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Identification of 2-bromo-3,4,5,6-tetrachloroaniline and its quantification in the color additives D&C Red Nos. 27 and 28 (phloxine B) using solid-phase microextraction and gas chromatography–mass spectrometry

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Abstract

The present work describes (a) the identification and characterization of a contaminant, 2-bromo-3,4,5,6-tetrachloroaniline (2BTCA), in the color additives D&C Red Nos. 27 and 28 (phloxine B) and (b) the determination of the extent and level of 2BTCA contamination in certified lots of these colors. For these purposes, 2BTCA (a compound not previously reported in the literature) and its positional isomer 4-bromo-2,3,5,6-tetrachloroaniline (4BTCA) were synthetically prepared. 4BTCA was used as the internal standard for the quantification of 2BTCA in the colors. Test portions from 35 certified lots of D&C Red Nos. 27 and 28 were analyzed for 2BTCA using a solid-phase microextraction–GC–MS method. Those lots were submitted for certification by both domestic (seven) and foreign (four) manufacturers during the past 4 years. Of the test portions analyzed, 22 (62.9%) contained 2BTCA in amounts ranging from 0.15 to 435.7 ppm with an average value of ~131.7 ppm. The remaining 13 (37.1%) test portions contained no detectable 2BTCA or less than 0.01 ppm, which is the limit of quantification of the present method. The analyses revealed substantial differences in the level of 2BTCA across lots from the same manufacturer as well as among different manufacturers. The wide range of 2BTCA levels found in the analyzed lots suggests that the presence of 2BTCA in D&C Red Nos. 27 and 28 may be avoided or significantly reduced during the manufacturing process. A direct correlation was observed between the presence of 2BTCA and that of 3,4,5,6-tetrachlorophthalic acid in analyzed batches of D&C Red Nos. 27 and 28. A chemical pathway that could explain the presence of 2BTCA in these color additives, and ways to avoid its formation, are also proposed.
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Keywords: Hunsdiecker reaction; Bromotetrachloroanilines; Anilines; D&C Red No. 27; D&C Red No. 28; Phloxines; Dyes

1. Introduction

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

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(R28, Color Index 45410, phloxine B, mainly **2**), are color additives used in the USA in drugs and cosmetics [1]. Their manufacture involves several steps: condensing tetrachlorophthalic acid (or its anhydride) with two equivalents of resorcinol; partially purifying the resulting 4,5,6,7-tetrachlorofluorescein (TCF) and then brominating it to yield the main component of R27, **1**; hydrolyzing **1** with sodium hydroxide, resulting in the main component of R28, **2** (Fig. 1). R27 and R28 are batch-certified by the US Food and Drug Administration (FDA) to ensure compliance with specifications in the Code of Federal Regulations (CFR) [1]. The specifications limit the level of regulated contaminants in those dyes. Excess levels of specified contaminants may result in the failure of a batch of R27 or R28 to meet certification requirements established by the FDA. Common sources of contamination lie in the presence of unreacted starting materials (e.g., tetrachlorophthalic acid, specified at ‘‘not more than 1.2%’’), of reaction intermediates, or of reaction byproducts (e.g., brominated resorcinol, specified at ‘‘not more than 0.4%’’). The impurities present in a low-grade starting material can be carried over to the final product during the manufacturing process and thus become another source for the contamination of the dye [2]. With the advent of new, more sensitive technologies, contaminants that are not specified in the CFR were identified and quantified in batches of R27 and R28 [2,3]. The toxicity of those contaminants is currently being assessed by the FDA and specifications limiting their presence in the color additives may be added to the CFR.

The present work describes the identification of a newly-found contaminant in R27 and R28, 2-bromo-3,4,5,6-tetrachloroaniline (2BTCA), and the determination of the extent and level of 2BTCA contamination in certified lots of these colors. For the determination of 2BTCA in batches of R27 and R28, a method was developed based on solid-phase microextraction (SPME), gas chromatographic separation and mass spectrometric detection. The technique (previously described in detail [4,5]) is based on the equilibrium process in which the analytes partition between a fiber coated with a polymeric film and the aqueous media. The adsorbed volatile and/or semi-volatile analytes are subsequently thermally desorbed into the injection port of a gas chromatograph, separated and analyzed using a mass spectrometer (SPME–GC–MS). The SPME method development was described in detail by Pawliszyn [6]. SPME was previously used for analysis of polyhalogenated aromatic compounds in aqueous media [7–10], including the analysis of hexachloro-

