ELSEVIER

Dyes and Pigments, Vol. 35, No. 2, pp. 101–110, 1997

PII: S0143-7208(96)00102-7 0143-7208/97 $17.00+0.00

Analysis of 1-Carboxy-5,7-Dibromo-6-Hydroxy-2,3,4-Trichloroxanthone in the color Additives D and C Red Nos. 27 and 28 (phloxine B) by Liquid Chromatography[1]

Adrian Weisz

Office of Cosmetics and Colors, U.S. Food and Drug Administration, Washington, DC 20204, USA

(Received 24 August 1996; accepted 25 September 1996)

ABSTRACT

High-performance liquid chromatography was used to analyse 1-carboxy-5,7-dibromo-6-hydroxy-2,3,4-trichloroxanthone (HXCA) in 34 commercial batches of U.S. certified color additives D&C Red Nos. 27 and 28 (phloxine B). Thirty-one samples were found to contain HXCA in amounts ranging from 0.01 to 0.47% with an average value of 0.12%. These values are one order of magnitude lower than those previously reported for the Japanese counterpart of D&C Red No. 28 (Food Red No. 104). The analyses also revealed significant differences in the level of HXCA across batches from the same manufacturer. The analyses suggest that the presence of HXCA in D&C Red Nos. 27 and 28 may be avoided in the manufacturing process. A theoretical account that could explain the origin of HXCA in these color additives is also proposed. © *1997 Elsevier Science Ltd*

Keywords: 1-Carboxy-5,7-dibromo-6-hydroxy-2,3,4-trichloroxanthone, high-performance liquid chromatography, color additives, D&C Red Nos. 27 and 28, phloxine B.

INTRODUCTION

D&C Red No. 27 (R27, mainly 2′,4′,5′,7′-tetrabromo-4,5,6,7-tetrachlorofluorescein, **1**, Colour Index 45410:1) and its disodium salt, D&C Red No. 28

[1]Presented at 109th AOAC International Annual Meeting and Exposition, Nashville, TN, 17–21 September 1995.

(R28, mainly **2**, Colour Index 45410), are U.S. certified color additives listed for use in drugs and cosmetics. They are batch-certified by the U.S. Food and Drug Administration (FDA) to ensure compliance with specifications described in the *Code of Federal Regulations* (CFR). [1] These colors are manufactured by the bromination of 4,5,6,7-tetrachlorofluorescein (TCF) followed by alkaline hydrolysis of the brominated product (Fig. 1). In the Japanese counterpart of D&C Red No. 28 (Food Red No. 104), the compound 1-carboxy-5,7-dibromo-6-hydroxy-2,3,4-trichloroxanthone (HXCA) was reported at levels ranging from 1 to *ca* 3%. [2] Thin-layer chromatography (TLC), UV and fluorescence spectrophotometry were used for the determination. Since current U.S. regulations for D&C Red Nos. 27 and 28 do not specify a limit for HXCA, determination of the extent and level of contamination in certified lots of these colors was of interest. In the present work, high-performance liquid chromatography (HPLC) with UV detection was used to analyse HXCA in commercial batches of D&C Red Nos. 27 and 28.

EXPERIMENTAL

Materials

The D&C Red Nos. 27 and 28 used in this study originated from batches submitted for certification in the past 5 years. Ammonium acetate (NH_4OAc), methanol and water were of chromatography grade. Ammonium hydroxide ($\geq 25\%$ NH_3 in water) was from Fluka (Buchs, Switzerland). The

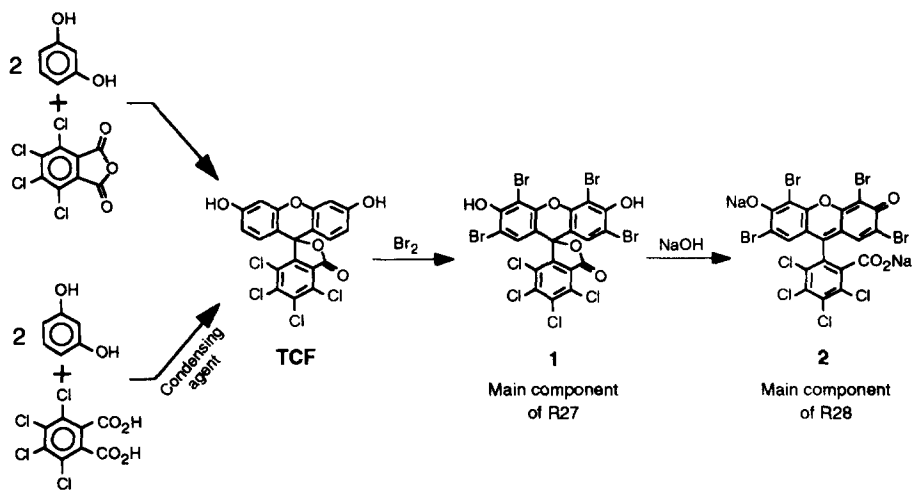

Fig. 1. Preparation of D&C Red Nos. 27 and 28 by bromination of 4,5,6,7-tetrachlorofluorescein (TCF).

