Journal of Chromatography A, 1218 (2011) 6156–6164

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Performance comparison of three types of high-speed counter-current chromatographs for the separation of components of hydrophilic and hydrophobic color additives

Adrian Weisz[a,*], Yoichiro Ito[b]

[a] Office of Cosmetics and Colors, Center for Food Safety and Applied Nutrition,USA Food and Drug Administration, College Park, MD 20740, USA
[b] Bioseparation Technology, Biochemistry and Biophysics Center, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA

ARTICLE INFO

Article history:
Available online 16 December 2010

Keywords:
High-speed counter-current chromatography
J-type coil planet centrifuge
Cross-axis coil planet centrifuge
Multilayer-coil column
Spiral-tube assembly column
HPLC
Color additives
FD&C Blue No. 2
D&C Red No. 17
Sudan II

ABSTRACT

The performance of three types of high-speed counter-current chromatography (HSCCC) instruments was assessed for their use in separating components in hydrophilic and hydrophobic dye mixtures. The HSCCC instruments compared were: (i) a J-type coil planet centrifuge (CPC) system with a conventional multilayer-coil column, (ii) a J-type CPC system with a spiral-tube assembly-coil column, and (iii) a cross-axis CPC system with a multilayer-coil column. The hydrophilic dye mixture consisted of a sample of FD&C Blue No. 2 that contained mainly two isomeric components, 5,5′- and 5,7′-disulfonated indigo, in the ratio of ~7:1. The hydrophobic dye mixture consisted of a sample of D&C Red No. 17 (mainly Sudan III) and Sudan II in the ratio of ~4:1. The two-phase solvent systems used for these separations were 1-butanol/1.3 M HCl and hexane/acetonitrile. Each of the three instruments was used in two experiments for the hydrophilic dye mixture and two for the hydrophobic dye mixture, for a total of 12 experiments. In one set of experiments, the lower phase was used as the mobile phase, and in the second set of experiments, the upper phase was used as the mobile phase. The results suggest that: (a) use of a J-type instrument with either a multilayer-coil column or a spiral-tube assembly column, applying the lower phase as the mobile phase, is preferable for separating the hydrophilic components of FD&C Blue No. 2; and (b) use of a J-type instrument with multilayer-coil column, while applying either the upper phase or the lower phase as the mobile phase, is preferable for separating the hydrophobic dye mixture of D&C Red No. 17 and Sudan II.

Published by Elsevier B.V.

1. Introduction

FD&C Blue No. 2 (B2, Indigotine, Color Index (C.I.) 73015) and D&C Red No. 17 (R17, Sudan III, C.I. 26100) are color additives used in food (B2), drugs (B2, R17), cosmetics (R17), and medical devices (B2, R17) in the United States. Before they may be used as color additives, B2 and R17 are subject to batch certification by the U.S. Food and Drug Administration (FDA) to ensure compliance with certain chemical specifications [1,2].

B2 is currently manufactured by sulfonating synthetic indigo with concentrated sulfuric acid, a process similar to that used in 1740 by Ludwig Barth who prepared "powder blue" (C.I. 75781) by sulfonating natural indigo [3,4]. The degree of sulfonation is dependent on the reaction conditions and during the manufacturing process of B2 results in mainly mono- and disulfonated components [5–7] (Fig. 1). B2 consists of a mixture primarily of the disodium salt of disulfonated indigo in positions 5 and 5′ (5,5′diSI) with up to 18% of the disodium salt of disulfonated indigo in positions 5 and 7′ (5,7′diSI) and up to 2% of the sodium salt of the monosulfonated indigo in position 5 (5SI) [1] (Fig. 1).

R17 is manufactured by coupling diazotized 4-aminoazobenzene with 2-naphthol [8]. The obtained product consists of a mixture primarily of 1-[[4-(phenylazo)phenyl]azo]-2-naphthalenol (Sudan III), up to 2% of an isomer of Sudan III (Sudan III iso), and up to 3% of 1-(phenylazo)-2-naphthol (Sudan I) [2] (Fig. 2).

In order to develop high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) methods of analysis for FDA batch certification, purified components as well as purified contaminants of these color additives are needed for use as reference materials. Purified B2 components and Sudan III iso are not available commercially. In the past, 5SI and 5,5′diSI were obtained by a synthetic method that included a lengthy purification step [5,6], while 5,7′diSI was obtained by separation from batches of B2 [6]. Sudan III iso was obtained previously in minute amounts by solvent precipitation and preparative TLC [9].

* Corresponding author. Tel.: +1 3014361145; fax: +1 3014362961.
E-mail address: adrian.weisz@fda.hhs.gov (A. Weisz).

0021-9673/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.chroma.2010.12.034

