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β-Hydroxynaphthoic acid synthesis monitoring by capillary zone electrophoresis and high-performance liquid chromatography Determination of polynaphthyl derivatives

Alma L. Revilla[a], Milan Vrchlabský[a], Otakar Humpa[b], Josef Havel[a,*]

[a]*Department of Analytical Chemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic*
[b]*Department of Organic Chemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic*

Abstract

Unidentified peaks were observed during the purity control of β-hydroxynaphthoic acid industrial samples using capillary zone electrophoresis (CZE) and HPLC methods. The origin of such peaks was supposed to be (1) a consequence of interaction between separated compounds and borate species, which might cause peak splitting, or (2) other side-products formed during the synthesis, such as polynaphthyl derivatives of β-hydroxynaphthoic acid and/or others. Both hypotheses were studied using CZE, HPLC, and TLC methods. The synthesis of 2,2′-dihydroxy-1,1′-binaphthyl-3,3′-dicarboxylic acid was done. The purified compound was use as standard to identify the unknown impurities in β-hydroxynaphthoic acid samples. To control the quality of the standard, NMR, HPLC and CZE were applied. It has been proved that the unidentified impurities were other sub-products from the synthesis and the formation of 2,2′-dihydroxy-1,1′-binaphthyl-3,3′-dicarboxylic acid was verified.

Keywords: Process monitoring; Hydroxynaphthoic acids; Carboxylic acids

1. Introduction

β-Hydroxynaphthoic acid (2-hydroxy-3-naphthalenecarboxylic acid, BON-acid) is obtained by the action of CO_2 on β-naphthol (βN) under pressure and at 280–290°C [1]. Checking the quality of BON-acid products requires the determination of the starting compound (βN) and also the other side-products formed during the synthesis. Known side products are: 2-hydroxy-1-naphthalenecarboxylic acid (21HN), 2-hydroxy-6-naphthalenecarboxylic acid (26HN), 2-hydroxy-3,6-naphthalenedicarboxylic acid (236HN) and 2,2′-dihydroxy-1,1′-dinaphtyl (DN).

In our previous work [2], a capillary zone electrophoresis (CZE) procedure for purity monitoring of BON-acid industrial samples was reported. We have found, however, that some of the samples contain, besides the expected side-products, also other unknown compounds (Fig. 1).

In this work, in order to determine the nature of the unknown peaks found in the previous work [2], two possibilities were taken into consideration: (1) the interaction of separated hydroxycarboxylic acids with borate polymeric species, which might be causing some artifacts as peak splitting described recently [3], or (2) the presence of the other side-products, as results of possible interactions between very reactive hydroxy and hydroxycarboxy aromatic compounds formed during the synthesis.

As was already reported [3], the use of borate buffer causes peak splitting of β-naphthol (up to

*Corresponding author.

