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

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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.

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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].

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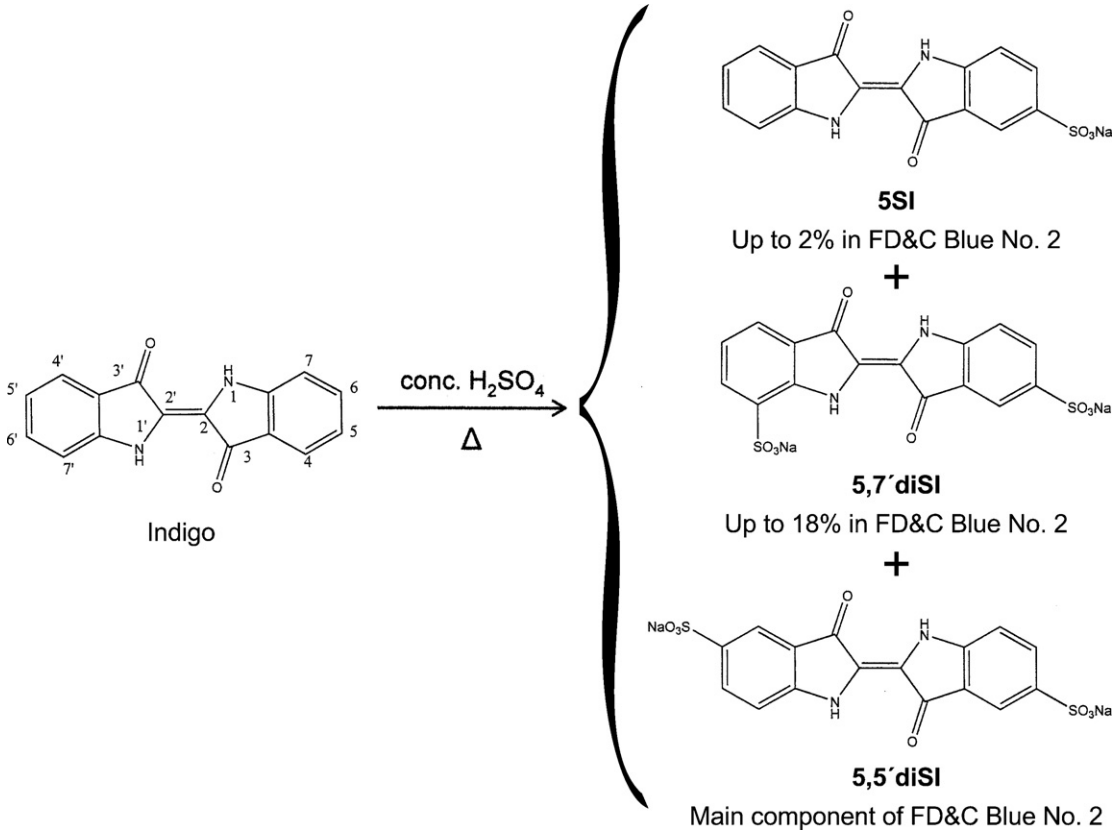

Fig. 1. Preparation of FD&C Blue No. 2 by sulfonating indigo.

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.

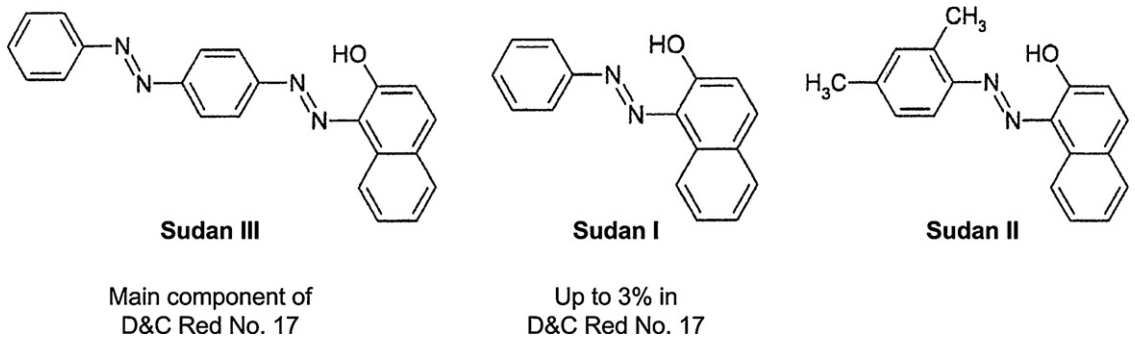

Fig. 2. Components of D&C Red No. 17 and the structure of Sudan II.

Table 1
Characteristics of the three counter-current chromatographs used in the present study.