materials used for the synthesis of HXCA were obtained from commercial suppliers and were used without further purification.

Synthesis of HXCA used as reference material

HXCA used as a reference material in this study was prepared by following the three-step sequence shown in Fig. 2. 2-(2,4-Dihydroxybenzoyl)-3,4,5,6-tetrachlorobenzoic acid, **3**, was prepared according to the method of Ullman and Schmidt [3] using resorcinol instead of phenol as the starting material. The crude product was washed with hot water (~70°C) and recrystallized from acetic acid–water (1:1), mp 229.5–231°C (lit. [4] 227°C). Bromination of **3** by a procedure described in the literature [4] resulted in 2-(3,5-dibromo-2,4-dihydroxybenzoyl)-3,4,5,6-tetrachlorobenzoic acid, **4**, a colorless crystalline material with mp 225–226°C. The cyclization of **4** to HXCA was achieved by the procedure described for the cyclization of the unbrominated analog **3**. [4] The resulting HXCA was a white powder that decomposed above 290°C. Its purity and identity were established by elemental analysis, positive ion chemical ionization (PICI) mass spectrometry, and HPLC analysis. The elemental analysis was performed by E+R Microanalytical Laboratory, Inc. (Corona, NY 11368, U.S.A.).

Analytical RP-HPLC

The system and method used were previously described. [5] The system consisted of a Model 8800 ternary pump, Model 8500 dynamic mixer, Model

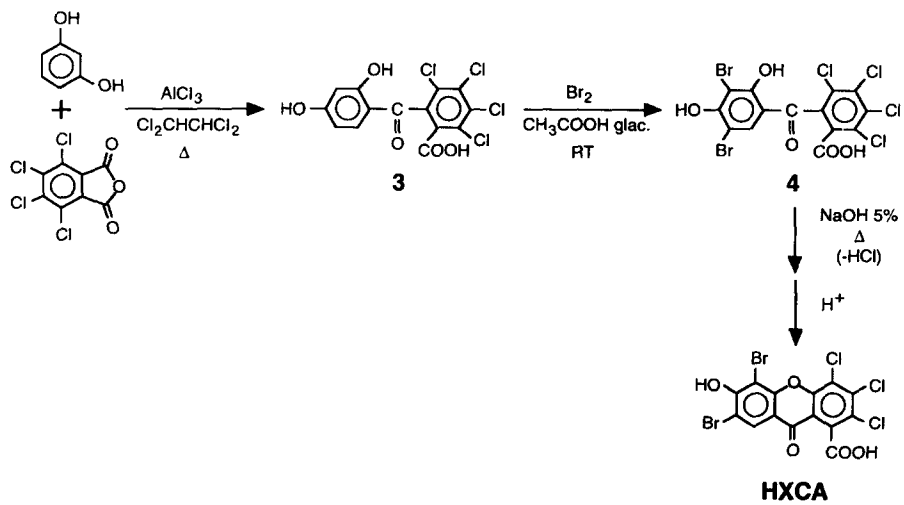

Fig. 2. Synthesis of HXCA for use as a reference material.

8780 autosampler, Model 4270 integrator (all Spectra-Physics, San Jose, CA, U.S.A.) and a Model 490 multiwavelength UV-Vis detector set at 254 nm (Waters Assoc., Milford, MA, U.S.A.). The autosampler was equipped with a Model 7010 injector (Rheodyne, Cotati, CA, U.S.A.) with a 20-μl sample loop. A Hypersil MOS-1 RPC-8 column (5-μm particle size, 250×4.6 mm i.d., Keystone Scientific, Bellefonte, PA, U.S.A.) was used.

The eluents were 0.1 M aqueous NH_4OAc and methanol. The column was eluted by using consecutive linear gradients of 25–90% methanol in 25 min, 90–100% methanol in 5 min, and 100% methanol for 5 min. The column was re-equilibrated with 25% methanol for 15 min. Other conditions were injection volume, 20 μl; full scale response, 0.128 absorbance units; and flow-rate, 1 ml/min.