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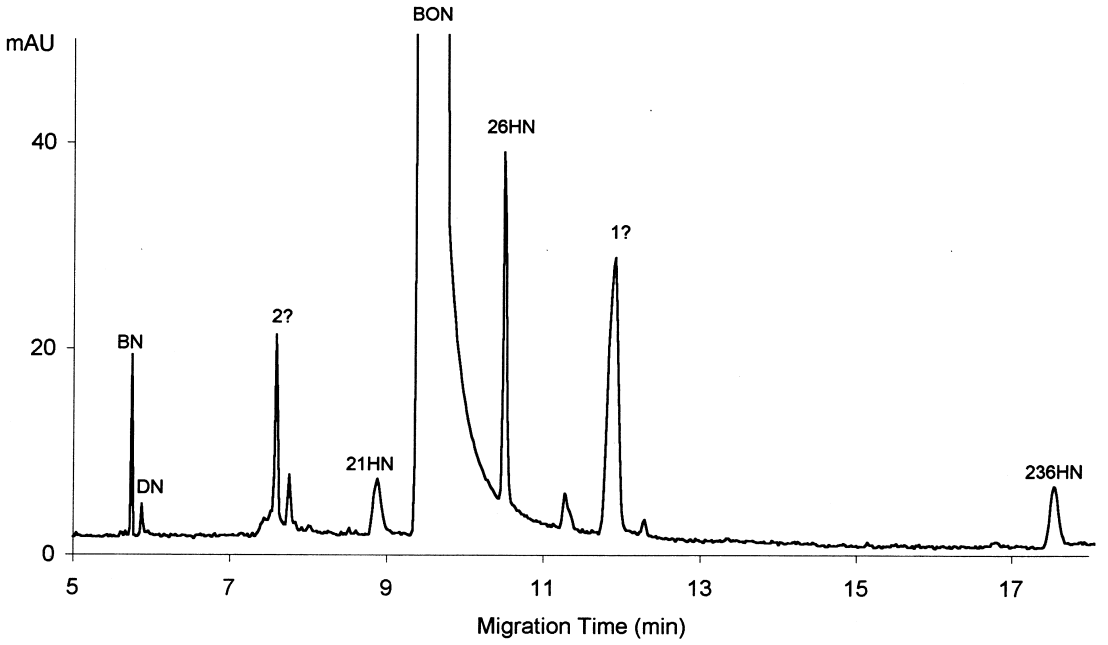


Fig. 1. Electropherogram for BON-acid industrial sample (BON-3 crude product). Sample concentration, 2 mg/ml. CZE conditions: 25 kV, 25°C, 5 s hydrodynamic injection. BGE: 20 m*M* boric acid+20 m*M* sodium tetraborate, pH 9.24. Detection at 230 nm.

three peaks). Because BON-acid and its isomers have structural similarities with βN, the possibility of the interaction between these compounds and borate polymeric species cannot be excluded. The interaction of 2-hydroxy-1-naphthalenecarboxylic acid with borate has already been proved by conductometric measurements [4].

The formation of polynaphthyl derivatives or other side-products seems to be the most probable explanation. The tendency to form dinaphthyl derivatives with the 1,1′ bond between two naphthalene rings was described already in the case of β-naphthol precursor [5–7] and even for β-hydroxynaphthoic acid [8]. It is interesting that 1,1′-binaphthyl-2,2′-diol can exist in a racemic mixture as diasteroisomers [(+)-2,2′-dihydroxy-1,1′-dinaphtyl and (−)-2,2′-dihydroxy-1,1′-dinaphtyl], and the same is true for binuclear derivative 2,2′-dihydroxy-1,1′-binaphthyl-3,3′-dicarboxylic acid (diBON) [8].

Moreover, under certain conditions these binaphthyl derivatives can form oxygen bridges with liberation of water molecules. The synthesis of 1,1′-dinaphthylen-2,2′-monooxide, hydroxydinaphthylen oxide and dinaphthylen dioxide has also been published [9–13]. Another process forming polynaphthyl derivatives was mentioned in Ref. [14], two hydroxyl or carboxyl groups of two BON molecules form a closed ring and γ-naphthoxanton was produced.

Among all these possibilities, the formation of diBON, binuclear derivative, under the reaction conditions for BON-acid synthesis is more probable than the formation of the other compounds. Therefore, the synthesis of diBON was performed and a NMR method was used for the identification and purity control. Comparative CZE, HPLC and TLC studies of a diBON sample and industrial products were done and the content of diBON in BON-acid real samples was determined.

2. Experimental

2.1. Chemicals

21HN, BON-acid, 23HN, 26HN, 236HN and diBON were obtained from Spolchemie (Ústí nad Labem, Czech Republic). βN, DN and organic solvents such as methanol were reagent grade (Merck, Darmstadt, Germany).

Sodium tetraborate, boric acid, sodium hydroxide

and boric acid were of analytical-grade purity (Lachema Brno, Czech Republic). The solution of tetrabutylammonium sulfate (20 m*M*) was prepared mixing tetrabutylammonium hydroxide (40%) and diluted sulfuric acid until pH 7. Double-distilled water from quartz still (Heraeus, Hanau, Germany) was used for the preparation of the solutions used in this study.