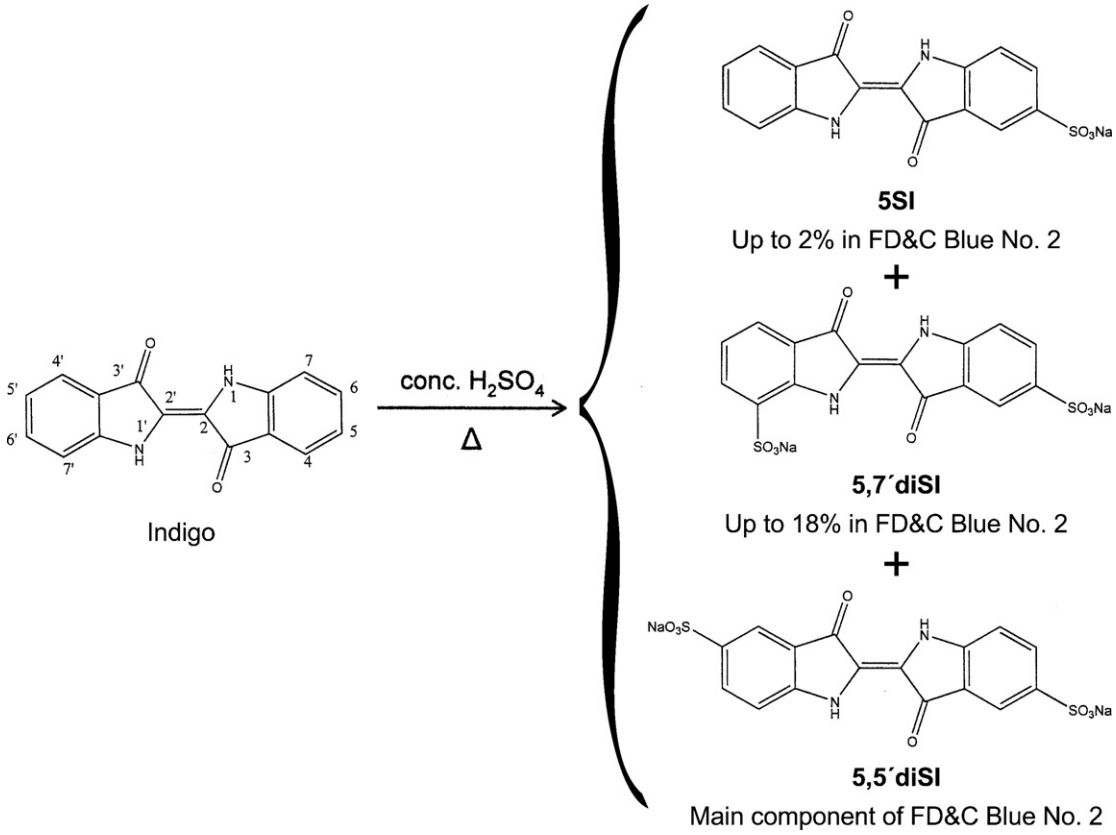

Fig. 1. Preparation of FD&C Blue No. 2 by sulfonating indigo.

High-speed counter-current chromatography (HSCCC) has been applied extensively to the separation of synthetic dyes [10–12]. HSCCC is a liquid-liquid partition technique that does not involve use of a solid support. One of the two immiscible liquid phases is retained in an Ito multilayer-coil column by centrifugal force while the other liquid phase is pumped through the rotating column. The principle of this technique, the instrumentation that it requires, the rationale for selecting a two-phase solvent system, and the implementation of an HSCCC separation procedure have been described in detail in earlier literature [13–15].

The present study assesses the effectiveness of using HSCCC to separate components from B2, a water-soluble dye, and from R17, a water-insoluble dye. The components of B2 and R17 were separated using three types of HSCCC instruments, each with a unique combination of a particular kind of column and a particular kind of centrifuge: (i) a J-type coil planet centrifuge (CPC) system with a conventional multilayer-coil column [16]; (ii) a J-type CPC system with a spiral-tube assembly column [17]; and (iii) a cross-axis CPC system with a multilayer-coil column [16]. For these separations, suitable polar and non-aqueous two-phase solvent systems were chosen. The separation performance of these three instruments was compared in terms of peak resolution, theoretical plate number, and retention of stationary phase.

2. Experimental

2.1. Materials

FD&C Blue No. 2 and D&C Red No. 17 test portions used in this study were from samples submitted to the FDA for batch certification. Acetonitrile (ACN), water, ammonium acetate (NH_4OAc) (all from Fisher Scientific, Fair Lawn, NJ, USA), and methanol (MeOH) (J.T. Baker, Phillipsburg, NJ, USA) were of chromatography grade. Hexanes (>99.9%, Fisher Scientific) and hydrochloric acid (HCl, 33–36%, J.T. Baker) were ACS reagent grade. *n*-Butanol (99.9%, Sigma–Aldrich, Milwaukee, WI, USA) and Sudan II (90%, Sigma–Aldrich) were used as-received.

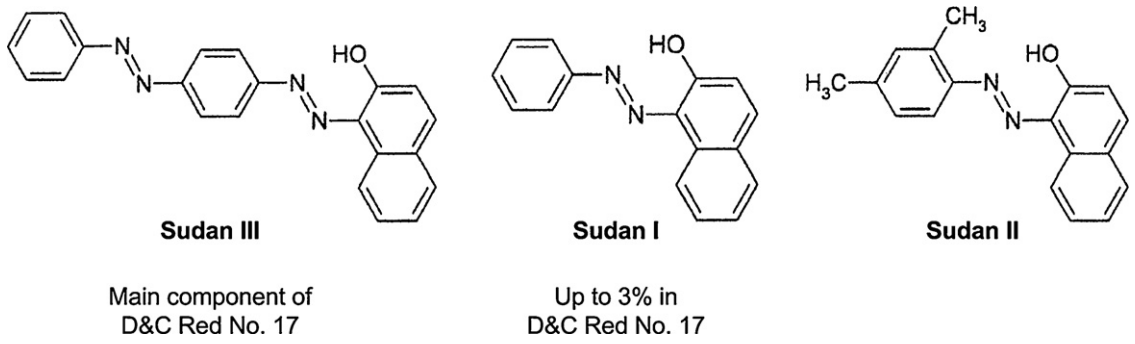

Fig. 2. Components of D&C Red No. 17 and the structure of Sudan II.