Food Chemistry 237 (2017) 733–742

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Determination of starting materials, intermediates, and subsidiary colors in the color additive Food Red No. 106 (Sulforhodamine B) using high-performance liquid chromatography

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ARTICLE INFO

Article history:
Received 1 December 2016
Received in revised form 13 May 2017
Accepted 16 May 2017
Available online 17 May 2017

Keywords:
Food Red No. 106
Sulforhodamine B
Subsidiary color
HPLC

ABSTRACT

The main subsidiary color of structure in Food Red No. 106 (R106) was identified to be a desethyl derivative (R106-SubA). High-performance liquid chromatography (HPLC) was performed for the quantitative determination of benzaldehyde-2,4-disulfonic acid, *N*,*N*-diethyl-*m*-aminophenol, leuco acid, pyrone acid, R106-SubA, etc. in R106. An ammonium acetate solution (20 mM) and acetonitrile:water (7:3) were used to stabilize the retention time of the HPLC analytes. The linearity of the calibration curves was in the range of 0.05–10 μg/mL, with good correlation coefficients ($R^2 > 0.9983$). The recoveries of impurities at levels 0.1%, 0.5% and 1% ranged from 94.2% to 106.6% with relative standard deviations of 0.1%–1.0%. While surveying commercial R106, the amounts obtained by area% determination were similar to those obtained by the calibration-curve determination. The area% determination by HPLC for the determinations of impurities in R106 is a simple and reliable method and can be applied in routine analysis.

1. Introduction

Food Red No. 106 (R106, Sulforhodamine B, Color Index No. 45100, Monosodium 6-[3,6-bis(diethylamino)xanthenium-9-yl]benzene-1,3-disulfonate) is authorized for use as a food coloring in Japan (Ministry of Health, Labour and Welfare, 2007); however it is prohibited for use as a food colorant in the United States, the European union (EU), and other countries. R106 is regulated as a cosmetic colorant as Acid Red 52 (European Commission, 1976) in the EU and is also used as a biological stain colorant by the name of Sulforhodamine B (Vichai & Kirtikara, 2006).

The manufacturing process of R106 comprises several steps: condensing benzaldehyde-2,4-disulfonic acid (BADS) and *N*,*N*-diethyl-*m*-aminophenol (DAP); partially purifying the resulting leuco acid (LA); dehydrating with concentrated H_2SO_4 to pyrone acid (PA); oxidizing with $K_2Cr_2O_7$, $KMnO_4$, or $FeCl_3$; and finally, desalting with NaCl to obtain R106 (Fig. 1; Horiguchi, 1968; Tanimura & Tanamoto, 2007).

During the manufacturing process, the unreacted starting materials, synthesized intermediates, and subsidiary color byproducts may be carried over into the final products. In Japan, food colorant products are inspected by a registered inspection institute under the Food Sanitation Act (Article 25) to ensure compliance with the purity in the specifications described in Japan's Specifications and Standards for Food Additives (Ministry of Health, Labour and Welfare, 2007). To investigate whether the commercial food colorant products meet the specifications, several specification tests are performed. The purity test using high-performance liquid chromatography (HPLC) to the determine unreacted raw materials and the products of side reactions is set in Coloring Matter Tests; this method is only used for Food Red No. 2 (Amaranth), Food Red No. 40 (Allura Red), Food Red No. 102 (New Coccine), Food Yellow No. 4 (Tartrazine), and Food Red No. 5 (Sunset Yellow FCF) in their monograms (Ministry of Health, Labour and Welfare, 2007). The purity tests for other food colorants including R106 are generally performed using paper chromatography (Ministry of Health,

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http://dx.doi.org/10.1016/j.foodchem.2017.05.084
0308-8146/