2.2. Solutions

Solutions of BON or diBON for CZE method 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 sonification using an ultrasonic cleaner (Branson, USA). The solutions were prepared daily.

Methanolic solutions of diBON and BON were prepared for HPLC in order to obtain a final concentration of 1 mg/ml. Posterior dilutions were done using double-distilled water.

For NMR analysis, the samples were prepared by dissolving 10 mg of the product in 0.5 ml of [$^{2}H_{6}$]dimethyl sulfoxide (DMSO)-d_6.

2.3. Apparatus and conditions

2.3.1. CZE

Electrophoretic measurements were performed using SpectraPhoresis 2000 (Thermo Bioanalysis, CA, USA) using uncoated fused-silica capillary (Avery Dennison, MA, USA), 70 cm (length to detector, 62.3 cm)×75 μm I.D., applied voltage, 25 kV, temperature, 25°C. Detection at 230 nm and/or high speed scan of the spectra were used throughout the work. The samples were injected by hydrodynamic injection (usually 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, 10 min with water and 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 use. The pH was measured using PHM 64 (Radiometer, Copenhagen, Denmark).

2.3.2. HPLC

A microcolumn chromatograph was build up with a high-pressure pump HPP 55001, a flow regulator to keep it in the range of 50–1000 μl/min, a CGC 150×1-mm column filled with Sepharon SGX C_{18} RPS, d_p=5 μm (Tessek, Prague), a UV–Vis detector UVM 6 (Dílny ČSAV, Prague) and a 4-μl cuvette. Isocratic mode (0.05 ml/min flow) was applied using, as mobile phase, methanol–0.03 *M* tetrabutylammonium (TBA) sulfate (65:35).

2.3.3. TLC

Silica gel 60 F_{254} plates (Merck, Germany), 20×20 cm, layer thickness 0.25 mm. A mixture of chloroform, methanol and acetic acid (50:20:1) was used as mobile phase.

2.3.4. NMR

^{1}H-, ^{13}C- and two-dimensional NMR spectra were recorded on a Bruker Avance DRX-500 spectrometer operating at frequencies of 500.13 MHz (^{1}H) and 125.76 MHz (^{13}C), equipped with a 5-mm QNP (H, C, F, P) probehead.

Spectra were measured as DMSO-d_6 solution at a temperature of 313 K. The chemical shifts were referenced to tetramethylsilane (TMS) used as internal standard; δ values are in ppm.

All the ^{1}H and ^{13}C-NMR signals were assigned on the basis of ATP, H, H-COSY, C-COSY, COLOC [15], HSQC [16] and HMBC [17], respectively.

Two-dimensional NMR experiments were carried out on a 5-mm triple-resonance probehead equipped with a gradient coil.

2.4. Synthesis of 2,2′-dihydroxy-1,1′-dinaphthyl-3,3′-dicarboxylic acid

The procedure was described in the literature [8]. A modified method was performed for the synthesis in Spolchemie laboratories:

2.4.1. Procedure

Fifty grams of pure BON acid were dissolved in 700 ml of 1 *M* potassium hydroxide. The solution was warmed up to boiling point and 10% $FeCl_3$ was continuously added (500 ml total). The reaction mixture was kept at 90°C for 6 h. Then it was

alkalized with 300 ml of 2 *M* potassium hydroxide and later $FeCl_3$ precipitate was removed by filtration. The solution was then acidified to pH 3 with hydrochloric acid, and thus binuclear BON-acid (diBON) precipitated. The product was washed with water and dried. The powder was dissolved in methanol, filtered and methanol evaporated to dryness (product designed as DIBON1). The crude product was then recrystallized from glacial acetic acid as purification step (product designed as DIBON2).

Another sample was prepared using the same procedure described above, but using a 1:1 mixture of βN and BON instead of pure BON (product designed as MIXBON).