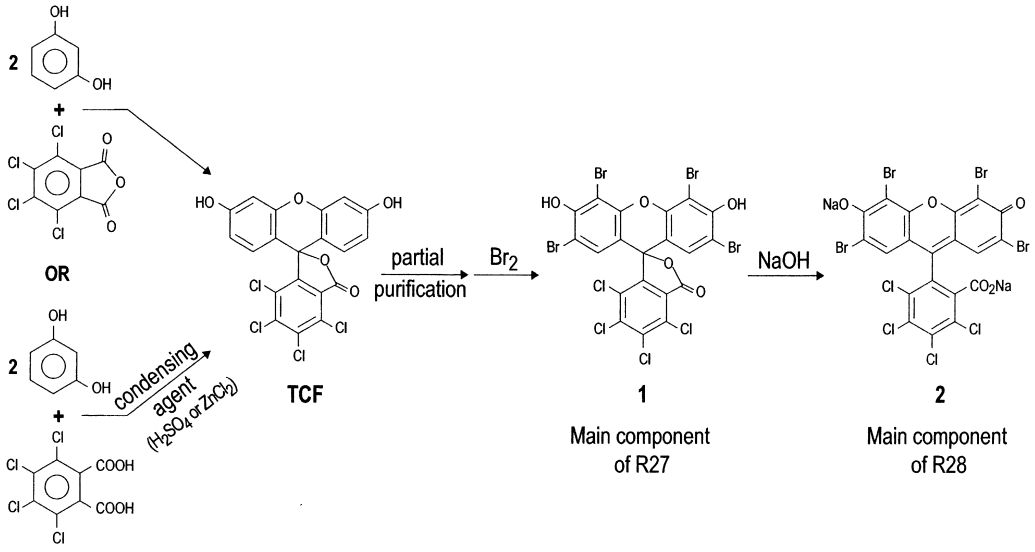

Fig. 1. Preparation of D&C Red Nos. 27 and 28 by condensation of 3,4,5,6-tetrachlorophthalic anhydride/acid with resorcinol followed by bromination of the condensation product 4,5,6,7-tetrachlorofluorescein (TCF).

benzene in R27 and R28 [2]. It was also used for the detection of halogenated aromatic amines in aqueous solutions [11,12]. R28 dissolves in water and R27 can be dissolved in a basic aqueous solution, and therefore, the 2BTCA content of both color additives can be analyzed by SPME–GC–MS.

2. Experimental

2.1. Materials and instrumentation

Most of the materials, instrumentation and procedures used were described previously [2]. The SPME holder, the SPME fiber [7 μm, 30 μm and 100 μm poly(dimethylsiloxane) (PDMS) coating] assembly, silanized SPME injection port sleeves (0.75 mm I.D. for Hewlett-Packard and Agilent gas chromatographs) and the glassware surface deactivating reagent (Sylon-CT, 5% dimethyldichlorosilane in toluene) were purchased from Supelco (Bellefonte, PA, USA). The 2-ml silanized vials (Target DP) capped with TEF/SIL septum screw caps were from National Scientific (Atlanta, GA, USA). The stirring bars (7 mm×2 mm) coated with PTFE were purchased from Cole-Parmer (Vernon Hills, IL, USA), Fisher Scientific (Pittsburgh, PA, USA) and Spectrum (Gardena, CA, USA). Prior to use, the water had been deionized with a Milli-Q water system from Milipore (Bedford, MA, USA). Bromine (99.5+%) was purchased from Aldrich (Milwaukee, WI, USA). 3,4,5,6-Tetrachloroaniline and 2,3,5,6-tetrachloroaniline (both 98%) were purchased through Fluka (Milwaukee, WI, USA) from Riedel-de Haen (Seelze, Germany). 2BTCA and 4-bromo-2,3,5,6-tetrachloroaniline (4BTCA) for use as standards were prepared as described below. 3,4,5,6-Tetrachlorophthalic acid (TCPHTHAc) used as a standard was purchased from Pfaltz and Bauer (Waterbury, CT, USA) and was recrystallized twice from water. Methylene chloride and ammonium hydroxide (28–30% NH_3 in water) were purchased from J.T. Baker (Phillipsburg, NJ, USA) and were used as received.

The GC–MS analyses were performed with an Agilent 6890N Network GC system interfaced with an Agilent 5973 mass-selective detector (Agilent Technologies, Wilmington, DE, USA). The gas chromatograph was equipped with a HP-5 MS (cross-linked 5% phenyl–methylsilicone) fused-silica capillary column, 30 m×0.25 mm I.D. with 0.25 μm film thickness. High-resolution mass spectra analyses were performed with a Micromass AutospecQ Mass spectrometer (Beverly, MA, USA) equipped with a Hewlett-Packard Model 5890 series 2 gas chromatograph.

2.1.1. Synthesis of 2BTCA and 4BTCA used as reference materials

2BTCA and 4BTCA used as reference materials in this study were prepared by brominating 3,4,5,6-tetrachloroaniline and 2,3,5,6-tetrachloroaniline, respectively, following the procedure described for the preparation of 4,4′-dibromobiphenyl [13] (Fig. 2). In a 7.5-cm diameter glass evaporating dish, 0.965 g (4.18 mmol) of 3,4,5,6-tetrachloroaniline powder was thinly spread. The dish was placed on a porcelain rack in a 16-cm I.D. desiccator surrounded by three 5-cm diameter glass evaporating dishes containing a combined total of ~6.5 g (2 ml, 40 mmol) of bromine. The desiccator was closed and the powder was left in contact with the bromine vapors overnight. The resulting light brown solid was then removed from the desiccator and allowed to stand on the evaporating dish open to the air in the hood for ~5 h. The purity and identity of the obtained 2BTCA, a compound not previously described in the literature, (1.2 g, light beige-colored powder, m.p. 224–226.5 °C) were established by elemental analysis and GC–MS (dissolved in CH_2Cl_2) (Fig. 3A). The same procedure was applied for the synthesis of 4BTCA [obtained 1.2 g, m.p. 236–238 °C (233–236 °C [14])] (Fig. 3B). The elemental analyses were performed by Complete Analysis Labs., E&R Microanalytical Division (Parsippany, NJ, USA).

