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Capillary zone electrophoresis of hydroxynaphthalenecarboxylic acids
Purity monitoring of β-hydroxynaphthoic acid in industry

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Abstract

A new capillary zone electrophoretic procedure has been developed for purity monitoring of 2-hydroxy-3-naphthalenecarboxylic acid as an important intermediate in the dyestuff and pharmaceutical industries. 2-Hydroxy-1-naphthalenecarboxylic, 2-hydroxy-6-naphthalenecarboxylic and 2-hydroxy-3,6-naphthalenedicarboxylic acids, β-naphthol and 2,2′-dihydroxy-1,1′-dinaphthyl were separated and determined as impurities at 0.02% relative levels. An uncoated capillary of 70 cm×75 μm I.D. was used. 20 m*M* Boric acid+20 m*M* borate of pH 9.2 was the background electrolyte. The CZE results were compared with those obtained by thin-layer chromatography.

Keywords: Hydroxynapthalenecarboxylic acids; Carboxylic acids

1. Introduction

Capillary zone electrophoresis (CZE) has become an important analytical tool for the determination of almost any kind of substance such as amino acids, proteins, drugs and single ions [1,2]. CZE has a great potential to be used also within the field of dyestuff chemistry for the analysis of dyes, their intermediate precursors and for product purity control. It is rapidly becoming an alternative, and in many cases a superior, technique to conventional methods of dye analysis such as HPLC [3]. From many applications of CZE for the determination of dyes and other compounds employed in the dye-manufacturing and dye-using industries we can refer, e.g., to [3–9].

β-Naphthoic acid (2-hydroxy-3-naphthalenecarboxylic acid, BON-acid) belongs to important precursors for the synthesis of dyestuffs and drugs. Moreover, the aqueous solution of its sodium salt has been used to solubilize riboflavin (vitamin B_2) [10]. BON-acid is obtained by the action of CO_2 on β-naphthol (βN) under pressure and at 280–290°C [10]. The reaction course can be followed determining unreacted βN, cf. scheme in Fig. 1.

Checking the quality of BON-acid products requires the determination of the starting compounds and also the other side-products formed during the synthesis. The side products are the two positional isomers of BON-acid (2-hydroxy-1-naphthalenecarboxylic acid, 2-hydroxy-6-naphthalenecarboxylic acid), 2-hydroxy-3,6-naphthalenedicarboxylic acid and the dimer of βN (2,2′-dihydroxy-1,1′-dinaphtyl).

Thin-layer chromatography (TLC) on silica gel plates has up to now been the most common method used for the purity check of the BON-acid quality [11]. On the other hand, CZE is a rapid method with high resolution efficiency. Electrophoretic mobility

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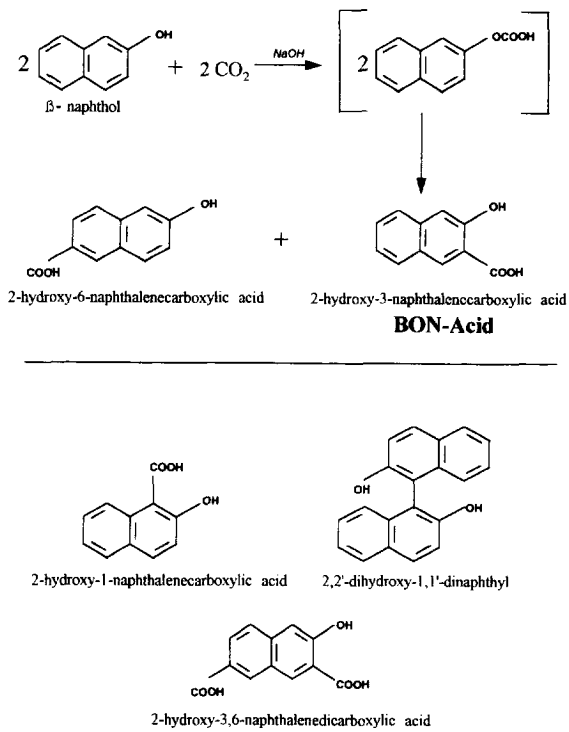

Fig. 1. Scheme of BON-acid synthesis and structures of various derivatives.

depends, besides many other factors, on the ratio of the net charge to the molecular mass and on the structure of the molecule, pK_a values, etc. Thus, for example, in the appropriate buffer solution there is even a possibility to separate molecules with the same charge and the same molecular mass but with a different position of the carboxyl-group in the molecule.