3. Results and discussion

After the synthesis of binuclear BON (DIBON1 and DIBON2), analytical methods were applied for its characterization and component determination. Also, the presence of unknown components was studied by microcolumn HPLC, TLC and compared with the CZE results.

3.1. Analytical control of diBON synthesis

The synthetic procedure and the quality of diBON product were checked and evaluated using NMR (^1H and ^{13}C), CZE, HPLC and TLC methods.

The sample DIBON1, as the crude product, was analyzed by CZE (Fig. 2a), micro-HPLC (Fig. 3b) and TLC. All three methods showed the presence of three components. The peak of the unreacted BON-acid was identified using standard. In the analysis of the DIBON2 sample only two components were observed, and unreacted BON-acid was also identified (Fig. 3b and d). The second component was later identified as diBON by NMR (see below). The sample DIBON2 was then used as 'standard' for further identifications.

For the sample MIXBON, five components were observed by CZE and HPLC (Figs. 2c and 3c). Three of the components were identified as BON, diBON and DN.

3.1.1. NMR analysis

The already mentioned products of synthesis were analyzed by ^1H and ^{13}C-NMR. Characterization of diBON was done using recrystallized BON-acid (BON-R) as a standard. Fig. 4 shows the structures

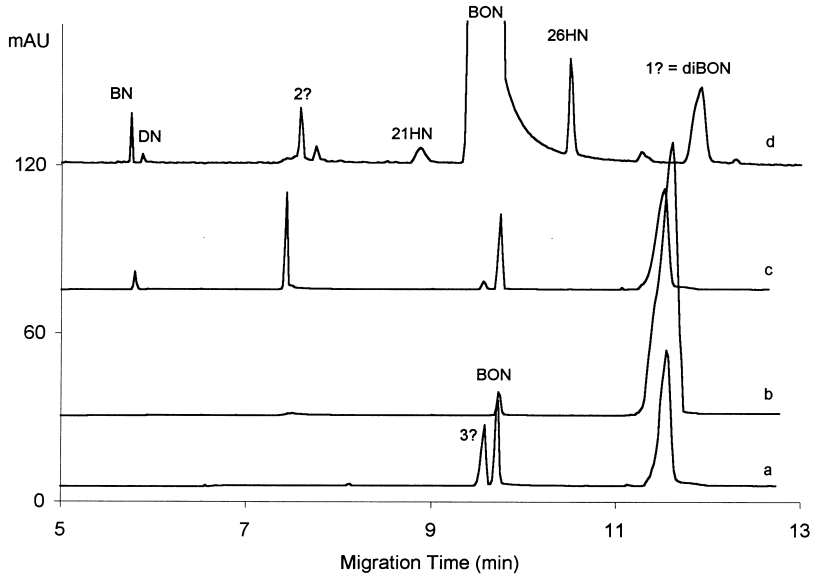


Fig. 2. CZE separation of (a) DIBON1, (b) DIBON2, (c) MIXBON, (d) BON-3. Sample concentration: 0.25 mg/ml. Other conditions as mentioned in Fig. 1.