2.2. Glassware treatment

All the glassware used for the analysis of 2BTCA in R27 and R28 was silanized prior to use, as described previously [7]. Silanized vials were commercially available (see Section 2.1). The volumetric flasks were filled with 5% solution of dimethyldichlorosilane in toluene (Sylon-CT). After a waiting period of 5–10 min, they were rinsed twice with

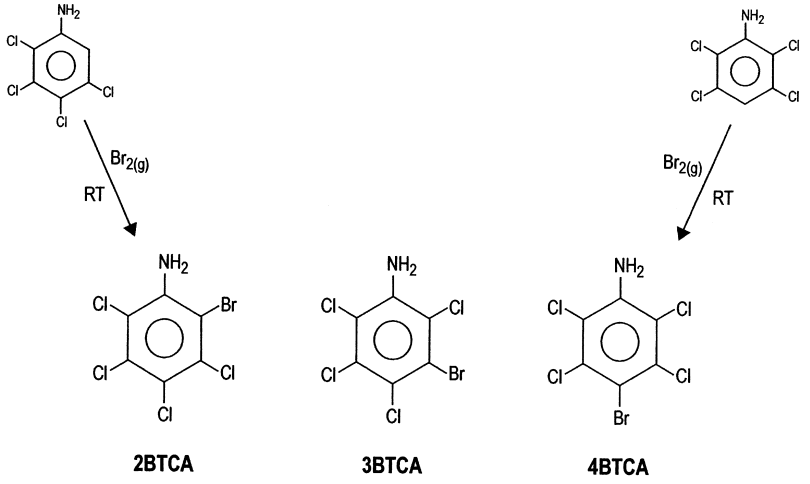

Fig. 2. Preparation of 2BTCA and 4BTCA.

toluene and twice with methanol followed by oven drying at 135 °C for 30 min.

2.3. Preparation of solutions to be analyzed

2.3.1. Preparation of the stock solutions

The stock solutions of 2BTCA [e.g., (A) 1.014±0.001 mg/ml, (B) 0.051±0.001 mg/ml] were prepared by dissolving 2BTCA [e.g., (A) 5.0701 mg] in methylene chloride (5 ml) in a 5-ml silanized volumetric flask. A portion of stock solution (A) was diluted to obtain solution (B). The stock solution of 4BTCA, 1.008±0.001 mg/ml, was prepared by dissolving 5.0402 mg 4BTCA in methylene chloride (5 ml) in a 5-ml silanized volumetric flask. These solutions were stored in the freezer (~−14 °C).

2.3.2. Preparation of the solutions for the calibration curve

For the 2BTCA calibration curve, approximately 50 mg of R28 (a batch of dye that was found to be free of 2BTCA by SPME–GC–MS) was placed in each of five 5-ml silanized volumetric flasks. 2BTCA stock solution (A) or (B) was added to the dry dye to eventually yield standard solutions containing 0.01, 0.1, 0.5, 1.0 and 3.0 ppm 2BTCA, respectively. A constant amount, 2.5 μl, of 4BTCA stock solution (1.008±0.001 mg/ml) was added to the dry dye in each of the five volumetric flasks. Approximately 2 ml of aqueous ammonia (~3% NH_3) was then added to each volumetric flask. The resulting mixtures were further dissolved by short sonication and agitation. The solutions were diluted to volume with aqueous ammonia (~3% NH_3). For the calibration curve, 1.5 ml of each of the above five standard solutions was analyzed by SPME–GC–MS as described in Sections 2.4 and 2.5.

2.3.3. Preparation of the dye solutions for SPME analyses

For the preparation of each of the 37 solutions of R27 and R28 analyzed, a test portion (approximately 50 mg) was placed in a 5-ml silanized volumetric flask and 2.5 μl of 4BTCA stock solution (1.008±0.001 mg/ml) was added onto the dry dye as an internal standard. (Note: prior to addition as an internal standard, the freezer-kept stock solution was warmed to room temperature and brought to the last recorded mass by adding methylene chloride). Approximately 2 ml of aqueous ammonia (~3% NH_3) was then added to the volumetric flask. The resulting solution was sonicated and mixed until all the dye dissolved. The solution was diluted to volume with aqueous ammonia (~3% NH_3, pH~11.8). Several of the analyzed samples were also prepared using only high-performance liquid chromatography (HPLC)-grade water (pH of resulting solution, ~7.7). In addition, some of them were prepared in aqueous NaOH solution at various pH values: 8.8; 10.3 and 11.8.

