1,5-NQ.

The spectra of the calibration solutions, obtained by the two methods are shown in Figure 8. The applied correction by both processes affect only the absorption bands positions below 295nm and does not affect the determination of the unsulfonated subsidiary colors concentrations.
Figure 8. The spectra of the calibration solution containing D&C Yellow No. 11 (c=4ppm)

The corrected spectra of the calibration solutions are presented in Figures 9 and 10.

Figure 9. The corrected spectra of Y11 calibration solutions (from Y10 matrix)

Figure 10. The corrected spectra of 1,5-NQ calibration solutions (from Y10 matrix)
3.2. The derivative spectra

Figure 11 shows the 1st order derivative spectra of the calibration solutions, obtained by the two methods, presented in Figure 7, where the concentration of the D & C Yellow No. 11 dye is 4ppm.

The differences corresponding to the two processes are of the order of 0.02 for Y11 and 0.0001 for 1,5-NQ.

The 1st order derivative spectra of D & C Yellow No. 11 and 1,5-Naphthyridinequinophthalone will be draw on the same graph, as in Figure 12.

The wavelengths at which the 1st order derivative of 1,5-NQ dye is zero and the 1st order derivative of D & C Yellow No. 11 dye is nonzero will be identify. The values \( \lambda = 294.75 \) nm and \( \lambda = 322.1 \) nm are found. The most appropriate value is \( \lambda = 294.75 \) nm.

For D&C Yellow No. 11 color, plot the calibration line \( D^1_{Y_{11}} = f(c) \) at this wavelength (Figure 13a).

![Figure 11](image1.png)

**Figure 11.** The first derivative spectra of the calibration solution containing D&C Yellow No. 11 (c=4ppm)

![Figure 12](image2.png)

**Figure 12.** The first derivative spectra of calibration solutions for D&C Yellow No.11 and 1,5-Naphthyridinequinophthalone
The calibration line for unsulfonated subsidiary color from the calibration data using least-squares linear regression is expressed as:

\[ y = mx + n \]  

(5)

where:
- \( y \) = the first derivative of absorbance at maximum values \( \text{nm}^{-1} \);
- \( x \) = the analyte concentration \( \text{mg} \cdot \text{L}^{-1} \);
- \( m \) = calculated slope of the calibration line \( \text{L} \cdot \text{mg}^{-1} \cdot \text{nm}^{-1} \);
- \( n \) = calculated intercept of the calibration line \( \text{nm}^{-1} \);
- \( r \) = correlation coefficient of data.

\[ D_l = 0.023 \cdot c + 0.0042; \quad r = 0.998 \]

\[ D_l = -0.0046 \cdot c - 0.0014; \quad r = 0.999 \]

Figure 13. The calibration line from first derivative spectra of unsulfonated subsidiary colors: a) D&C Yellow No.11; b) 1,5-Naphthyridinequinophthalone

The line parameters are calculated: the slope \( m_{Y11} \) and the intercept \( n_{Y11} \). These values presented in Table 1 are used to determine the concentration of the D & C Yellow No. 11 dye in the analyzed samples.

|  | \( m_{Y11}(\text{L} \cdot \text{mg}^{-1} \cdot \text{nm}^{-1}) \) | \( n_{Y11}(\text{nm}^{-1}) \) | \( r_{Y11} \) |
|---|---|---|---|
|  | 0.023 | 0.0042 | 0.9984 |

At the wavelength \( \lambda = 294.75 \text{ nm} \):

\[ D_{1\text{NO}} = 0 \quad \text{and} \quad D_1 = D_{1\text{Y11}} = m_{Y11} \cdot c_{Y11} + n_{Y11} \]  

(6)

The D&C Yellow No.11 color concentration will be:

\[ c_{Y11} = \frac{(D_{1\text{Y11}} - n_{Y11})}{m_{Y11}} \]  

(7)

The wavelengths at which the 1st order derivative of D & C Yellow No. 11 dye is zero and the 1st order derivative of 1,5-NQ dye is nonzero will be identify. The value \( \lambda = 319.95 \text{ nm} \) are found. For 1,5-NQ color, plot the calibration line \( D_{1\text{NO}} = f(c) \) at this wavelength (Figure 13b).

The line parameters are calculated: the slope \( m_{1\text{NO}} \) and the intercept \( n_{1\text{NO}} \). These values presented in Table 2 are used to determine the concentration of the 1,5-NQ dye in the analyzed samples.
Table 2. The parameters of the calibration line for 1,5-Naphthyridinequinophthalone.

| $m_{NQ}$ (L·mg⁻¹·nm⁻¹) | $n_{NQ}^i$ (nm⁻¹) | $r_{NQ}$ |
|--------------------------|-------------------|----------|
| -0.0046                  | -0.0016           | -0.9986  |

At the wavelength $\lambda = 319.95$ nm:

$$D_{Y_{11}} = 0 \text{ and } D_{NQ}^i = m_{YNQ}^i \cdot c_{NQ} + n_{NQ}^i \quad (8)$$

The 1,5-NQ color concentration will be:

$$c_{NQ} = \frac{(D_{NQ}^i - n_{NQ}^i)}{m_{NQ}^i} \quad (9)$$

4. Conclusions
The determination of the concentrations of the unsulphonated subsidiary colors in D & C Yellow No. 10 batches is very important because of the restrictions on their maximum admissible concentrations imposed by the Code of Federal Regulations. Small concentrations (of the order of ppm) of the compounds require the use of high precision and fineness methods, such as spectrophotometry.

The concentrations of compounds in a mixture can’t be determined spectrophotometrically when their absorption bands are very close position.

Derivative spectrophotometry allows the determination of their concentrations using first order or higher order derivatives. For two-component mixtures, derivative spectrophotometry is based on the measurement of the first derivative of the mixture of two colorants at the wavelength for which one of them has the first derivative equal to zero.

The presence of the third soluble compound in both water and dichloromethane does not alter the determination of the concentrations of the unsulphonated compounds D&C Yellow No.11 and 1,5-Naphthyridinequinophthalone. This compound does not have absorption in the spectral region used to determine the concentrations of the two unsulphonated compounds.

If the concentrations of D & C Yellow No. 11 and 1,5-NQ from the D & C Yellow No. 10 analyzed certified batches do not fall within the required restrictions then those batches are rejected.

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