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Preparative purification of 4-hydroxy-1-naphthalenesulfonic acid sodium salt by high-speed counter-current chromatography

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Abstract

Among the many applications of 4-hydroxy-1-naphthalenesulfonic acid sodium salt (HNSA, CAS No. 6099-57-6) is its use as a common intermediate in the manufacture of dyes used as color additives. In order to develop an HPLC method to analyze HNSA in such additives, it was necessary to obtain pure HNSA as a reference material. This compound is only commercially available in “technical” or “practical” grade (~70-85% purity). Unsatisfactory results were encountered during the attempts to eliminate impurities using published preparative purification methods.

The current work demonstrates the successful application of high-speed counter-current chromatography (HSCCC) to the preparative purification of “practical” grade HNSA. The purifications of 0.55 g and 1.00 g portions were achieved by using a solvent system that consisted of n-butanol/water (1:1), acidified with 0.2% trifluoroacetic acid. In contrast to the procedure involved in previous HSCCC separations of aromatic sulfonates, it was not necessary to add a ligand and a retainer to the solvent system for these separations. The resulting yields were 320 mg and 485 mg of HNSA, respectively, of over 99% purity. Elemental analysis, HPLC (at 240 nm), UV and high-resolution MS analyses confirmed the identity and purity of the HNSA obtained.

Keywords

Counter-current chromatography; Color additive intermediate; Purification; 4-Hydroxy-1-naphthalenesulfonic acid sodium salt

1. Introduction

4-Hydroxy-1-naphthalenesulfonic acid sodium salt (HNSA, commonly known as Neville and Winther’s acid; CAS No. 6099-57-6) is an intermediate used extensively as a coupling component in the manufacture of azo dyes [1,2]. One of those dyes (Ponceau SX) is batch-certifiable as FD&C Red No. 4 for use as a color additive in the USA as long as the batch meets certain chemical specifications described in the US Code of Federal Regulations (CFR) [3]. To determine compliance with these requirements, the dyes are subjected to an analytical process of batch certification by the US Food and Drug Administration (FDA) [4-6]. In order

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to develop an HPLC method to analyze HNSA in such additives, it was necessary to obtain pure HNSA as a reference material, but this compound is only commercially available in “technical” or “practical” grade (~70-85% purity). The source of the impurities present in HNSA may be the impure starting materials used in the multi-step manufacturing process and/or the reaction by-products obtained during this process (Figure 1) [7]. The difficulty of eliminating the impurities by preparative methods was evident when various published purification methods were implemented with unsatisfactory results [8-10].

In the present study, high-speed counter-current chromatography (HSCCC) [11-13] was chosen to purify samples of “practical” grade HNSA. HSCCC is a liquid-liquid partition technique that does not involve use of a solid support. One of the liquid phases (the stationary phase) is retained in a multilayered coil column by centrifugal force while the other liquid phase (the mobile phase) is pumped through the column. The principles of this technique [11-13] as well as its step-by-step implementation and application to the separation and purification of various compounds have been discussed previously [14]. HSCCC has been applied to the separation of components from dyes [15] and to the purification of sulfonated dye byproducts [16]. Previously, sulfonated dyes have been separated by a HSCCC technique only when a ligand (i.e., an ion-exchange reagent or an ion-pairing reagent) and a retainer (e.g., HCl, H₂SO₄) were added to the solvent system in order to enhance the partitioning of the sulfonated dye components into the organic stationary phase [15,17]. The current work demonstrates the preparative purification of “practical” grade HNSA using HSCCC without the addition of a ligand and retainer.

2. Experimental

2.1. Materials

The 1-naphthol-4-sulfonic acid sodium salt used in this study was purchased from Acros Organics (Practical grade, now Fisher Scientific, Pittsburgh, PA, USA) and Aldrich (Technical grade ~ 70%, Milwaukee, WI, USA). The 1-naphthol-4-sulfonic acid was from ChemPacific [Technical grade (mislabelled as 97%), Baltimore, MD, USA]. *n*-Butanol (A.C.S.-reagent grade) and methanol and water (both HPLC-solvent grade) were all from J.T. Baker (Phillipsburg, NJ, USA). Trifluoroacetic acid (TFA, ≥98%) was from Fluka (Buchs, Switzerland) and ammonium acetate (NH₄OAc, HPLC grade) was from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. High-Speed Counter-current Chromatography

2.2.1. Instrumentation—The separation was performed with a commercial J-type high-speed CCC system (Model CCC-1000, Pharma-Tech Research, Baltimore, MD, USA) that consisted of a column (three multilayer-coils connected in series made of 1.6 mm I.D. PTFE tubing with a total capacity of ~325 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 the stationary phase, sample solution and mobile phase into the column without introducing air in the system; (b) a UV detector model Uvicord SII with a 275 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).