The aim of this work was to study the electrophoretic migration behavior of hydroxynaphthalenecarboxylic acids and to work out a CZE procedure for the determination of impurities in BON-acid final products.

2. Experimental

2.1. Chemicals

2-Hydroxy-1-naphthalenecarboxylic acid (21HN), 2-hydroxy-3-naphthalenecarboxylic acid (BON-acid, 23HN), 2-hydroxy-6-naphthalenecarboxylic acid (26HN), 2-hydroxy-3,6-naphthalene dicarboxylic acid (236HN), β-naphthol (βN) and 2,2′-dihydroxy-1,1′-dinaphthyl (DN) were obtained from Spolchemie (Ústí nad Labem, Czech Republic).

Solutions of the BON-acid samples were prepared by dissolving 50 mg samples in water with addition of 1 ml 1 *M* NaOH and filling up to 25 ml in a measuring flask. The solutions were subjected to sonication using an ultrasonic cleaner (Branson, USA). The solutions were prepared daily.

Sodium tetraborate (borate), boric acid and sodium hydroxide were of analytical-grade purity (Lachema, Brno, Czech Republic). Mesityl oxide was from Fluka (Buchs, Switzerland).

Double distilled water from a quartz still (Heraeus, Hanau, Germany) was used for the preparation of the solutions used in this study.

2.2. Apparatus and conditions

Electrophoretic measurements were performed using SpectraPhoresis 2000 (Thermo Bioanalysis, CA, USA) using an uncoated fused-silica capillary, 70 cm (length up to detector 62.3 cm)×75 μm I.D. (Avery Dennison, MA, USA), applied voltage 25 kV, temperature of 25°C. Detection at 230 nm and/or high speed scan of the spectra was used throughout the work. The samples were injected by hydrodynamic injection (5 s), using a vacuum 1.5 p.s.i. relative to ambient pressure; 1 p.s.i.=6894.76 Pa.

Prior to use the capillary was washed for 5 min with 1 *M* NaOH at 60°C, 5 min with 0.1 *M* NaOH at 60°C and then 10 min with water at 30°C. Finally, the capillary was rinsed for 10 min with the background electrolyte (BGE) at 25°C. Before each measurement, the capillary was washed with the working electrolyte.

Buffer solutions were filtered through glass crucible S4 filters (Cavalier, Czech Republic) and degassed before the use. The pH was measured using PHM 64 (Radiometer, Copenhagen, Denmark).

Thin-layer chromatography was performed on TLC pre-coated silica gel 60 F_{254} plates (Merck, Germany), 20×20 cm, layer thickness 0.25 mm. Chloroform–methanol–acetic acid (50:20:1) was used as the mobile phase.

3. Results and discussion

3.1. Optimization

We started our research for optimum analytical conditions by comparing the single injections of BON-acid and the pure derivatives at pH 9.3 in 10 m*M* borate+10 m*M* boric acid electrolyte buffer. We decided to try these buffer conditions based on our previous work [12] reporting the separation of α- and β-naphthol.

A standard mixture (model sample) of all components suggested to be present in industrial BON-acid products was prepared from pure components. The model sample containing all the derivatives of hydroxynaphthalenecarboxylic acids, i.e., 21HN, 26HN, 236HN, βN, DN and the target compound BON-acid was used to search the best conditions for the separation of individual components.

Several other buffers were tried to reach the separation. BGE such as phosphate and germanate, showed noisy results, low sensitivity and poor reproducibility for CZE separation of the samples tested. On the other hand, borate–boric acid buffer (10 m*M* boric acid+10 m*M* borate) of pH 9.2 was found useful for the separation of naphthalenecarboxylic acids, only βN and DN were badly resolved. Increasing the concentrations of both components of the buffer up to 20 m*M*, achieved the separation of βN and DN (Fig. 2).