Instrument	Planetary motion	Possible samples for separation	Partition efficiency	Stationary phase retention	Availability
HSCCC with conventional multilayer coils	J-Type planetary motion: the column holder revolves around the central axis of the centrifuge while rotating about its own axis at the same angular velocity in the same direction	A broad range of hydrophobic and hydrophilic compounds, except for extremely polar compounds such as proteins and polysaccharides	High	High retention for non-polar to moderately polar systems, but almost no retention for polymer phase systems	Commercially available
HSCCC with spiral tube assembly coils	J-Type planetary motion	Universally applicable, including polar peptides, proteins, nucleic acids and polysaccharides	Moderate	High retention for all solvent systems, including polymer phase systems	Commercially available
HSCCC with a cross-axis coil planet centrifuge (CPC)	XL-Type planetary motion: the column revolves around the vertical centrifuge axis while rotating about its horizontal axis at the same angular velocity	Almost universally applicable	Moderate	Retains all solvent systems, but low retention for polymer phase systems	Not commercially available

2.2. High-speed counter-current chromatography

2.2.1. Instrumentation

Separations were performed using three different HSCCC instruments. The general characteristics of the three instruments are tabulated in Table 1. Several details are given below:

J-type HSCCC with multilayer coils (Fig. 3(A)): The separations were performed with a commercial J-type HSCCC system (Model CCC-1000, Pharma-Tech Research, Baltimore, MD, USA) that consisted of a column (three multilayer coils connected in series and made of 1.6 mm i.d. polytetrafluoroethylene (PTFE) tubing with a total capacity of ~320 ml) mounted on a rotating frame (centrifuge), a speed controller, and a Model 300 LC pump (Scientific Systems, State College, PA, USA). To this system we added (a) a right-angle flow-switching valve (Upchurch Scientific, Oak Harbor, WA, USA) to conveniently introduce into the column the stationary phase, sample solution, and mobile phase without introducing air into the system [18]; (b) a UV detector, model Uvicord SII with a 254-nm UV lamp (Pharmacia LKB, Uppsala, Sweden); (c) a chart recorder (Kipp & Zonen, Delft, The Netherlands) for monitoring the effluent; and (d) a Foxy fraction collector (Isco, Lincoln, NE, USA).

J-type HSCCC with spiral-tube assembly coils (Fig. 3(B)): The separations were performed with the system described above except that it was fitted with three spiral-tube assembly coils (CC Biotech LLC, Rockville, MD, USA) [17] for use as the column. Each coil consisted of a spiral tube support wound with 1.6 mm i.d. PTFE tubing. Since the respective weights of the three spiral-tube assembly coils were different from each other, some adhesive tape was wrapped around each spiral tube support to bring them all to the same weight in order to balance the centrifuge system. The total capacity of the column made of the three spiral-tube assembly coils was ~250 ml.

HSCCC with a cross-axis coil planet centrifuge (Fig. 3(C)): The separations were performed using a prototype cross-axis CPC (the axis of the coil rotation is perpendicular to the centrifuge axis [16]) built by Pharma-Tech Research. The column consisted of four multilayer coils connected in series and made of 1.6 mm i.d. PTFE tubing with a total capacity of ~250 ml. The relevant parameters [16] of the instrument are as follows: radius, r, of the multilayer-coil holder, ~4.5 cm; distance between the two axes, R, ~ 5 cm; and measure of the lateral shift of the multilayered-coil holder along its axis, L, ~7.6 cm. The instrument was connected as described above.

2.2.2. Selection of two-phase solvent systems and samples

In order to assess the performance of these three high-speed counter-current chromatographs, the following two-phase solvent systems with a significant difference in hydrophobicity were selected: 1-butanol/1.3 M HCl (1:1, v/v), as a representative hydrophilic system, and hexane/acetonitrile, as a representative hydrophobic low viscosity system. For each solvent system, a test mixture that contained two components was chosen based on their suitable partition coefficient $K_{\text{upper phase}}/K_{\text{lower phase}}$ ($K_{\text{UP/LP}}$) values and separation factors K_2/K_1 (where $K_2 > K_1$). The two components of B2, 5,5′diSI and 5,7′diSI, were chosen for the 1-butanol/1.3 M HCl solvent system, and a mixture of R17 (Sudan III) and Sudan II for the hexane/acetonitrile solvent system. It should be noted that due to the low solubility of Sudan I (the minor component in R17), its dimethylated analog, Sudan II (Fig. 2), was substituted in its place

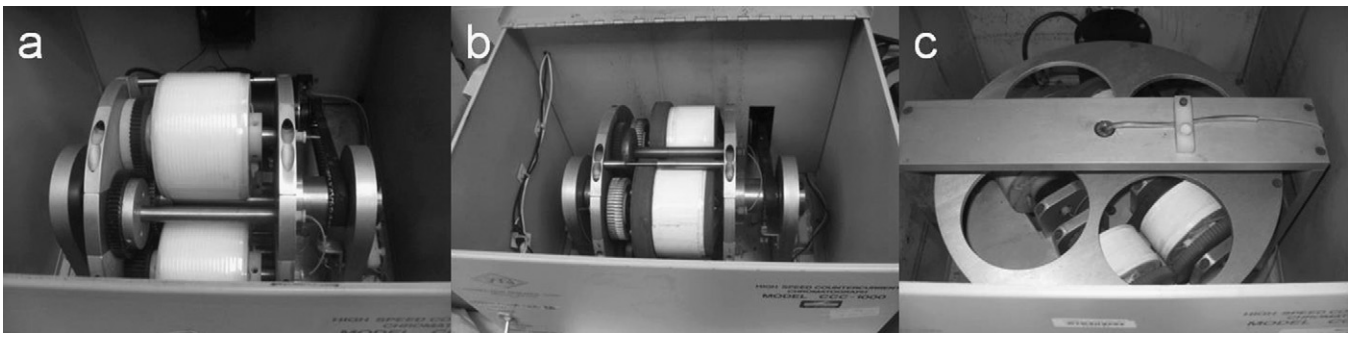

Fig. 3. Photographs of the counter-current chromatographs used in the present study: (A) conventional J-type instrument with three multilayer coils, (B) J-type instrument fitted with three spiral-tube assembly coils, and (c) cross-axis CPC instrument with four multilayer coils.