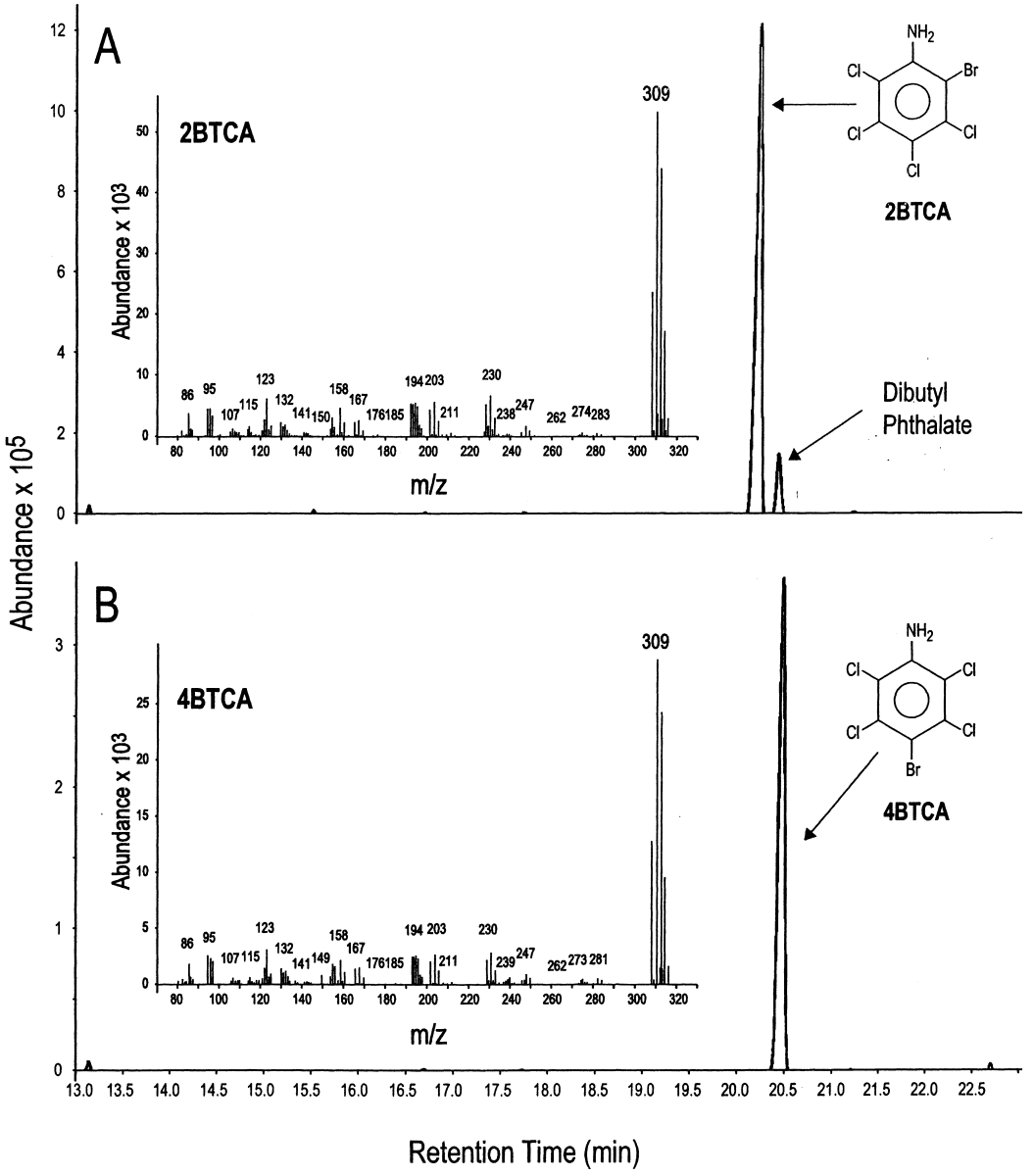

Fig. 3. SPME–GC–MS analyses and the mass spectra of synthetic (A) 2BTCA (for the presence of dibutyl phthalate as a contaminant see text) and (B) 4BTCA, used as reference materials in the present study.

2.4. Extraction procedure

A 2-ml silanized vial containing an unused PTFE-coated magnetic stir bar was filled with 1.5 ml of the 4BTCA spiked dye solution (see Section 2.3.3). The metallic needle of the SPME holder was inserted into the vial through the septum, and the SPME fiber was submerged in the sample while the sample was vigorously stirred at room temperature (25±1 °C). A Model 4805, Micro-V magnetic stirrer (Cole-Parmer, Chicago, IL, USA) was used with the speed controller set at MAX (~1100 rpm). Only the fiber (not the metallic needle itself) was in contact with the sample solution. After 25 min of exposure (adsorption step),

the fiber was retracted into the SPME needle and immediately inserted into the heated GC injector (desorption step) for GC–MS analysis.