Thus, as optimal CZE conditions, BGE containing 20 m*M* boric acid+20 m*M* tetraborate at pH 9.2; voltage of 25 kV and temperature of 25°C was recommended.

3.2. Thin-layer chromatography

TLC is often applied as the industrial controlling procedure for the synthesis of BON-acid. Results of TLC analysis (R_F values) of BON-acid BASF (BON-B) separation are shown in Table 1. The separated spots were extracted and analysed by CZE under the optimal conditions in order to compare these result with those obtained with the model sample. It was observed that in TLC separation BON-acid was tailing, that is why the BON-acid was also detected in the separated spots of 236HN, A and B.

Comparing the results of these two methods, peaks of some other impurities were found by CZE in addition to those obtained by TLC. Moreover, CZE is a faster method to separate all the compounds present in the reaction mixture and a more easily

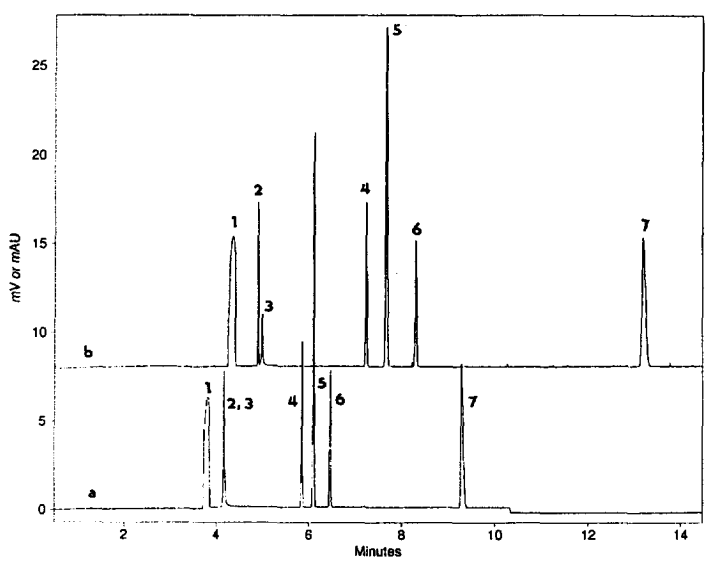

Fig. 2. Separation of the model sample: (1) mesityl oxide 0.1% (v/v), (2) βN, (3) DN, (4) 21HN, (5) BON-acid, (6) 26HN, (7) 236HN. CE conditions: pH 9.2, injection time 5 s, voltage 25 kV, temperature 25°C. Curves: (a) BGE: 10 m*M* boric acid+10 m*M* borate, (b) BGE: 20 m*M* boric acid+20 m*M* borate.

Table 1
TLC R_F values of BON-acid and its impurities

Substance	A[a]	B[a]	236HN	BON-acid	21HN	26HN	βN	DN
R_F	0.1	0.125	0.219	0.419	0.519	0.637	0.731	0.750

Conditions: pre-coated silica gel 60 F_{254} plates, chloroform–methanol–acetic acid (50:20:1).
[a] Unknown spots.

quantification of the components is possible (see Section 3.3).

3.3. Real sample analysis

Under the optimal conditions the analysis of two real industrial samples of BON-acid products was performed (Fig. 3). Some unknown peaks were also found. The quantity of the known impurities was determined by the standard addition method. The results from the determination of impurities for the BON-acid samples are given in Table 2. The detection limits for the impurities (Table 3) were calculated with a signal-to-noise ratio (S/N) equal to three.

The analysis of recrystallized BON-acid prepared in the industrial laboratory has shown (Fig. 4b) that DN and βN are not present. Unknown substances with the migration time around 7 min were also no more present. On the other hand, the other impurities