Table 2
Solvent systems used and partition coefficient (K) measurements for HSCCC separations of components in hydrophilic and hydrophobic dye mixtures.

Compound	Solvent system	$K_{UP/LP}$*
Sudan II	Hexane/ACN	0.7
Sudan III	Hexane/ACN	0.4
5,5′diSI	1-Butanol/1.3 M HCl	0.4
5,7′diSI	1-Butanol/1.3 M HCl	0.9

* UP: upper phase; LP: lower phase.

to better meet the purposes of this work. The K values of the sample components in the corresponding two-phase solvent systems are shown in Table 2.

2.2.3. Separation procedure

The HSCCC separations were performed following the general directions previously described [13–15]. After equilibration in a separatory funnel, the two phases of each solvent system were separated and used as the stationary and mobile HSCCC phases. Each of the three instruments was used for two experiments with the hydrophilic dye mixture and two with the hydrophobic dye mixture, for a total of 12 experiments. In one set of experiments, the lower phase (LP) was used as the mobile phase, and in the other set of experiments, the upper phase (UP) was used as the mobile phase. A hydrophilic sample consisted of ~300 mg of B2 dissolved in 12 ml of the 1-butanol/1.3 M HCl (1:1, v/v) solvent system (6 ml LP and 6 ml of UP). A hydrophobic sample consisted of a mixture of ~5 mg of R17 and ~1 mg of Sudan II dissolved in 10 ml of the hexane/acetonitrile solvent system (5 ml UP and 5 ml of LP). The sample solutions were filtered through Uniprep 0.45-μm glass microfiber filter units (Whatman, Clifton, NJ, USA) prior to use. Each separation was initiated by using the LC pump to fill the entire column with the stationary phase. Next, a syringe was used to load a sample solution into the column. The mobile phase was then pumped into the column (at 1 ml/min for the hydrophilic separations or at 2 ml/min for the hydrophobic separations) while the column was rotated at 800 rpm. When the J-type instrument with multilayer-coil column or the cross-axis CPC system was used, the elution was in head-to-tail mode if LP was the mobile phase or in tail-to-head elution mode if UP was the mobile phase [13]. When the J-type instrument with spiral-tube assembly column was used, the elution was as follows: if the LP was the mobile phase, it was pumped into the inner head terminal; if the UP was the mobile phase, it was pumped into the outer tail terminal [17,19,20]. The effluent was monitored with a UV detector at 254 nm (the recorder was set at 20 min/cm) and in some experiments the effluent was collected in fractions (2 or 4 ml/tube) using a fraction collector. The fractions representing the same HSCCC peak were pooled and analyzed using HPLC. After the elution of the second compound of each mixture, the experiments were stopped and the column contents (V_c) were collected into a graduated cylinder by applying pressurized air. The volume of the stationary phase retained in the column (V_s) was measured after the two phases separated. The % retention of the stationary phase was calculated as follows:

$$S_F = \left(\frac{V_s}{V_c}\right) \times 100$$

The theoretical plate number (N) and peak resolution (R_S) of the separation were calculated in a way similar to that used for other liquid chromatographic techniques [15,21]:

$$N = \left(\frac{4R}{W}\right)^2$$

$$R_S = 2\frac{(R_2 - R_1)}{(W_1 + W_2)}$$

where R represents the distance (mm) from the origin of the chromatogram to the middle of the specified peak, and W represents the peak width (mm) at the baseline of the specified peak.

2.3. High-performance liquid chromatography

In previous studies, the components of FD&C Blue 2 were analyzed by gravity-column chromatography followed by spectrophotometric determination [22] or by various HPLC methods [23–25] that eluted the B2 components in 25–30 min. Color components of D&C Red No. 17 were separated by TLC and determined by visible spectrophotometry [26]. The improved HPLC methods developed for the present study, and described below, separate the B2 components in 3.3 min and the R17 and Sudan II mixture in 10 min.

An aliquot (25 μl) from the HSCCC-collected fractions was diluted with 2 ml of acetonitrile, and the solution was filtered through a Uniprep 0.45-μm glass microfiber filter unit (Whatman, Clifton, NJ, USA) prior to analysis by HPLC.

The HPLC analyses were performed with a Waters Alliance 2690 separation module (Waters, Milford, MA, USA). The eluents were (A) 0.2 M NH_4OAc in water/methanol (95:5, v/v) and (B) acetonitrile. The same column (Kinetex C-18, 2.6 μm particle size, 100 Å pore size, 100 mm × 4.6 mm i.d., Phenomenex, Torrance, CA, USA) was used for analyzing components in the B2 and R17 mixtures. To analyze the B2 components, the column was eluted using a linear gradient of 0–60% acetonitrile in 4.07 min, followed by 60% acetonitrile for 2.03 min, 60–100% acetonitrile in 0.42 min, and 100% acetonitrile for 1.83 min. The column was re-equilibrated with 0% acetonitrile for 2 min. To analyze the R17 components, the column was eluted using a linear gradient of 0–60% acetonitrile in 4.07 min, followed by 60% acetonitrile for 2.03 min, 60–80% acetonitrile in 0.42 min, and 80% acetonitrile for 6.08 min. The column was re-equilibrated with 0% acetonitrile for 3 min. The effluent was monitored with a Waters 996 photodiode array detector set at 254 nm. Other conditions included: flow-rate, 1.5 ml/min; column temperature, 35 °C; injection volume, 5 μl.