2.5. GC–MS method, analysis and quantification

The desorption step was achieved by exposing the SPME fiber to the hot (280 °C) GC injector for 2 min with the GC injector purge flow turned off. The injector purge was then turned on and the fiber was left in the injection port for an additional 3 min to remove any residual contaminants from the fiber (see also Results and discussion). After that period of time, the fiber was retracted into the SPME holder which was removed from the injector and it was ready for the next extraction. The GC conditions were as follows: initially, the oven temperature was maintained at 60 °C for 2 min; then it was increased to 165 °C at a rate of 15 °C/min; then to 190 °C at 2 °C/min; and finally, to 300 °C at 40 °C/min, where it was held for 1 min. Helium was used as the carrier gas at a flow-rate of 40 cm/s. The temperature of the MS transfer line was 280 °C. The MS source temperature was 230 °C. The MS quadrupole temperature was 150 °C. The MS operating conditions were as follows: ionization was performed by electron ionization at 70 eV; the mass spectrometer was scanned over the range m/z 80–380; the threshold was set at 150; the solvent delay was set to 13 min. The total time required for the GC–MS analysis of each sample was ~25 min. A deionized water blank was analyzed prior to the first dye sample of the day to verify that the SPME fiber and the GC column had no detectable amounts of 2BTCA. The 2BTCA present in R27 and R28 was quantified using the ratio of the integrated peak area from the reconstructed ion chromatogram (RIC) of the m/z 309 ion for 2BTCA to the corresponding area of m/z 309 ion for the internal standard, 4BTCA. A linear regression calibration curve was obtained for 2BTCA using standard solutions (see Section 2.3.2) at five concentration levels ranging from 0.01 to 3 ppm. The curve resulted from plotting the ratio of 2BTCA (m/z 309) response to 4BTCA (internal standard, m/z 309) response against the level of 2BTCA concentration. The dilution factor was taken into account when the concentration of 2BTCA in the sample was calculated.

2.6. HPLC determination of 3,4,5,6-tetrachlorophthalic acid in D&C Red Nos. 27 and 28

Analytical reversed-phase HPLC was performed with a Waters Alliance 2690 separation module (Waters, Milford, MA, USA). The eluents were 0.1 *M* NH_4OAc in water–methanol (95:5) and methanol. The column (Hypersil MOS-1 RPC-8, 5 μm particle size, 250×4.6 mm I.D., Keystone Scientific, Bellefonte, PA, USA) was eluted by using consecutive linear gradients of 25–90% methanol in 25 min, 90–100% methanol in 5 min, followed by 100% methanol for 5 min. The column was re-equilibrated with 25% methanol for 15 min. The effluent was monitored with a Waters 996 photodiode array detector set at 254 nm. Other conditions included: injection volume, 20 μl; and flow-rate, 1 ml/min.

Solutions of R27 and R28 were prepared for HPLC analysis at a concentration of approximately 10 mg/ml in aqueous NaOH (0.008 *M*). A portion (0.5 ml) of the color solution was filtered through a Mini-UniPrep 0.45-μm pore-size syringeless filter device (Whatman, Clifton, NJ, USA) prior to chromatography. TCPHTHAc was quantified by using a five-point calibration curve prepared with external standards chromatographed separately from the samples. The data points ranged from 0.010 to 1.506% (w/w) for TCPHTHAc. The instrument response was linear over this range.

3. Results and discussion

In a previous study, during GC–MS analyses for the determination of hexachlorobenzene (HCB) in chloroform extracts of batches of R27 and R28 [15], the GC chromatograms consistently contained a peak that represented another contaminant that eluted several minutes later than HCB. The electron ionization (EI) mass spectrum of the contaminant showed the molecular ion at m/z 307 to be composed of an isotopic cluster. The cluster was characteristic in terms of peak intensities and isotope abundance ratios, of a compound with a halogen composition of Cl_4Br (Fig. 3A). High-resolution measurements of the m/z 307 ion showed that its composition is

$C_6H_2N^{79}Br^{35}Cl_4$ (measured m/z 306.8126, calculated for $C_6H_2N^{79}Br^{35}Cl_4$ 306.8125). The total number of rings and double bonds (r+db) of a molecule of the formula $C_6H_2NBrCl_4$ is equal to 4 [16]. From these data and from the mass spectral fragmentation pattern, the most plausible structures for the new contaminant were the three positional isomers, 2BTCA, 3-bromo-2,4,5,6-tetrachloroaniline (3BTCA) and 4BTCA, shown in Fig. 2. 2BTCA and 4BTCA were prepared as described in the Experimental section. Due to the unavailability of the starting material, no attempts were made to prepare 3BTCA. 4BTCA had a different retention time than the R27/R28 contaminant, while the retention time of 2BTCA was identical to that of the contaminant under various GC conditions (Fig. 4). In addition, 2BTCA and the contaminant have identical collision-induced dissociation (CID) mass spectra. These