2.2.2. Separation procedure—The HSCCC purifications were performed following the general directions previously published [14,18]. The solvent system was chosen so that the value of the partition coefficient of the components, $K_{\text{Upper Phase}/\text{Lower Phase}}$ ($K_{\text{UP/LP}}$) would be in the vicinity of 1. The two-phase solvent system consisted of *n*-butanol-water (500 ml:

500 ml) to which 2 ml of TFA (0.2% v/v) was added, bringing the pH of the solvent system to ~ 1.6. The “practical” grade HNSA in this solvent system had a $K_{UP/LP}$ of 1.0. After equilibration in a separatory funnel the two phases were separated before use, resulting in 550 ml of upper organic phase and 450 ml of lower aqueous phase. The organic UP was used as the stationary phase and the aqueous LP was used as the mobile phase. The separation was initiated by filling the entire column with the stationary phase using the LC pump, and then loading the HNSA sample dissolved in a mixture of stationary and aqueous phases in the ratio of 1:1 (e.g., 8 ml:8 ml for a 550 mg sample portion or 16 ml:16 ml for a 1.0 g sample portion; less than these volumes would not result in two phases) using a syringe. The mobile phase was then pumped into the column at 2 ml/min while the column was rotated at 850 rev./min in head to tail elution mode. The effluent was monitored with a UV detector at 275 nm (the recorder was set at 20 min/cm) and collected in fractions (4 ml/tube) using a fraction collector. The fractions collected were analyzed using HPLC..

2.3. High-performance liquid chromatography

Analytical reversed-phase HPLC was performed with a Waters Alliance 2690 Separation Module (Waters, Milford, MA, USA). The eluents were 0.1M aqueous NH_4OAc and methanol. The column (Hypersil MOS-1 RPC-8, 5 μm particle size, 250×4.6 mm I.D., Keystone Sci., Bellefonte, PA, USA) was eluted by using a linear gradient of 10-100% methanol in 30 min, followed by 100% methanol for 10 min. The column was re-equilibrated with 10% methanol for 10 min. The effluent was monitored with a Waters 996 photodiode array detector set at 240 nm. Other conditions included: flow-rate, 1 ml/min; column temperature, 25°C; injection volume, 20 μl .

A 20 μl aliquot from the CCC collected fractions was diluted to 1 ml with water/methanol (7:3) and filtered through an AUTOVIAL 5 syringeless filter of 0.45- μm pore size (Whatman, Sanford, ME, USA) prior to chromatography. The concentration of the samples analyzed in Fig. 2 and Fig. 3 was 1 mg/ml.

3. Results and discussion

The analytical HPLC analyses of commercially-available HNSA purchased from three different sources are presented in Fig. 2. These analyses show that the main component (HNSA) is accompanied by impurities that vary in level and/or identity across batches. The present work involved the batch that is represented in Fig. 2B. The UV (275 nm) chromatogram of the eluate obtained from the HSCCC separation of 0.55 g test portion of this material is shown in the lower part of Fig. 3. The separation was completed in ~ 3h. The solvent front (first fraction containing mobile phase) emerged at fraction 53, and the retention of the stationary phase, measured after the separation, was 15.6% of the total column volume. The retention was lower than the calculated value for this separation due to carry over in some of the collected fractions. The UV chromatogram obtained consisted of two major peaks. The eluates corresponding to these peaks were collected in fractions 54 – 66 and 71 – 90. Fractions 54 – 66 (hatched area on the left in Fig. 3B) contained the two major impurities contaminating the commercial HNSA. Fractions 71 – 90 (hatched area on the right in Fig. 3B) contained a single component whose HPLC peak (Fig. 3B) corresponded to the retention time of HNSA in Fig. 3A. The elution volume of a compound with K in the vicinity of 1 is expected to occur at fraction 81, which is the middle of the collected peak. The HNSA isolated from these combined fractions (0.34 g, white amorphous material) was over 99% pure by HPLC at 240 nm, Fig. 3B, and elemental analysis (Complete Analysis Laboratories, Inc., Parsippany, NJ). The purified HNSA was characterized by elemental analysis [found (%) C 49.03, H 3.06, S 12.86; calculated (%) for $\text{C}_{10}\text{H}_7\text{O}_4\text{SNa}$, C 48.78, H 2.87, S 13.02] and negative-ion ESI high-resolution MS (measured mass for the ion $[\text{M}-\text{H}]^-$ m/z 223.00629, calculated for $\text{C}_{10}\text{H}_7\text{O}_4\text{S}$ 223.00705) and its $K_{UP/LP}$

value is 1.19 (by HPLC) in the solvent system used. In another experiment, the separation of 1.0 g of the material was performed to demonstrate that purification of larger quantities of “practical” grade HNSA is possible by this method. The separation resulted in 0.49 g of over 99% pure HNSA. To demonstrate the generality of this method, a portion (0.53 g) of the more contaminated commercial batch shown in Fig. 2A was purified, resulting in HNSA of over 98% (by HPLC, not shown).

4. Conclusion

It has been shown that high-speed counter-current chromatography is an effective preparative method for separating HNSA from the impurities in a batch of commercial HNSA. The present method could readily be extended to obtain high-purity HNSA from other commercially-available batches.

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Figure 1.
Preparation of 4-hydroxy-1-naphthalenesulfonic acid sodium salt (HNSA).

Figure 2.
Analytical HPLC of commercial sample portions of HNSA obtained from three different sources.

Figure 3.

Preparative purification of "practical" grade HNSA by high-speed CCC. (A) HPLC analysis of the original material, (B) high-speed counter-current chromatogram of the separation of a 0.55-g portion of material and HPLC analyses of the separated components.