3. Results and discussion

The performance of the three HSCCC instruments was assessed on the basis of their use for separation of hydrophilic and hydrophobic dye mixtures. The hydrophilic dye mixture consisted of a sample of FD&C Blue No. 2 that contained ~78% 5,5′diSI, 11.3% 5,7′diSI, and ~0.7%5SI. The HPLC analysis of a test portion of the original B2 sample is shown in Fig. 4(A). The hydrophobic dye mixture consisted of a sample of D&C Red No. 17 to which was added ~17% Sudan II. The HPLC analysis of the hydrophobic dye mixture is shown in Fig. 5(A).

Using each of the three HSCCC instruments, two experiments were performed with each of the two dye mixtures. The LP of the solvent system was used as the mobile phase in one set of experiments and the UP was used as the mobile phase in the other set of experiments.

Fig. 6 and Table 3 present the results obtained for the two sets of experiments that investigated the separation of the hydrophilic dye mixture. For each experiment, Table 3 shows the partition efficiency expressed in terms of theoretical plate number (N) and peak resolution (R_S), as well as the % retention of the stationary phase (S_F). When the LP was the mobile phase, the J-type HSCCC system with multilayer-coil column and the J-type HSCCC system with spiral-tube assembly column yielded higher peak resolution than was found in the other experiments. This result can be explained by speculating that the LP, as the mobile phase, combined with the J-type instrument's configuration, provided greater interfacial area between the two phases. That greater interfacial area is, in turn,