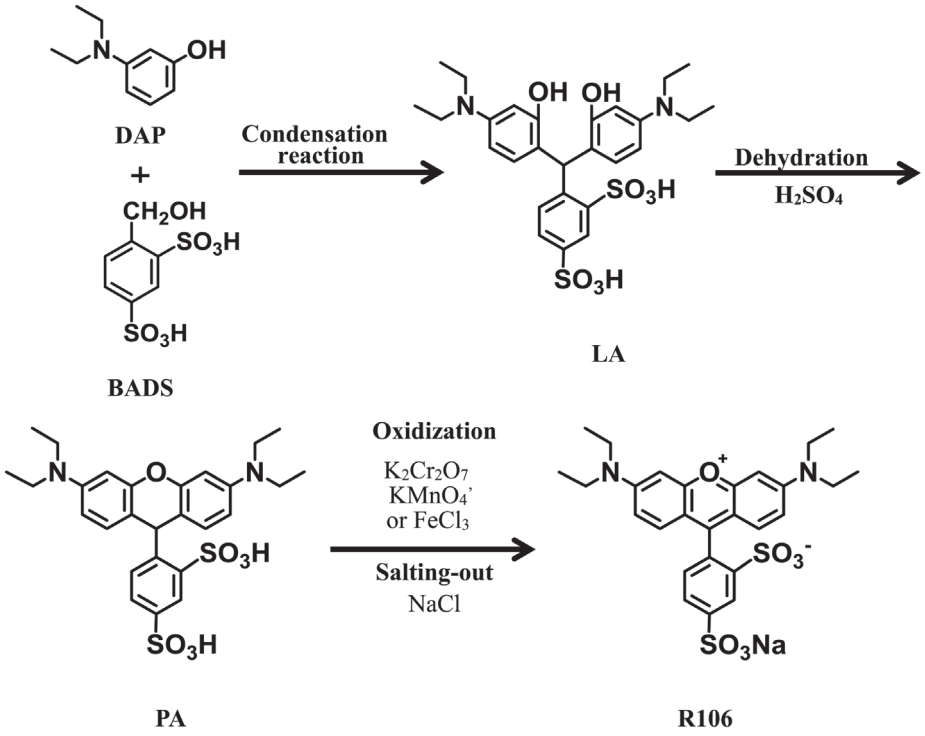

Fig. 1. Synthesis scheme of Food Red No. 106.

Labour and Welfare, 2007). There are no requirements for impurity levels in R106. The acceptance criterion is determined only by observing of one spot for R106 on the paper chromatogram (Ministry of Health, Labour and Welfare, 2007). This method is considered time-consuming for equilibration of mobile phase condition and development of analyte, inaccurate, and to have poor resolution (Joint FAO/WHO Expert Committee on Food Additives, 2006, Tanimura & Tanamoto, 2007). Furthermore, this method cannot detect non-coloring substances such as the starting materials and intermediates due to the limitations of visual observation (Ministry of Health, Labour and Welfare, 2007).

To separate of the impurities in R106, high-speed counter-current chromatography (Oka, Ikai, Kawamura, Hayakawa, & Yamada, 1991), pH-zone refining counter-current chromatography (Oka et al., 2002), and thin-layer chromatography and HPLC (Ochi, Okuda, & Fujii, 2016) have been used. The desethylated derivative subsidiary color of R106 was suggested by fast atom bombardment mass spectrometry (Oka et al., 1991), and two unknown dyes detected by TLC in pickled vegetable samples containing R106 and New Coccine were also reported (Ochi et al., 2016). These dyes are brominated R106 and its desethyl derivative, generated mainly due to bromine contamination during the manufacturing process. Paper chromatography, thin-layer chromatography, and column chromatography are still used to determine the intermediates and/or subsidiary color additives (Joint FAO/WHO Expert Committee on Food Additives, 2006; Ministry of Health, Labour and Welfare 2007; The United States Pharmacopeial Convention, 2017). In order to simplify and modernize the methods used to analyze the components of food colors, an HPLC method has been successfully used determine intermediates and/or subsidiary color additives and certify food color (Tsuji, Amakura, Okada, & Tonogai, 2001; Tsuji, Nakano, Furukawa, Yoshii, & Tonogai, 2005; Mai et al., 2006; Vu, Rickard, Sullivan, Richfield-fratz, & Weisz, 2011; Weisz & Krantz, 2014). Although the determining the starting materials, intermediates, and subsidiary colors in the R106 products for quality control is very important for food safety assurance, no reports on the determining of impurities including the unreacted starting materials, intermediates, and subsidiary colors in R106 products by HPLC have been published, there is no structural information about the main subsidiary color in R106, either.

In the present study, we analyzed the structure of the main subsidiary color in the commercial R106 products and developed an accurate and simple HPLC method for simultaneous determination of the unreacted starting materials, intermediates, and subsidiary colors. Moreover, we tried to apply area% calculation for the determination of the unreacted starting materials, intermediates, and subsidiary colors by HPLC without standard materials instead of purity test by paper chromatography. Furthermore, a survey of the commercial R106 products was performed to determine their unreacted starting materials, intermediates, and subsidiary color contents.

2. Materials and methods

2.1. Materials

The starting materials for R106, BADS of >84.0% purity (by HPLC), and DAP of >99% purity (by HPLC) and the intermediate products LA of >99% purity (by HPLC) and PA of >97.5% purity (by HPLC) were kindly provided by Daiwa Dyestuff Mfg. Co., Ltd. (Saitama, Japan). R106 standard (R106-Std; >97% purity by HPLC) was a reference standard for Japan's Specifications and Standards for Food Additives purchased from the Pharmaceutical and Medical Device Regulatory Science Society of Japan (Osaka, Japan). Ammonium acetate was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and acetonitrile for HPLC was purchased from Merck (Darmstadt, Germany).