Solutions of D&C Red Nos. 27 and 28 were prepared for HPLC analysis by dissolving approximately 20 mg of dye in a solution that consisted of 9.6 ml of methanol–water (50:50, v/v) and 0.4 ml of 5% aqueous ammonia. Two milliliters of the color solution was filtered through a UniPrep 0.45-μm glass microfiber syringeless filter unit (Whatman, Clifton, NJ, U.S.A.) prior to chromatography.

Linearity

HXCA was quantified by using a five-point calibration curve prepared according to the external standard procedure by analysing separate similar amounts of D&C Red No. 27 (Sample 1 from Table 1) spiked with HXCA. The data points ranged from 0.013 to 1.006% by weight for HXCA. The instrument response was linear over this range.

RESULTS AND DISCUSSION

Chromatograms of solutions of D&C Red No. 27 with and without added HXCA are shown in Fig. 3. Test portions from 34 certified lots of D&C Red Nos. 27 and 28 were analysed for HXCA using HPLC (Fig. 4 shows three examples). These lots represent domestic (A–C,E,G,I in Table 1) and foreign (D–France; F,H–Japan) manufacturers that requested certification for these color additives in the past 5 years. The study includes one lot of D&C Red No. 27 (Sample 1 in Table 1) that was used in the animal feeding studies upon which FDA based its safety evaluation of D&C Red Nos. 27 and 28. As shown in Table 1, 31 samples (91.2%) contained 0.01–0.47% HXCA, with an average value of 0.12%. HXCA was not found in three of the 34 samples analysed. Notably, the levels of HXCA varied even for batches produced by the same manufacturer. Figure 5 shows chromatograms of two

TABLE 1
1-Carboxy-5,7-dibromo-6-hydroxy-2,3,4-trichloroxanthone (HXCA) Found in Certified Lots of D&C Red Nos. 27 and 28 by HPLC

Sample	*Manufacturer*	*Color additive*	*HXCA found (%)*
1[a]	AA-4623	R27	NF[b]
2	A	R28	0.03
3	A	R27	NF
4	A	R28	0.05
5	A	R28	NF
6	A	R28	0.02
7	A	R28	0.27
8	A	R28	0.02
9	B	R28	0.01
10	B	R28	0.02
11	B	R28	0.02
12	B	R28	0.03
13	B	R28	0.13
14	B	R28	0.29
15	B	R28	0.07
16	C	R28	0.02
17	C	R28	0.02
18	C	R27	0.01
19	C	R27	0.29
20	C	R27	0.47
21	C	R28	0.06
22	C	R28	0.02
23	D	R27	0.03
24	D	R27	0.02
25	D	R27	0.01
26	D	R27	0.24
27	E	R27	0.03
28	E	R27	0.13
29	E	R27	0.11
30	F	R27	0.23
31	F	R27	0.25
32	G	R28	0.36
33	H	R27	0.09
34	I	R27	0.23

[a]From toxicology test batch.
[b]Not found.

batches of D&C Red No. 28 produced consecutively by the same manufacturer, one (Sample 7) containing an appreciable amount of HXCA and the other (Sample 8), containing trace level of this contaminant.

HXCA is a by-product in the synthesis of D&C Red Nos. 27 and 28. It may be produced from the reaction of equimolar amounts of resorcinol and tetrachlorophthalic anhydride, or by basic decomposition of TCF or D&C Red No. 27 as shown in Fig. 6.

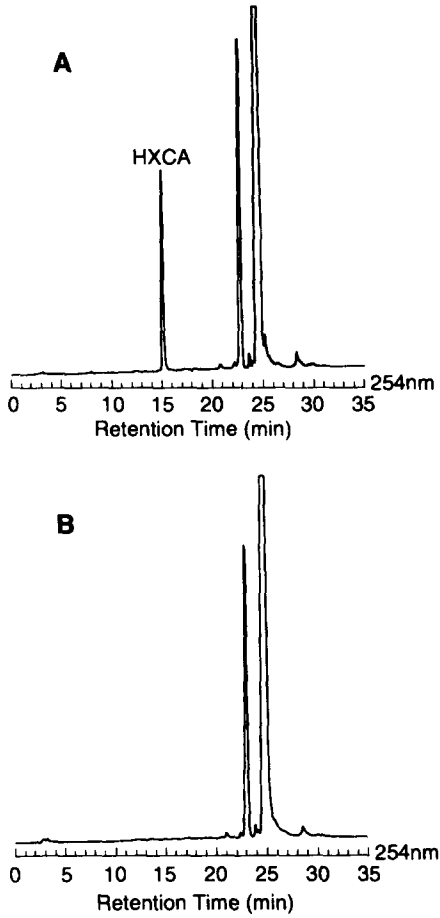

Fig. 3. HPLC chromatograms of (A) color blank fortified with HXCA and (B) unfortified color blank (sample 1 in Table 1).