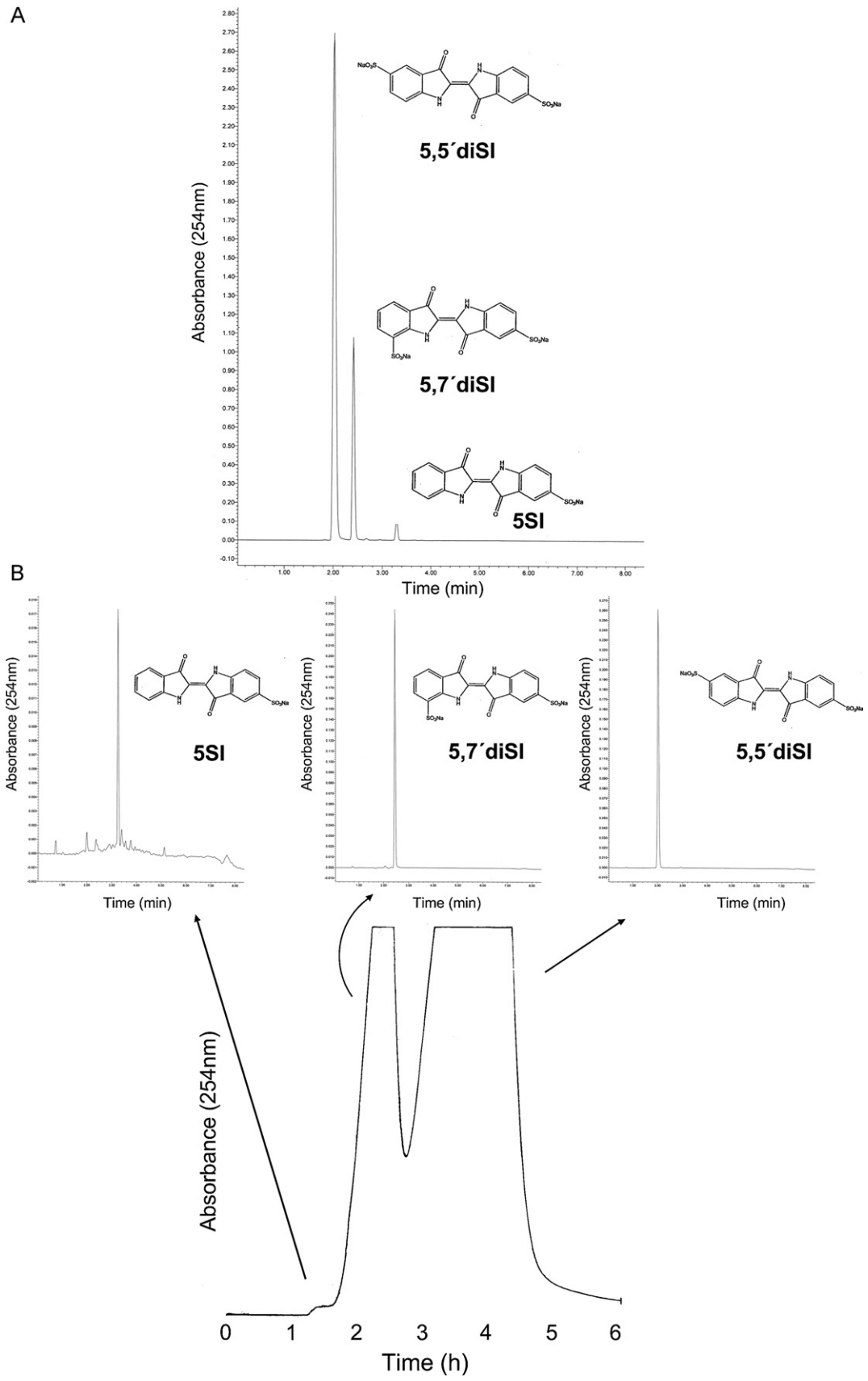

Fig. 4. Separation of a 0.3-g test portion of FD&C Blue No. 2 using a J-type HSCCC instrument with a spiral-tube assembly column (Fig. 3(B)) and UP as the mobile phase. (A) HPLC analysis of the original mixture. (B) Chromatogram of the HSCCC separation and HPLC analyses of the separated components.

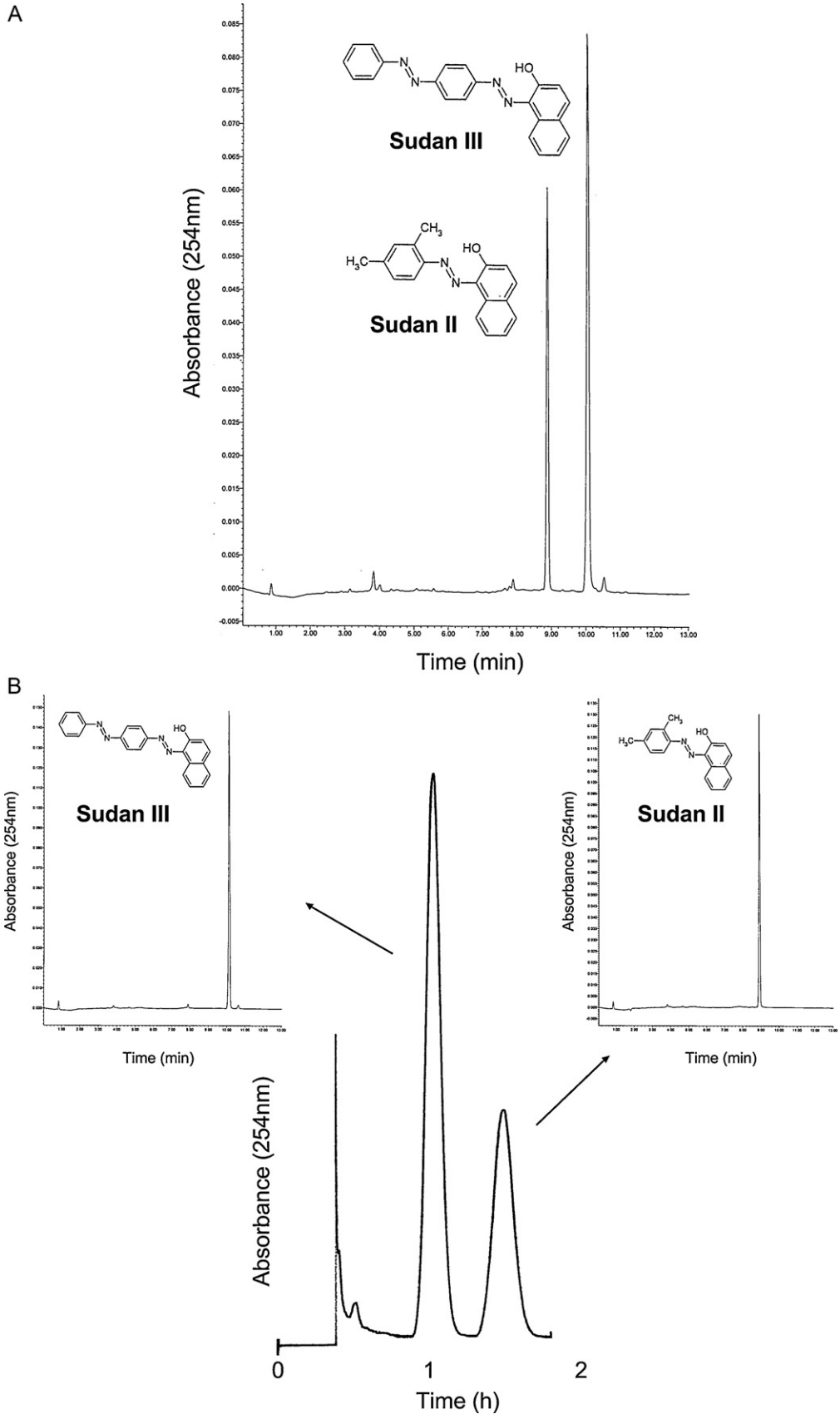

Fig. 5. Separation of a test portion of D&C Red No. 17 (~5 mg) spiked with Sudan II (~1.2 mg) using a J-type HSCCC instrument with a spiral-tube assembly column (Fig. 3(B)) and LP as the mobile phase. (A) HPLC analysis of the original mixture. (B) Chromatogram of the HSCCC separation and HPLC analyses of the separated components.

