Determination of organic impurities in D&C Red No. 6, D&C Red No. 7, and D&C Yellow No. 10 using solid phase extraction, ultra-performance liquid chromatography, and mass spectrometry

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Introduction

D&C Red No. 6 (**R6**) is the disodium salt of 3-hydroxy-4-[(4-methyl-2-sulfophenyl)azo]-2-naphthalenecarboxylic acid. D&C Red No. 7 (**R7**) is the calcium salt of the same dye anion (Figure 1).

D&C Yellow No. 10 (**Y10**) is the disodium salt of 2-(2-quinolyl)-1,3-indandione disulfonic acid (Figure 2).

R6, **R7**, and **Y10** straight colors and their lakes (insoluble pigments formed by precipitating the straight colors onto insoluble substrata) are approved for use as color additives in drugs and cosmetics. These color additives are subject to batch certification by the U.S. Food and Drug Administration (FDA) to ensure compliance with their Code of Federal Regulation (CFR) listing requirements.[1]

R6 and **R7** have a specification for *p*-toluidine (**PT**, **Figure 1**) of not more than 15 ppm. **PT** is an unsulfonated aromatic amine that may be present in the starting material PTMS.[2] **Y10** has a specification for its starting material, which is certifiable as D&C Yellow No. 11 (**Y11**, **Figure 2**) of not more than 4 ppm.

Currently FDA uses chloroform extraction techniques and high-performance liquid chromatography (HPLC) with photodiode array (PDA) detection for determining these organic impurities in the straight colors.[3-5] The lakes are not analyzed.

The present study reports solid phase extraction (SPE) techniques combined with (1) ultra-performance liquid chromatography (UPLC) and quantitation by tandem mass spectrometry (LC-MS/MS) for determining **PT** in **R6** and **R7** straight colors and lakes and (2) UPLC with PDA detection (LC-PDA) for determining **Y11** in **Y10** straight colors and lakes.

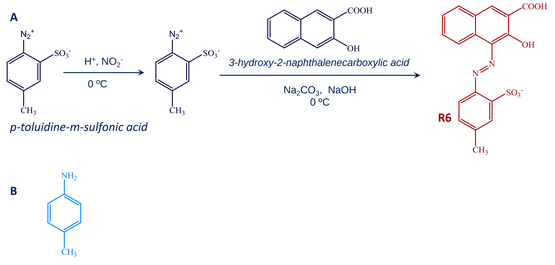


Figure 1 (A) Manufacture of **R6**; (B) **PT**, impurity in **R6** and **R7**.

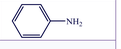
Monosulfonic acids (>85%)
R_1=SO_3^-, R_2= H
R_2= SO_3^-, R_1= H

Disulfonic acid (<15%)
R_1=R_2=SO_3^-

Figure 2 Manufacture of **Y10**.

Materials & Methods

Table 1 MS parameters.

Analytes	Precursor ion	Daughter ions	Cone voltage (eV)	Collision voltage (eV)	Structure
PT	107.9	93.2	35	14	
ANL[a]	94.0	77.0	40	15	
MS Parameter Scan type: MRM Ion source: ESI Polarity: Positive Capillary voltage: 3 kV Source Temperature: 125°C			Desolvation temperature: 450°C Desolvation gas flow: 700 L/h Cone gas flow: 60 L/h Dwell time: 0.1 sec [a]Aniline: internal standard		

Materials:

- Strata SCX cation-exchange cartridges (500 mg/6mL) for **PT** in **R6** and **R7**
- Strata-X reversed phase cartridges (500 mg/6mL) for **Y11** in **Y10**
- SPE manifold system
- Whatman 0.2 µm PTFE syringeless filters

Analytical reference materials:

- **PT**, 99.7%
- **Y11** – certified sample recrystallized from acetonitrile (elemental analysis: found C 79.16%, H 4.26%, N 5.20; calculated C 79.11%, H 4.06%, N 5.13%)
- Aniline (**ANL**, 99.8%) – used as internal standard for LC-MS/MS
- Matrices – **R7** lake and **Y10** lake samples from certified batches previously found to be free of the analytes

Instrumentation:

- Waters Acquity UPLC system with tandem quadrupole (TQD) mass spectrometer and PDA detector
- XBridge Phenyl column (2.1x75 mm id, 2.5 µm) for **PT** in **R6** and **R7**
- Acquity UPLC BEH C18 (2.1x50 mm id, 1.7 µm) for **Y11** in **Y10**

Chromatography Conditions:

For PT in R6 and R7
Eluents: (A) 0.1% formic acid/water, pH ~ 2.7 (B) acetonitrile
Gradient (%A): 1 min 90-70%, 0.1 min 70-60%, 1.9 min 60-50%, 0.1 min 50-90%, hold 0.9 min 90%.
Flow rate: 0.6 mL/min
Column temperature: 40 ºC ± 5 ºC
Injection volume: 10 µl
Run time: 4 min

For Y11 in Y10
Eluents: (A) 0.02 M $NH_4(CH_3COO)$/water, pH ~ 6.7 (B) acetonitrile
Gradient (%A): hold 1 min 95%, 1 min 95-85%, 1.5 min 85-65%, 0.5 min 65-10%, and 2 min 10-95%.
Flow rate: 0.45 mL/min
Column temperature: 35 ºC ± 5 ºC
Injection volume: 5 µL
Run time: 7 min

Sample Preparations:

Extraction solutions:

*Extraction solution-1 for **R6** and **R7** straight colors and lakes:*

- 0.05 M H_2SO_4 and 5 mg/mL $Li_2C_2O_4$ in 50% ethanol/water

*Extraction solution-2 for **Y10** straight colors and lakes:*

- 1% CH_3CN in water for straight colors or 1% CH_3CN with 4 mL NH_4OH for lakes

Calibration and survey sample solutions:

Stock and internal standard solutions:

- 7.5 µg/mL **Y11**, 1 µg/mL of **PT**, and1 µg/mL of **ANL** in acetonitrile

R6 and R7 solutions:

- 100 mg **R7** lake matrix and 1.0 mL **ANL** in 25 mL extracting solution-1 spiked with 0.25, 0.5, 0.75, 1.0, 1.25, or 2.0 mL **PT** stock solutions
- 100 mg **R6** and **R7** samples in 25 mL of extracting solution with 1.0 mL **ANL**

Y10 solutions:

- 750 mg **Y10** lake matrix and 4 mL of NH_4OH in 50 mL of extracting solution-2 spiked with 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, or 0.6 mL **Y11** stock solutions
- 750 mg Y10 samples in 50 mL extracting solution-2

Solid Phase Extraction

For PT in R6 and R7
Condition: 5 mL methanol
Equilibrate: 5 mL 5% H_2SO_4/water
Load: 5 mL sample
Wash: 5 mL 5% H_2SO_4/water
Wash: 5 mL water
Wash: 5 mL 90% methanol/water
Dry: 10 min under vacuum
Elute: 3.0 mL 5% NH_4OH/ methanol into 5 mL volumetric flask
Add: 100 µL conc. formic acid,
dilute to volume with water, filter into LC vial for LC-MS/MS analysis (Table 1)

For Y11 in Y10
Condition: 5 mL methanol
Equilibrate: 5 mL water
Load: 10 mL sample
Wash: 10 mL 30% methanol/water
Dry: 10 min under vacuum
Elute: 10 mL CH_3CN
Evaporate: 40 °C
Reconstitute: 2 mL 50% CH_3CN/water, filter into LC vial for LC-PDA analysis ($\lambda_{Y10,Y11}$: at 410 nm)

Results and Discussion

Six-point calibration curves were prepared for each analyte.

PT was quantified by LC-MS/MS using a **R7** lake matrix spiked with the analyte. The calibration data points ranged from 2.5-20 ppm. The instrument response was linear over the calibration range (see **Figure 2**).

Y11 was quantified by LC-PDA using a **Y10** lake matrix spiked with the analyte. The calibration data points ranged from 0.5-6.0 ppm. The instrument response was linear over the calibration range (see **Figure 3**).

The limit of detection (LOD) and the limit of quantification (LOQ) for each method were calculated from the calibration data and are shown in **Tables 2 and 3**. The precision of each method, expressed as relative standard deviation (RSD), was determined at three different concentration levels by analyzing three separately prepared solutions (see **Tables 2 and 3**).