Both **3** and **5** were previously isolated from commercial samples of TCF.[6] Compound **4** is an impurity limited by the CFR to no more than 0.7% in D&C Red Nos. 27 and 28. [1] Compound **5** and HXCA were identified earlier by TLC as decomposition products of TCF and phloxine, respectively, in strong alkaline solution. [7] Fig. 7 shows the HPLC chromatogram of the decomposition products obtained when a certified batch of D&C Red No. 27 dissolved in 50% aqueous NaOH was heated at 135°C for 30 min. HXCA was the major component obtained under these conditions. These results suggest that contamination of D&C Red Nos. 27 and 28 by HXCA may result from the alkaline treatments involved in the manufacturing process. Possible pathways for its formation are outlined in Fig. 6.

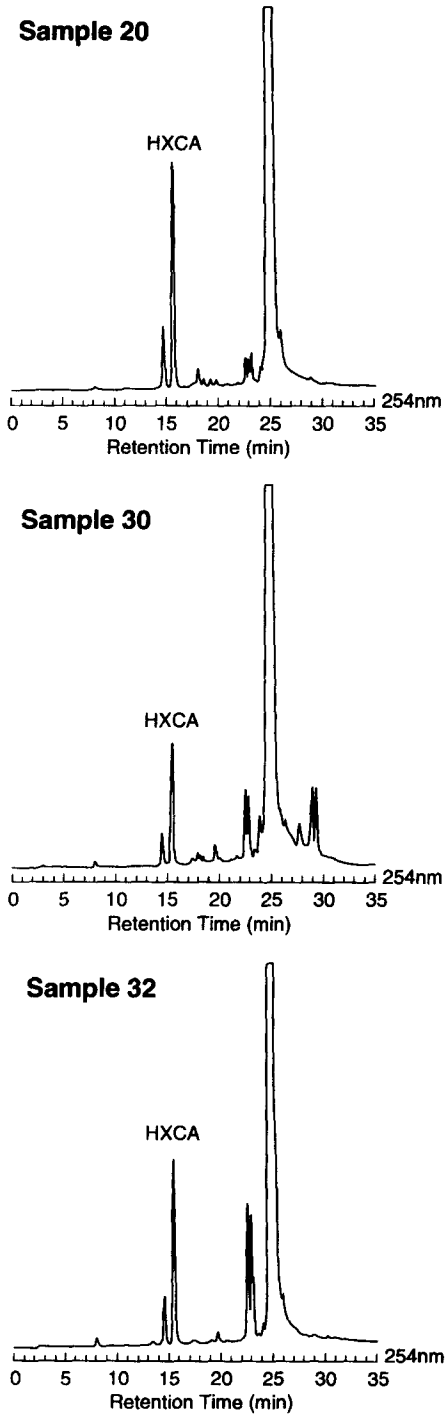

Fig. 4. HPLC chromatograms of commercial samples of D&C Red Nos. 27 and 28.

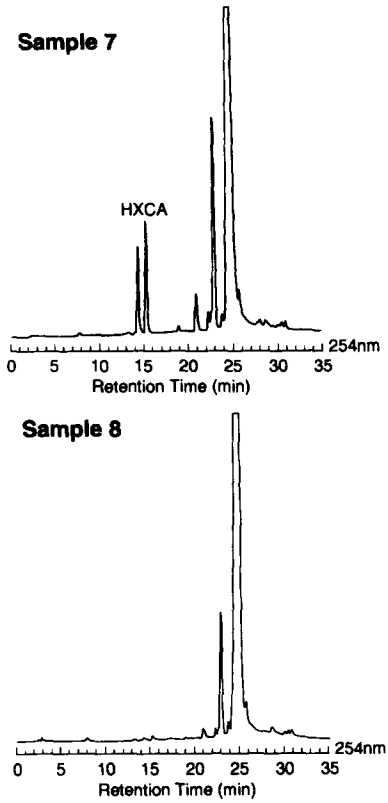

Fig. 5. HPLC chromatograms of samples from two batches of D&C Red No. 28 submitted consecutively for certification by the same manufacturer.