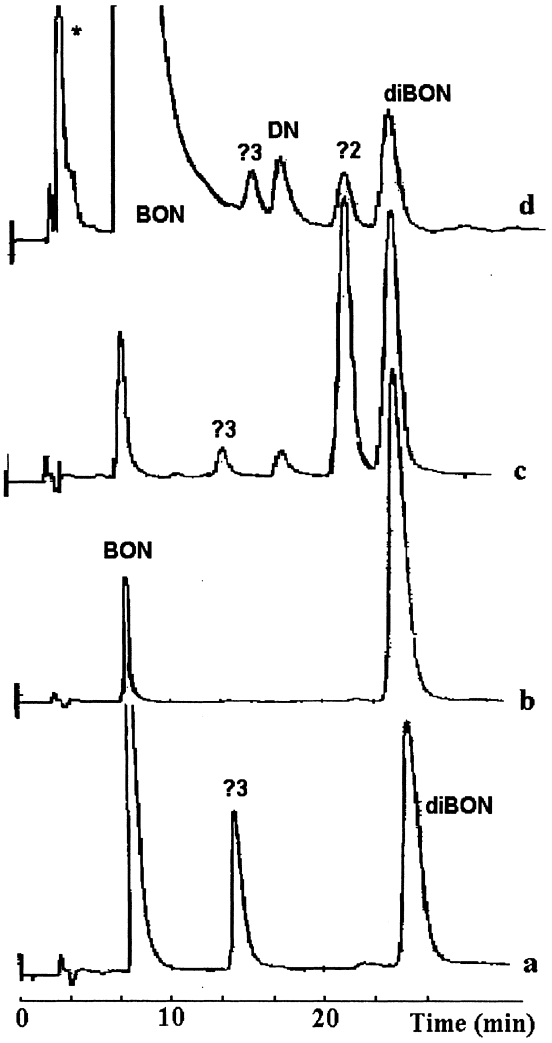


Fig. 3. HPLC separation of (a) DIBON1, (b) DIBON2, (c) MIXBON, (d) BON-3. Sample concentration around 2 mg/ml.

of BON and diBON and the numbering used in NMR analysis, while Fig. 5 shows the NMR spectra for (a) DIBON2, (b) DIBON1 and (c) BON-R samples.

The ^{1}H-NMR spectra show a notable difference between mono- and binuclear BON (Fig. 4). The results obtained from NMR can be summarized as follows:

3.1.2. BON mononuclear

^{1}H-NMR (500 MHz, DMSO-d_6, 313 K, TMS): δ=11.5 (s, *COOH*), 8.56 (s, 1H, *H-4*), 7.97 (d, 1H, *H-5*), 7.77 (d, 1H, *H-8*), 7.55 (m, 1H, *H-7*), 7.36 m, 1H, *H-6*), 7.34 (s, 1H, *H-1*); ^{13}C-NMR (125.7 MHz, DMSO-d_6, 313 K, TMS): δ=171.5 (*COOH*, s), 156.0 (*C-2*, s), 137.2 (*C-8a*, s), 132.5 (*C-4*, d), 129.2 (*C-7*, d), 129.0 (*C-5*, d), 126.7 (*C-4a*, s), 125.9 (*C-8*, d), 123.8 (*C-6*, d), 115.3 (*C-3*, d), 110.8 (*C-1*, d).

3.1.3. BON binuclear

^{1}H-NMR (500 MHz, DMSO-d_6, 313°K, TMS): δ=11.3 (s, *COOH*), 8.76 (s, 2H, *H-4*), 8.08 (m, 2H, *H-5*), 7.36 (m, 4H, *H-6+7*), 7.03 (m, 2H, *H-8*); ^{13}C-NMR (125.7 MHz, DMSO-d_6, 313 K, TMS): δ=172.1, 154.1, 136.5, 132.6, 129.8, 129.2, 126.7, 124.0, 123.6, 116.4, 114.8.

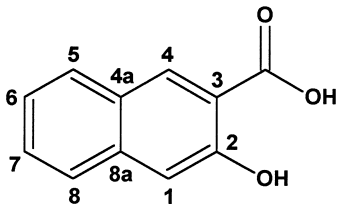
The results from DIBON2 sample confirmed the presence of 7% mononuclear BON and 93% binuclear diBON. For DIBON1 sample, three components were detected. Besides the signal of BON and diBON another frequence was detected.