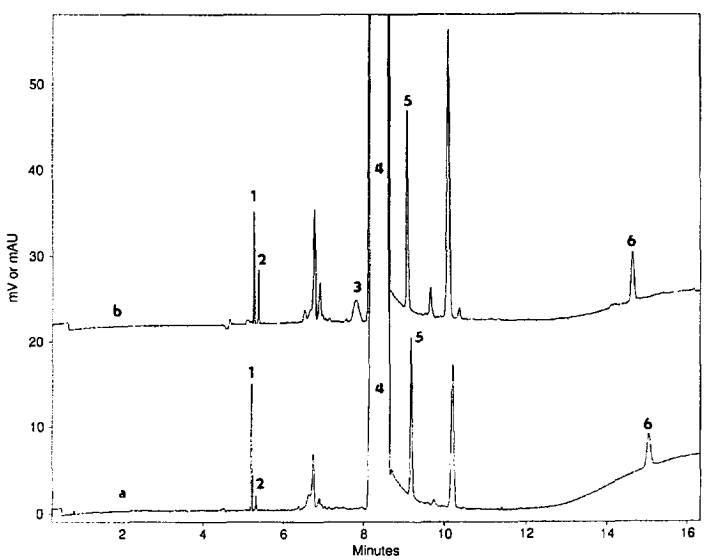

Fig. 3. Analysis of BON-acid industrial products (comparison of various BON-acid samples). (1) βN, (2) DN, (3) 21HN, (4) BON-acid, (5) 26HN, (6) 236HN. CE conditions: BGE 20 mM boric acid+20 mM borate, pH 9.2; temperature 25°C, voltage 25 kV. Curves: (a) BON-B, (b) BON-P.

Table 2
Results of CZE analysis: content of impurities (% relative) in BON-acid products

Substance	βN	DN	21HN	26HN	236HN
BON-B	0.15±0.02	0.02±0.02	–[a]	0.17±0.07	0.15±0.03
BON-P	0.22±0.02	0.08±0.04	0.02±0.02	0.53±0.12	0.33±0.08

[a] No peak observed.

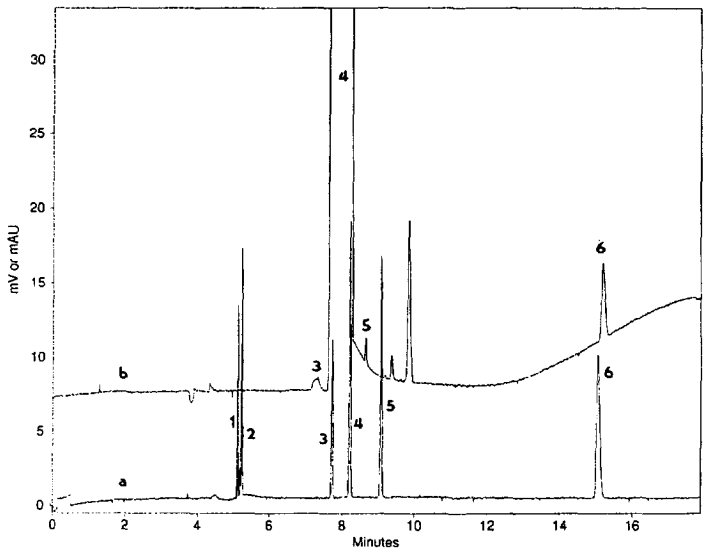

Fig. 4. Comparison of recrystallized BON-acid and model sample electropherograms. (1) βN, (2) DN, (3) 21HN, (4) BON-acid, (5) 26HN, (6) 236HN. CE conditions: BGE 20 m*M* boric acid+20 m*M* borate, pH 9.2; temperature 25°C, voltage 25 kV. Curves: (a) model sample, (b) recrystallized BON-acid.

(migration time 9–10 min) were still present. The identification of the unidentified compounds is continuing. One of these unidentified peaks probably could be the dimer of BON-acid.

4. Conclusions

A procedure to determine the purity of BON-acid by CZE was developed. Lower analysis time was reached with CZE than those reported by TLC. Using the CZE procedure it was possible to follow the synthesis and purification done by the industry. Even in the recrystallized sample, impurities are present. The identification of these two unknown substances is under investigation.

Table 3
Detection limits of BON-acid impurities determined by CZE

Substance	Detection limit (μg/ml±S.D.)	Detection limit (% relative)
βN	0.044±0.006	0.002
DN	0.056±0.009	0.003
21HN	0.045±0.010	0.002
26HN	0.033±0.008	0.016
236HN	0.042±0.004	0.021

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