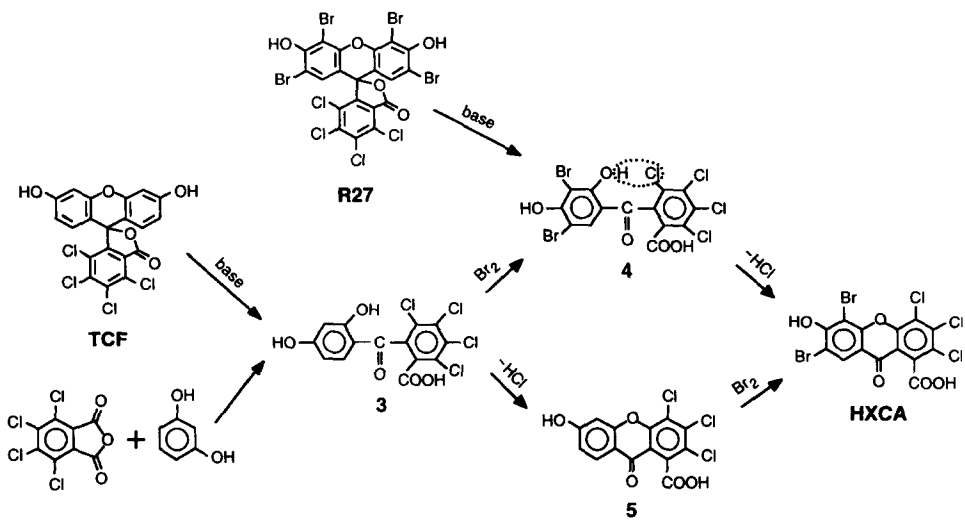

Fig. 6. Possible pathways for the formation of HXCA as a by-product during the manufacturing of D&C Red Nos. 27 and 28.

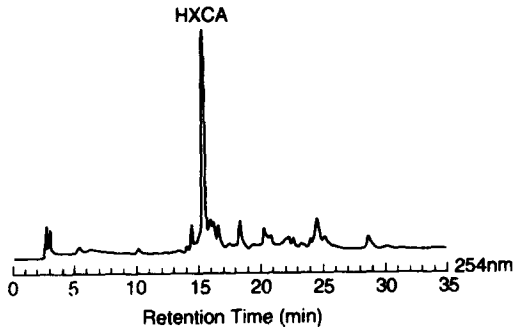

Fig. 7. HPLC chromatogram of the decomposition products of D&C Red No. 27 in alkaline solution (see text for experimental details).

CONCLUSIONS

This study confirms the presence of HXCA in batches of D&C Red Nos. 27 and 28 submitted for FDA batch certification. However, the amount found is one order of magnitude lower than that previously reported for the Japanese counterpart of D&C Red No. 28 (Food Red No. 104).[2] The range of HXCA levels found in batches submitted for certification (e.g. Table 1 and Fig. 5) suggest that the formation of HXCA may be avoided in the manufacturing process.

ACKNOWLEDGEMENTS

The author would like to thank Mr Denis Andrzejewski for performing the mass spectrometric analysis of HXCA and Dr Sandra J. Bell for reviewing the manuscript.

REFERENCES

1. *Code of Federal Regulations*, Title 21, Parts 74.1327-74.1328, U.S. Government Printing Office, Washington, DC, 1995.
2. Kamikura, M., *Shokuhin Eiseigaku Zasshi*, 1970, **11**, 242; *Chemical Abstracts*, 1971, **75**, 22460a.
3. Ullman, F. and Schmidt, W., *Berichte*, **52** (1919) 2098.
4. Orndorff, W. R. and Adamson, W. A., *Journal of the American Chemical Society*, **40** (1918) 1235.
5. Weisz, A., Langowski, A. J., Meyers, M. B., Thieken, M. A. and Ito, Y., *Journal of Chromatography*, **538** (1991) 157.

6. Weisz, A., Andrzejewski, D., Shinomiya, K., and Ito, Y., in *Modern Countercurrent Chromatography*, ACS Symposium Series 593, ed. W. D. Conway and R. J. Petroski, Chap. 16. American Chemical Society, Washington, DC, 1995, pp. 203–217.
7. Kamikura, M., *Shokuhin Eiseigaku Zasshi*, 1968, **9**, 348; *Chemical Abstracts*, 1969, **70**, 79128z.