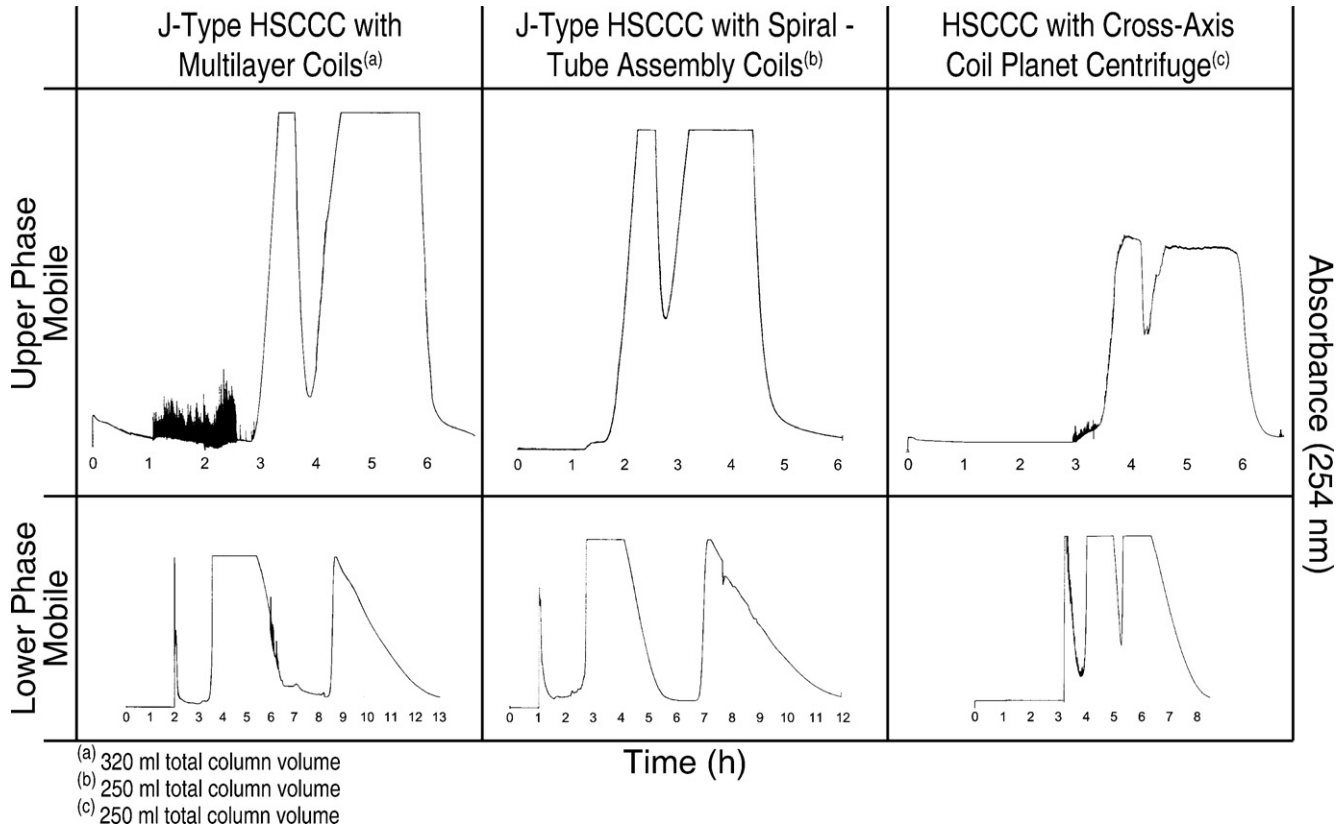

Fig. 6. HSCCC separations of the two main components of the hydrophilic dye FD&C Blue No. 2 using three different types of instruments. The experiments shown in the top row were performed with the upper phase as the mobile phase. Those shown in the bottom row were performed with the lower phase as the mobile phase. For details see Section 2.2.

because the UP had an affinity for the walls of the PTFE coils and formed a thin film over the internal surface of the tubing. The major component 5,5′diSI eluted first and the minor component 5,7′diSI eluted second when the LP was the mobile phase, and the order was reversed when the UP was the mobile phase, as shown in Fig. 4(B).

Fig. 7 and Table 4 present the results of the two sets of experiments for the separation of the hydrophobic dye mixture. Table 4 shows the separation performance parameters for each experiment. Both J-type instruments provided high retention of the stationary phase and high peak resolution when either the UP or the LP was the mobile phase. The major component of R17 (Sudan III) eluted first and Sudan II eluted second when the LP was the mobile phase, and the order was reversed when the UP was the mobile phase, as shown in Fig. 5(B).

The poor retention of the stationary phase when using the cross-axis CPC for both dye mixtures (Tables 3 and 4) is due to the construction of our prototype instrument even though it tends to form sharp peaks that have high theoretical plate number. The parameters R (which influences the retention of the stationary phase) and L (which influences the mixing in the column), described in Section 2.2.1, are shorter than optimal. The parameters should be nearly equal in length, measuring ~10–12 cm [27], but in this instrument, they are 5 and 7.6 cm, respectively.

Although in our present study the spiral-tube assembly column yields lower partition efficiency than does the multilayer-coil column (Tables 3 and 4), it still has an important advantage. Specifically, it can provide a satisfactory level of stationary phase retention for very polar solvent systems such as 1-butanol–acetic acid–water (4:1:5, v/v) [20], 2-butanol–water, and polyethylene glycol–potassium phosphate polymer phase systems [28], all of which cannot be efficiently applied to the conventional multilayer-coil column.