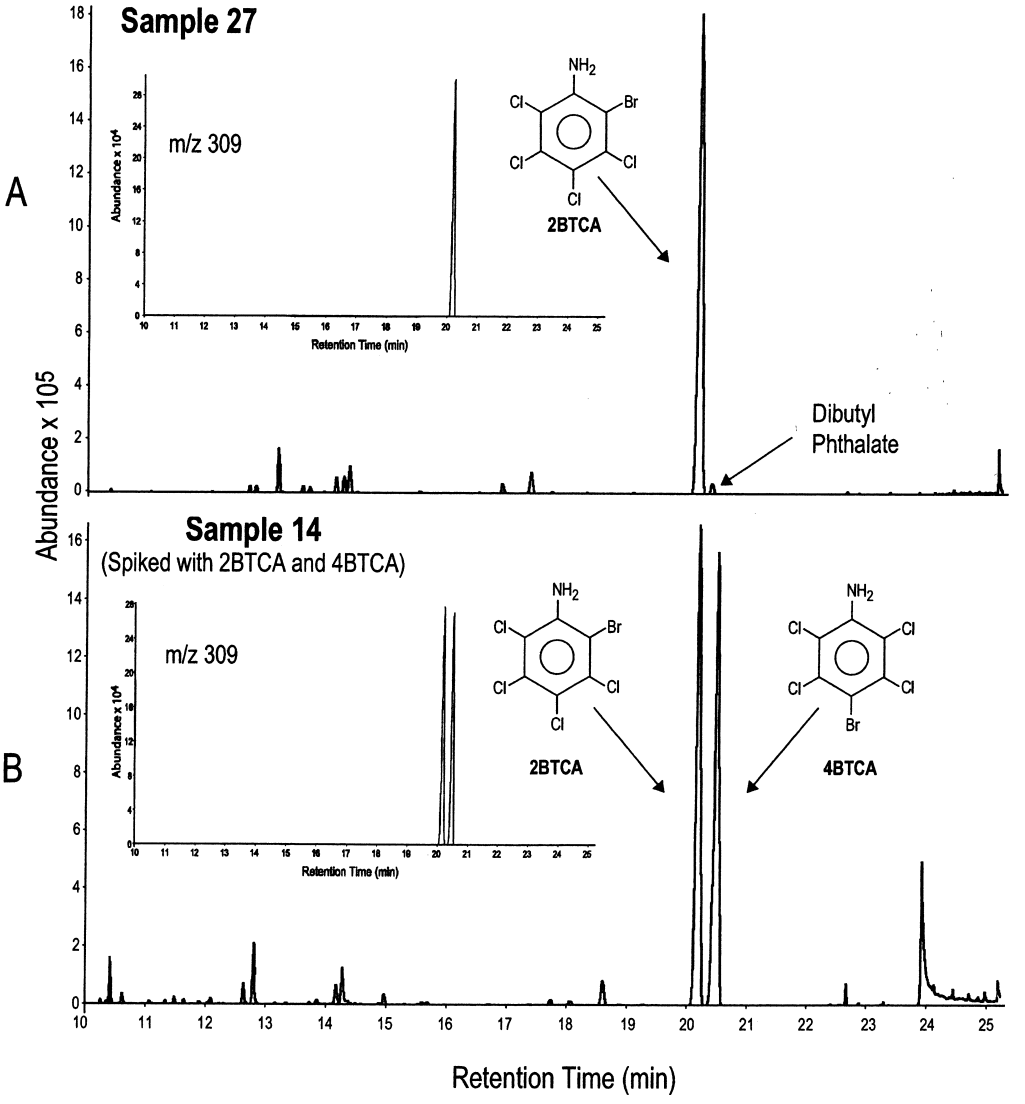

Fig. 4. SPME–GC–MS total ion chromatograms and reconstructed ion chromatograms for m/z 309 ion of (A) a batch of D&C Red No. 28 (sample 27 in Table 1) (for the presence of dibutyl phthalate as a contaminant see text), and (B) a batch of D&C Red No. 28 (that does not contain 2BTCA, sample 14 in Table 1) spiked with 2BTCA and 4BTCA (0.756 μg each, in 1.5 ml dye solution).

results and the presence of four continuous chlorine atoms in 2BTCA (not in 3BTCA or 4BTCA) as in the R27/R28 starting material (3,4,5,6-tetrachlorophthalic anhydride or acid) point to 2BTCA as the contaminant. A possible route for the formation of 2BTCA during the manufacturing process of R27 and R28 is proposed in Fig. 5. Unreacted 3,4,5,6-tetrachlorophthalic anhydride, if present in TCF, may be opened with aqueous ammonia (which sometimes is the base used for the partial purification of TCF) to the ammonium salt of the carboxyamide **3**, which may undergo a Hofmann rearrangement in the presence of Br_2 and base, resulting in the 2-carboxy-3,4,5,6-tetrachloroaniline, **4**. The latter intermediate may give rise to 2BTCA by a Hunsdiecker (see Refs. [17,18]) or a Simonini (see Ref. [19]) type of bromodecarboxylation. The metal ions needed for this reaction may be provided by the remnant traces of $ZnCl_2$, which is used as a condensing reagent (due to its high affinity for water) in the reaction between resorcinol and tetrachlorophthalic acid/anhydride [20] (Fig. 1). The sequence of the latter two reactions may be reversed and 2BTCA may be formed via the intermediacy of the bromoamide **5**.