3.2. Identification of diBON on industrial BON sample

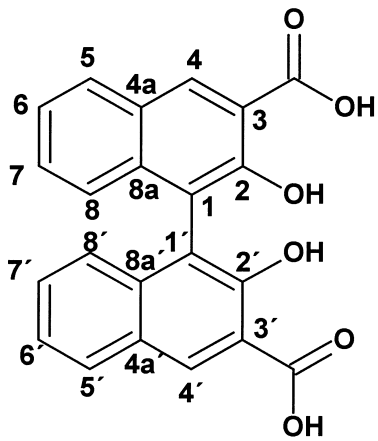
CZE analysis of DIBON2 under the same conditions as those used for BON real sample analysis [2] was done. Hydrodynamic injection (1 s) and high-speed scanning were applied. Comparing the electropherogram of industrial BON (Fig. 2d) and DIBON2 (Fig. 2b), the peaks of mononuclear BON (migration time, t_m=9.7 min) and binuclear diBON (t_m=11.3 min) were identified. The absorption spectra (Fig. 6) corresponding to BON and diBON (obtained via fast-scanning during the electrophoresis), show only small differences between both compounds (λ_{max}. 232 and 235 nm for BON and diBON, respectively).

The analysis of the diBON sample by CZE has proved that the unknown peak 1? from Fig. 1 is certainly diBON. It was not necessary to identify the compound corresponding to peak 2? in Fig. 1 because it was not present after the recrystallization of BON-acid.

Using a mobile phase with great eluent strength (methanol–0.03 *M* TBA, 65:35), binuclear derivative (DN), BON and diBON were identified in the industrial BON sample (Fig. 3d). The other unknown



a



b

Fig. 4. Chemical structures of (a) BON, (b) diBON.

peaks (labelled as 2? and 3?) were also detected. Nevertheless, the other impurities (21HN, 26HN, 236HN and BN) were eluted in this system in a common peak before that of BON.

Comparing CZE and HPLC results, it can be remarked that the resolution of DN from BN was more critical in CZE where the differences in mobilities were rather small. In any case, for diBON and BON, the resolution was similar for both methods.

TLC separation of BON-acid industrial sample and DIBON1 was also done. The presence of BON (R_F=0.419), 26HN (R_F=0.219), 236HN (R_F=0.637), BN (R_F=0.731), DN (R_F=0.750), besides diBON (R_F=0.12) and an unidentified compound (R_F=0.35), was confirmed for BON-acid. Contrary to HPLC results, diBON shows the highest affinity to the polar silica gel, while the other binuclear species (DN) were moving with the front.

3.3. Determination of diBON in BON-acid real samples

As was mentioned above, the DIBON2 preparative could be used as a standard to identify diBON in the industrial samples of BON-acid and, moreover, to quantify later on the content of diBON in those samples. Four industrial samples were evaluated. The latest one (BON-R) was recrystallized in glacial acetic acid trying to remove impurities.

The results from the determination of DN and diBON in the industrial BON-acid samples by CZE are summarized in the Table 1. It should be remarked that BON 1–3 were crude products, and BON-R was recrystallized in glacial acetic acid as purification step.

3.4. Borate: peak splitting

On the other hand, the possible peak splitting of the studied compounds due to borate buffer was also studied, but contrary to peak splitting observed earlier [3], no sign of peak splitting was found in this case. Moreover, HPLC and TLC results confirmed the nature of the unidentified peaks as side-products of the synthesis.