Table 3
Theoretical plates, peak resolution, and retention of stationary phase obtained for the separation of hydrophilic dye components using three types of counter-current chromatographs.

Separation performance parameters		J-Type HSCCC with multilayer coils*		J-Type HSCCC with spiral tube assembly coils		HSCCC with a cross-axis coil planet centrifuge	
		LP Mobile	UP Mobile	LP Mobile	UP Mobile	LP Mobile	UP Mobile
Theoretical plates	5,5'diSI	27	70	29	60	212	119
	5,7'diSI	109	267	8	169	80	562
Peak resolution		1.5	1.0	1.3	1.0	0.7	1.0
Retention of stationary phase after separation (%)		51.6	55.0	68.0	64.0	22.0	28.0

* Adjusted for 250 ml total column volume.

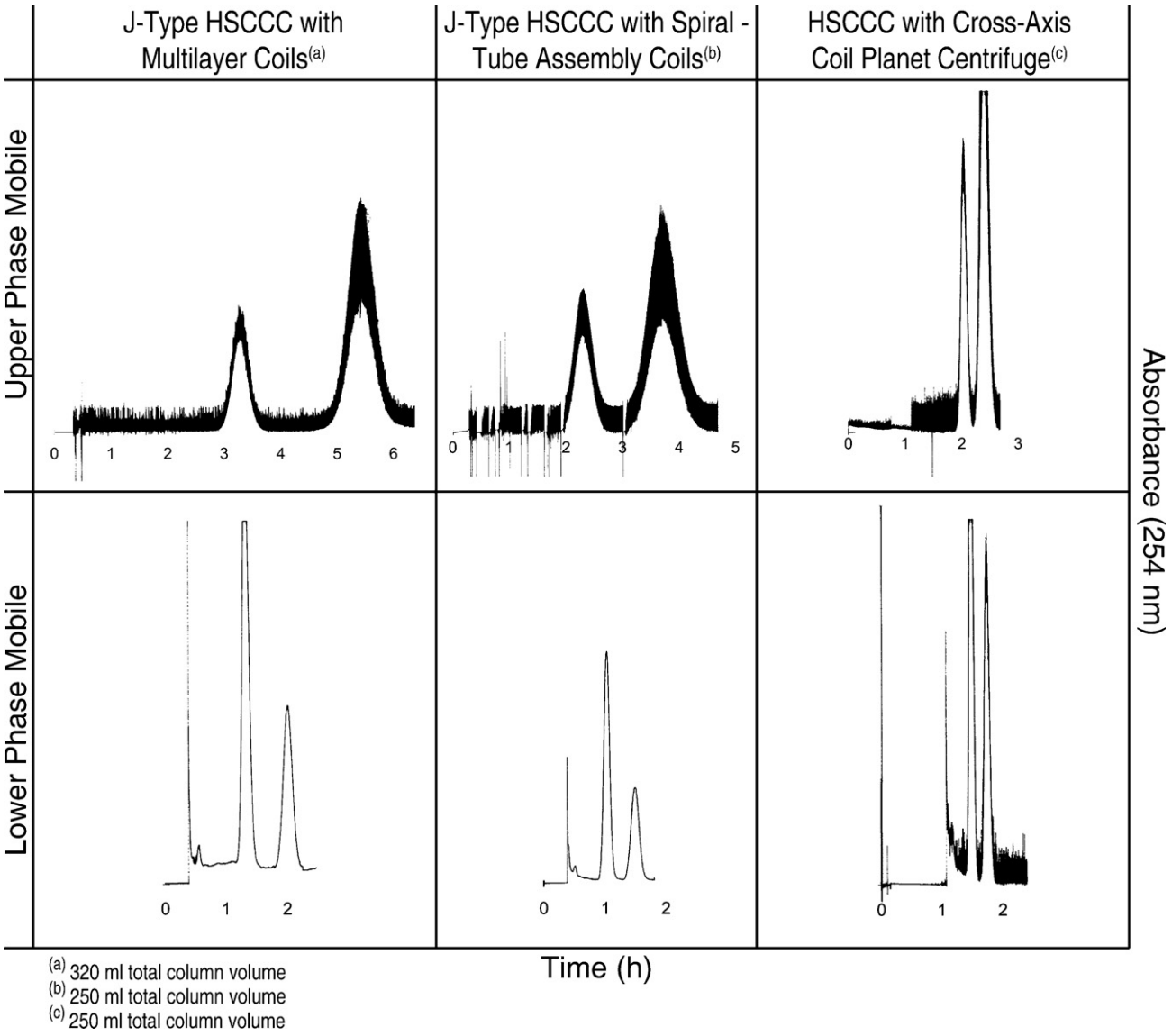

Fig. 7. HSCCC separations of the two main components of the hydrophobic mixture of D&C Red No. 17 and Sudan II using three different types of instruments. The experiments shown in the top row were performed with the upper phase as the mobile phase. Those shown in the bottom row were performed with the lower phase as the mobile phase. For details see Section 2.2.

Table 4
Theoretical plates, peak resolution, and retention of stationary phase obtained for the separation of hydrophobic dye components using three types of counter-current chromatographs.