The SPME method used to analyze 2BTCA in batches of R27 and R28 was based on the method developed previously for the analysis of HCB in those dyes [2]. For quantification of 2BTCA, the ideal internal standard would have been [$^{13}C_6$]2BTCA. Because such an isotopomer was not available, the most closely related alternative was the 4BTCA isomer that has GC and MS behavior similar to that of 2BTCA. The dye samples were prepared in ammonium hydroxide (~3% NH_3, pH approximately 11.8). At that pH level, the main components of R27 and R28 are dissociated in the aqueous solution and do not interfere with the extraction of 2BTCA (which is in the undissociated form) by the PDMS-coated fiber. Comparable quantitative results were obtained when the dye samples were prepared in sodium hydroxide solutions at pH 10.3 and 11.8. When R28 was dissolved in either a sodium hydroxide solution at pH 8.8 or only in water (pH approximately 7.7), the quantity of 2BTCA adsorbed on the fiber was drastically reduced (~1/10), possibly due to the smaller amount of undissociated 2BTCA present in the solution at those pH levels [6]. To obtain reproducible results, as noted previously [2], the internal standard (4BTCA dissolved in methylene chloride) was added directly onto the dry dye in the volumetric flask. To avoid contamination from the PTFE-coated magnetic stir bar, which was shown earlier to possibly retain small amounts of polychlorinated compounds after use [21,2], a new stir bar was used for each analysis. However, the PTFE coating of all the stir bars used leached the plasticizer dibutyl phthalate (identified by a match in the Wiley mass spectral data base). The GC retention

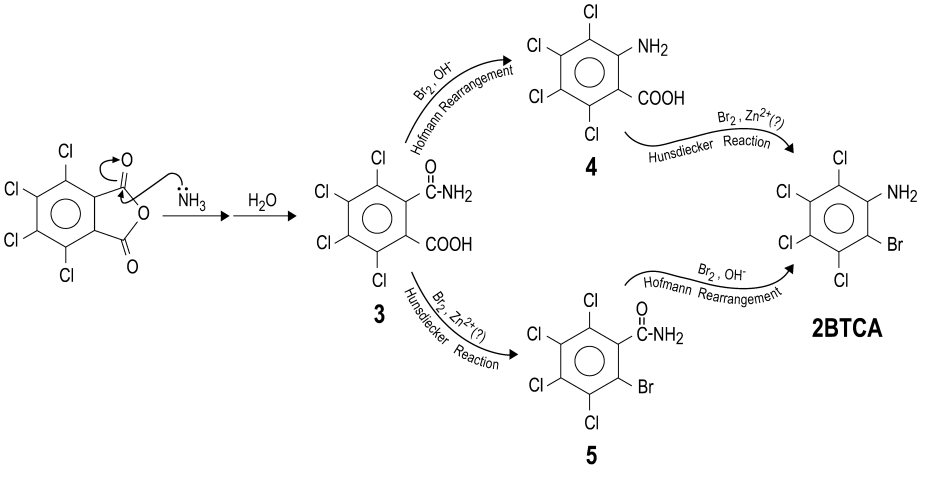

Fig. 5. Possible pathways for the formation of 2BTCA as a by-product from 3,4,5,6-tetrachlorophthalic anhydride during the manufacturing of D&C Red Nos. 27 and 28.

time of the phthalate was close to that of 4BTCA. Efforts to eliminate this contaminant before use of the stir bar, such as vigorous stirring for 30 min in ethanol or methylene chloride, or sonication for 20 min in ethanol, were unsuccessful. Its removal was not pursued farther since the dibutyl phthalate (M_r 278) does not produce a m/z 309 ion and thus did not affect the quantitative RIC analyses of 2BTCA. After this work was completed, we received custom-made pyrex coated stir bars (7 mm×2 mm, Spectrum). Use of those stir bars greatly reduced the presence of dibutyl phthalate. A 25-min extraction time was found to be suitable for the extraction of the 2BTCA from dyes. A shorter extraction time (i.e., 15 min) was not sufficient for 2BTCA to reach equilibrium between the aqueous phase and the SPME fiber. A longer extraction time (i.e., 45 min) did not increase the amount of 2BTCA adsorbed by the fiber's PDMS coating.

After analysis of samples with high levels of 2BTCA (i.e., over 100 ppm), it is necessary to run 2–3 water blanks prior to the next analysis in order to return to a clean background. One of the reviewers suggested that this ''memory effect'' can be eliminated by using an SPME fiber with a thinner film of PDMS. In fact, we found that use of a 30-μm thickness PDMS fiber provides adequate 2BTCA response with the added advantage of not retaining 2BTCA after the desorption step (no ''memory effect''). Use of a thinner PDMS film (7 μm PDMS fiber) decreases the sensitivity of the method.