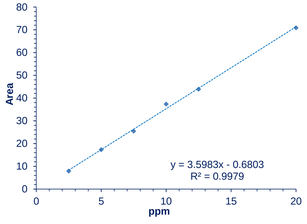


Figure 2 Calibration curve for the quantitative determination of **PT** in **R6** and **R7** using LC-MS/MS.

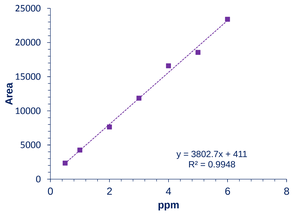


Figure 3 Calibration curve for the quantitative determination of **Y11** in **Y10** using LC-PDA.

Table 2 Validation data for **R6** and **R7** LC-MS/MS and for **Y10** LC-PDA methods.

Analyte	Retention time (min)	Equation	R^2	Linear range (ppm)	LOD (ppm)	LOQ (ppm)	RSD (%)	Recovery (%)
PT	0.65	Y=3.598x-0.6803	0.998	2.5-20	0.076	0.23	4.3-9.5	110
Y11	4.22	Y=3802.7x+411	0.995	0.5-6	0.11	0.32	2.5-6.8	98

Results and Discussion

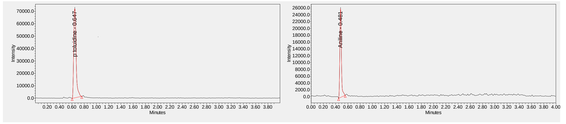


Figure 4 MRM ion chromatograms of **PT**, transition m/z 107.9 → 93.2 and **ANL**, transition m/z 94 → 77 for extracted **R7** sample fortified with 10 ppm **PT** and 10 ppm **ANL**.

Table 4 Preliminary survey of **PT** in certified lots of **R6** and **R7**.

Color additive[a]	Manufacturer	PT LC-MS/MS	PT Current[b]
R7 lake[c]	A	0.57	NA[d]
R7	B	3.0	8.7
R7	C	0.36	6
R6 lake	C	0.97	NA
R6	A	1.4	<5
R6	D	0.75	<5
R7 lake	C	0.71	NA
R7	C	0.99	8
R7	C	1.1	7
R7	E	0.68	11
R6	B	1.0	NF[e]
R6 lake	F	0.67	NA

a Batches certified in 2006-2014.
b HPLC with PDA detection.
c Matrix sample.
d Not analyzed (NA).
e Not found (NF).

Table 5 Preliminary survey of **Y11** in certified lots of **Y10**.

Color additive[a]	Manufacturer	Y11 LC-PDA	Y11 Current[b]
Y10 lake[c]	A	<LOQ[e]	NA[d]
Y10	B	9.1	7.5
Y10	C	5.6	5.1
Y10	D	487	460
Y10 lake	E	0.46	NA
Y10	F	5.6	4.7
Y10 lake	G	0.52	NA
Y10 lake	A	0.33	NA
Y10 lake	H	0.55	NA

a Batches certified in 1997-2010.
b HPLC-PDA after chloroform extraction.
c Matrix sample.
d Not analyzed (NA).
e Limit of quantification
f Not found (NF).

Conclusions

SPE extraction combined with LC-MS/MS for the determination of **PT** in **R6** and **R7** straight colors and lakes and LC-PDA for the determination of **Y11** in **Y10** straight colors and lakes both may be applicable for use in routine batch certification. The new methods are rapid (4 min and 7 min total analysis cycle, respectively) with significantly lower quantitation limits.

The **PT** results for **R6** indicate that the new LC-MS/MS is more sensitive than the current method. The **PT** results for **R7** are all lower than the results obtained by the current method. The new **Y11** results are very consistent with the results obtained by the current method. A comprehensive survey of **R6**, **R7** , and **Y10** samples submitted for certification is in progress.

References

1. Code of Federal Regulations, Title 21, Sections 74.1710, 74.2710, and 74.1710, U.S. Government Printing Office, Washington, DC, 2014.
2. Code of Federal Regulations, Title 21, Sections 74.1306(b), 74.1307(b), and 74.170(b), U.S. Government Printing Office, Washington, DC, 2014
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