Separation performance parameters	J-Type HSCCC with multilayer coils*		J-Type HSCCC with spiral tube assembly coils		HSCCC with a cross-axis coil planet centrifuge	
	LP Mobile	UP Mobile	LP Mobile	UP Mobile	LP Mobile	UP Mobile
Theoretical plates → Sudan III	292	466	484	152	1820	1296
Theoretical plates → Sudan II	378	396	334	125	1369	1296
Peak resolution	1.9	2.9	1.8	1.5	1.4	1.6
Retention of stationary phase after separation (%)	83.7	83.9	72.3	80.9	32.7	20.0

* Adjusted for 250 ml total column volume.

4. Conclusions

This work has shown that use of a J-type HSCCC instrument with either a multilayer-coil column or a spiral-tube assembly column, while applying the LP as the mobile phase, is the preferred method for separating the hydrophilic components of FD&C Blue No. 2. Furthermore, the results suggest that it is advisable to use a J-type instrument with a multilayer-coil column, applying either the UP or the LP as the mobile phase, to separate hydrophobic mixtures such as D&C Red No. 17 and Sudan II. This configuration provides

higher retention of the stationary phase and, when combined with a low viscosity solvent system, results in high peak-resolution and theoretical plate number.

References

[1] Code of Federal Regulations, Title 21, Part 74.102, U.S. Government Printing Office, Washington, DC, 2010.
[2] Code of Federal Regulations, Title 21, Part 74.1317, U.S. Government Printing Office, Washington, DC, 2010.
[3] Colour Index, vol. 4, 3rd ed., The Society of Dyers and Colourists, 1971, p. 4639.
[4] J. Wisniak, Indian J. Hist. Sci. 39 (1) (2004) 75.
[5] M.X. Sullivan, B. Cohen, W.M. Clark, U.S. Publ. Health Rep. 38 (1923) 1669.
[6] J.H. Jones, L.S. Harrow, K.S. Heine Jr., J. Assoc. Off. Agric. Chem. 38 (1955) 949.
[7] J.E. Bailey Jr., J. Travis, Dyes Pigments 6 (1985) 135.
[8] E. Thorpe, A Dictionary of Applied Chemistry, vol. 1, Longmans, London, 1921, p. 472.
[9] M. Kuzukawa, J. Okada, R. Kanbe, Y. Ikeda, K. Yoshimura, R. Hayakawa, K. Matsunaga, Eisei Kagaku 36 (1990) 497.
[10] A. Weisz, in: Y. Ito, W.D. Conway (Eds.), High-Speed Countercurrent Chromatography, Chemical Analysis, vol. 132, Wiley, New York, 1996, p. 337 (Chapter 12).
[11] A. Weisz, Y. Ito, in: I.D. Wilson, E.R. Adlard, M. Cooke, C.F. Poole (Eds.), Encyclopedia of Separation Science (III), vol. 6, Academic Press, London, 2000, p. 2588.
[12] A. Weisz, E.P. Mazzola, Y. Ito, J. Chromatogr. A 1216 (2009) 4161.
[13] Y. Ito, New York in: Y. Ito, W.D. Conway (Eds.), High-Speed Countercurrent Chromatography, Chemical Analysis, vol. 132, Wiley, 1996, p. 3 (Chapter 1).
[14] Y. Ito, J. Chromatogr. A 1065 (2005) 145.
[15] W.D. Conway, Countercurrent Chromatography: Apparatus, Theory & Applications, VCH, New York, 1990.
[16] Y. Ito, in: J.-M. Menet, J.-M. Menet, D. Thiebaut (Eds.), Countercurrent Chromatography, Chromatographic Science Series, vol. 82, Dekker, New York, 1999, p. 87 (Chapter 3).
[17] Y. Ito, R. Clary, J. Powell, M. Knight, T.M. Finn, J. Liq. Chromatogr. Rel. Technol. 31 (2008) 1346.
[18] A. Weisz, A.L. Scher, Y. Ito, J. Chromatogr. A 732 (1996) 283.
[19] M. Knight, Y. Ito, T.M. Finn, J. Liq. Chromatogr. Rel. Technol. 31 (2008) 471.
[20] Y. Ito, R. Clary, J. Powell, M. Knight, T.M. Finn, J. Liq. Chromatogr. Rel. Technol. 32 (2009) 2013.
[21] J.E. Haky, in: J. Cazes (Ed.), Encyclopedia of Chromatography, Dekker, New York, 2001, p. 711.
[22] M. Singh, J. Assoc. Off. Anal. Chem. 53 (1970) 250.
[23] M. Singh, J. Assoc. Off. Anal. Chem. 58 (1975) 48.
[24] J.E. Bailey, J. Assoc. Off. Anal. Chem. 63 (1980) 565.
[25] S. Tsuji, M. Nakano, M. Furukawa, K. Yoshii, Y. Tonogai, Shokuhin Eiseigaku Zasshi (J. Food Hyg. Soc. Jpn.) 46 (2005) 116.
[26] J.C. Molitor, J. Assoc. Off. Agric. Chem. 50 (1967) 1198.
[27] Y. Ito, H. Oka, J. Slemp, J. Chromatogr. 463 (1989) 305.
[28] Y. Ito, Chem. Eng. Proc. 49 (2010) 782.