For the quantification of 2BTCA, a calibration curve was used. It was obtained by plotting the peak-area ratio of 2BTCA to 4BTCA against the level of 2BTCA concentration. The data points ranged from 0.01 to 3 ppm. Over this range, the SPME–GC–MS method shows good linearity with a correlation coefficient for the linear regression of 0.995. Each point was determined from the integrated areas of the m/z 309 ions (molecular ions) of 2BTCA and 4BTCA. Each determination was made in triplicate (three different aliquots of each calibration point) by either manual or auto integration. The precision of the method in terms of relative standard deviation (RSD), measured at medium (50 ppm) concentration of 2BTCA, is 1.1% and it was determined by analyzing five vials of the same sample. The limit of detection (LOD) was determined by spiking a portion of 2BTCA-free dye with known quantities of 2BTCA and 4BTCA standards and analyzing them by the SPME–GC–MS method. Using a signal-to-noise ratio of 5:1, the LOD was found to be 0.001 ppm of 2BTCA and the limit of quantification (LOQ) was 0.01 ppm. A lower level of detection and quantification can be achieved by using a less-wide scan range or the selected ion monitoring MS mode.

Test portions from 35 certified lots of D&C Red Nos. 27 and 28 were analyzed for 2BTCA using the SPME–GC–MS method described above. The lots were submitted for certification by both domestic (A, B, C, D, E, F, K in Table 1) and foreign companies (G—Japan, H—UK, I—France, J—India) during the past 4 years. The study also included two lots of D&C Red No. 27 (''toxicology test batches'', samples 1 and 2 in Table 1) that were used in the animal feeding studies upon which the FDA based its safety evaluation of D&C Red Nos. 27 and 28. Of the test portions analyzed, 22 (62.9%) contained 2BTCA in amounts ranging from 0.15 ppm to 435.7 ppm with an average value of ~131.7 ppm. The remaining 13 (37.1%) test portions contained no detectable 2BTCA or less than 0.01 ppm, which is the limit of quantification of the present method. Notably, 21 samples (60.0%) had over 30 ppm of 2BTCA and 8 samples (22.9%) had over 100 ppm of 2BTCA. These latter samples had a substantially higher level of 2BTCA than that found in the ''toxicology test batches'', which contained no detectable 2BTCA or <0.01 ppm (Table 1). A direct correlation was observed between the presence of 2BTCA and that of TCPHTHAc in analyzed batches of R27 and R28 (Table 1). When TCPHTHAc was in negligible quantities in the dye, no quantifiable 2BTCA was found. This further substantiates the proposed pathway for the formation of 2BTCA in R27 and R28 (Fig. 5).

4. Conclusions

This study demonstrates the presence of 2BTCA, a newly-detected polyhalogenated aromatic amine contaminant, in lots of D&C Red Nos. 27 and 28 submitted for FDA certification. The wide range of 2BTCA levels found in lots submitted for certifica-

Table 1
2-Bromo-3,4,5,6-tetrachloroaniline (2BTCA) and 3,4,5,6-tetrachlorophthalic acid (TCPHTHAc) found in certified batches of color additives D&C Red Nos. 27 and 28 using SPME–GC–MS

Sample No.	Manufacturer	Color additive[a]	2BTCA found (ppm)	TCPHTHAc found (%)
1	AA-4623[b]	R27	ND[c]	0.02
2	AA-8945[b]	R27	<0.01	0.02
3	A	R28	50.2	0.29
4	A	R28	100.0	0.37
5	A	R28	38.5	0.44
6	A	R28	96.1	NM[d]
7	A	R28	37.9	0.41
8	B	R28	ND	0.01
9	B	R27	222.0	0.14
10	B	R28	52.8	0.59
11	B	R28	29.5	0.57
12	C	R28	<0.01	0.09
13	C	R27	435.7	NM
14	C	R28	ND	0.07
15	C	R28	ND	0.10
16	C	R27	ND	0.01
17	C	R27	420.2	0.15
18	D	R27	0.15	0.04
19	E	R28	179.4	0.43
20	E	R27	ND	0.02
21	E	R27	ND	0.01
22	E	R28	ND	0.08
23	E	R28	ND	0.02
24	E	R28	ND	0.01
25	F	R28	<0.01	0.11
26	F	R28	ND	0.08
27	G	R28	48.7	0.29
28	G	R28	146.8	0.41
29	G	R28	153.3	0.41
30	G	R28	89.7	0.35
31	H	R27	60.4	0.10
32	H	R28	391.4	0.31
33	H	R28	95.6	0.22
34	I	R28	60.5	0.40
35	I	R27	57.9	0.10
36	J	R28	ND	0.09
37	K	R28	43.5	0.34

[a] Color batches certified between 1998 and 2001.
[b] From toxicology test batch.
[c] Not detected.
[d] Not measured.

tion (‘‘not detected’’–435.7 ppm; Table 1 and Fig. 4) suggests that the contamination with 2BTCA may be avoided or significantly decreased through appropriate changes in the color-manufacturing process. Such changes may involve use of TCF that is free of TCPHTHAc, or purifying TCF, prior to its bromination, with a base other than aqueous ammonia. The suggested chemical pathway for the formation of 2BTCA during the preparation of these colors could provide at least an initial basis of investigation toward that end. Current regulations for D&C Red Nos. 27 and 28 do not specify a limit for 2BTCA. FDA might consider the need to limit the 2BTCA levels in these color additives. In that case, the

SPME–GC–MS method presented here could be applied, after further optimization, to the enforcement of future 2BTCA specifications in the batch certification of these colors.

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