4. Conclusions

The presence of impurities in BON-acid products

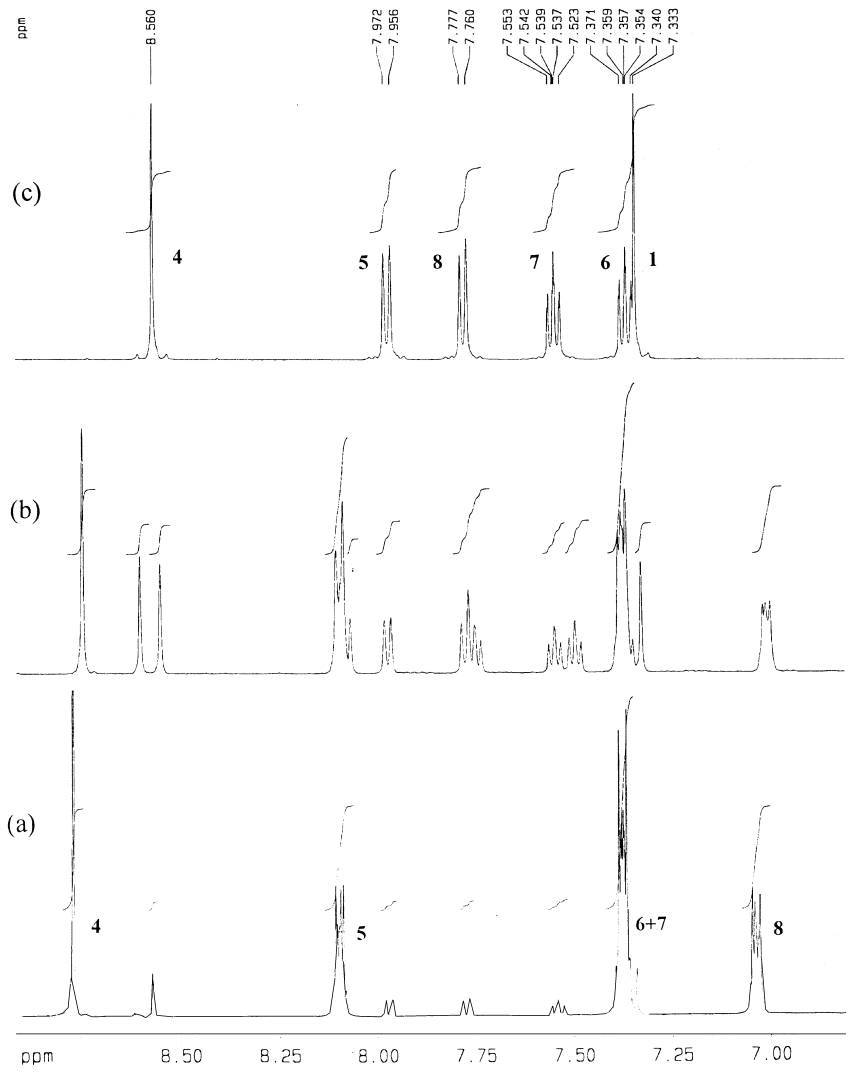


Fig. 5. Comparison of the ^{1}H spectra recorded for (a) DIBON2, (b) DIBON1, (c) BON-R.

was confirmed by TLC and HPLC methods. It was proved that impurities are not related to the use of borate buffer in CZE. The nature of the unknown impurities in BON-acid real samples has been verified after synthesizing diBON compound, and its posterior analysis. Separation and quantification of all the β-hydroxynaphthoic acid impurities is much better by CZE than by using HPLC or TLC methods. NMR was found to be an important tool to check and determine the structure and composition of the synthesized compounds.

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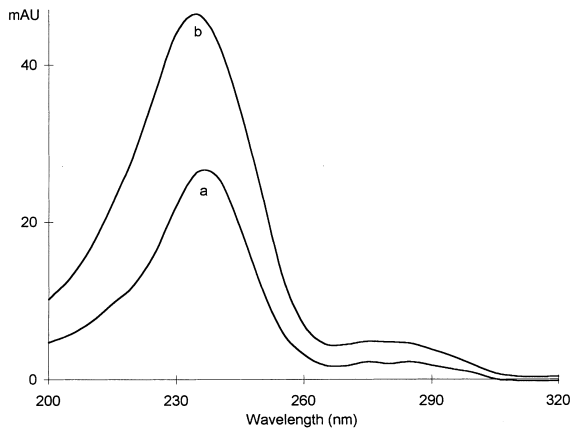

Fig. 6. Absorption spectra for (a) BON, (b) diBON. Spectra measured during electrophoresis experiments. Experimental conditions as mentioned in Fig. 1.

Table 1
Determination of polynaphthyl derivatives in BON-acid industrial samples by CZE

BON Sample	BON-1	BON-2	BON-3	BON-R
diBON (%)	0.98	0.50	0.89	0.25
DN (%)	0.01	0.02	0.06	—

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