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Simultaneous determination of a pyridine-triphenylborane anti-fouling agent and its estimated degradation products using capillary zone electrophoresis

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Abstract

A commercial organoborane compound, pyridine-triphenylborane (PTPB), is often applied to ship hulls as an anti-fouling agent. We developed capillary zone electrophoresis (CZE) with direct UV detection for the simultaneous determination of PTPB and its estimated degradation products: diphenylborinic acid (DPB), phenylboronic acid (MPB), and phenol. The limits of detection (LODs) for PTPB, DPB, MPB, and phenol were, respectively, 25, 30, 50, and 29 μg/l at a signal-to-noise ratio of three. At concentrations of 0.5 mg/l, values of the relative standard deviation (RSD, *n*=6, intra-day) of peak area were obtained, respectively, for PTPB, DPB, MPB, and phenol, as 4.1, 4.1, 4.7, and 3.4% for peak heights 3.6, 3.2, 1.7, and 1.4%, and for migration times 1.1, 1.1, 1.0, and 0.73%. The analytes were detected within 14 min. Simple photodegradation experiments were conducted to verify the usefulness of the proposed method for additional PTPB degradation investigations.

Keywords: Capillary zone electrophoresis; Diphenylborinic acid; Phenol; Phenylboronic acid; Pyridine-triphenylborane

1. Introduction

An anti-fouling agent is usually applied to ship hulls to prevent worsening of fuel consumption rates resulting from the buildup of marine organisms, such as barnacles and bivalves, which become attached to the surfaces of ship hulls. Organotin compounds have been used to date as effective anti-fouling agents. However, the International Maritime Organization (IMO) prohibited, in principle, the use of organotin compounds as anti-fouling agents from September 2008 because they are harmful to non-target marine organisms [1, 2]. More than 20 chemical substances including Sea-Nine 211, Diuron, and Irgarol 1051, have been used as anti-fouling agents in place of organotin compounds [3]. Characteristics of an ideal anti-fouling agent are the following: the efficacy of an anti-fouling agent is long-lasting, and it is easily degradable to compounds that are less toxic to marine organisms shortly after dissolving in seawater. One anti-fouling agent, pyridine-triphenylborane (PTPB), is frequently used in some Asian countries because of its effectiveness [4]. A few reports describe the degradation process of PTPB, its degradation products, and their toxicities to marine organisms [4–6]. However, they remain poorly understood because of the lack of an analytical method for both PTPB and its estimated degradation products. High performance liquid chromatography (HPLC) [7, 8] and HPLC–mass spectrometry (MS) [9] are conventionally used for determination of PTPB. However, no analytical method is available to determine PTPB and its estimated degradation products—diphenylborinic acid (DPB), phenylboronic acid (MPB), and phenol—simultaneously.

This study developed a procedure for simultaneous determination of PTPB, DPB, MPB, and phenol using capillary zone electrophoresis (CZE). The following analytical conditions were examined: effects on analytical performance of rinsing a capillary with 0.1 M sodium hydroxide, applied voltage, pH of the background electrolyte (BGE), wavelength used for detection, and the injection period of a sample solution. Simple degradation experiments using acetonitrile solutions containing PTPB were also conducted to verify the usefulness of the proposed method for additional PTPB degradation investigations. From a practical point of view, in the PTPB degradation experiments, PTPB would be dissolved in seawater and remaining PTPB and its degradation products are extracted using a solid phase extraction procedure. Takahashi *et al.* [8] has already established the extraction procedure. The eluate is finally evaporated to near dryness and the residue is dissolved with acetonitrile containing 1v/v% pyridine. Therefore, we used acetonitrile solutions containing PTPB for the simple degradation experiments. That is to say, PTPB samples dissolved in acetonitrile were put in the open air for 8 days to examine the PTPB degradation. The sample solutions were analyzed using the CZE method established in this study immediately after preparation and then after 8 days. Results show that the concentrations of DPB, MPB, and phenol increased concomitantly with decreased PTPB concentration. Results show that the CZE method is useful to elucidate the PTPB degradation process and its degradation products.

2. Experimental

2.1. Apparatus

The capillary electrophoresis (CE) apparatus used throughout this study had a UV-visible absorbance detector (270A-HT; Perkin-Elmer, Foster City, CA, USA). A polyimide-coated fused-silica capillary electrophoresis column was used (75 μm I.D. × 375 μm O.D; GL Sciences, Tokyo, Japan). The total length of the column (L_{tot}) was 72 cm; its effective length (L_{det}) was 50 cm. The peak area, peak height, and migration time were measured using a chromato-integrator (D-2500; Hitachi, Tokyo, Japan). This experiment used a pH meter (F-22; Horiba, Kyoto, Japan).

2.2. Reagents

All reagents were of analytical reagent grade; they were used as received. Pyridine-triphenylborane (PTPB), diphenylborinic acid (DPB), and phenylboronic acid (MPB) were obtained from Hokko Chemical, Tokyo, Japan. Phenol was obtained from Nacalai Tesque, Kyoto, Japan. The structures of these compounds are presented in Fig. 1. The stock solutions (1000 mg/l) for PTPB, DPB, MPB, and phenol were prepared in acetonitrile. It has been verified that PTPB was unstable not only in water but also in organic solvents, such as acetone, acetonitrile, and methanol [8]. To keep the original concentrations for longer time, 1v/v% pyridine (Nacalai Tesque) was added to the stock solutions for PTPB, DPB, and MPB. The stock solutions were then covered with aluminum foil and kept in a refrigerator to

prevent their degradation. Standard solutions used for the examination of analytical conditions and preparation of the calibration graphs were prepared by subsequent dilution of the stock solutions with acetonitrile. The pH of the background electrolyte (BGE, 20 mM sodium tetraborate) was adjusted using 1 M sodium hydroxide (Nacalai Tesque) or 1 M hydrochloric acid (Wako Pure Chemical Industries, Osaka, Japan). The BGE was filtered through a 0.45 μm membrane filter (Advantec Toyo Kaisha, Tokyo, Japan) before use. Distilled, demineralized water, obtained from an automatic still (WG220; Yamato Kagaku, Tokyo, Japan) and a Simpli Lab-UV high-purity water apparatus (Nihon Millipore, Tokyo, Japan) was used for all experiments.

2.3. Procedure

A new capillary was washed with 1 M sodium hydroxide for 40 min and then with water for 10 min. Before the first analysis of each day, the capillary was washed with water for 5 min, 1 M sodium hydroxide for 5 min, and water for 10 min. The thermostat was maintained at 30°C. The following optimum analytical conditions were established: the capillary was washed with 0.1 M sodium hydroxide for 3 min; then the capillary was filled with BGE by vacuum for 3 min (each step was run automatically); a BGE, 20 mM sodium tetraborate was adjusted to pH 10.0 with 1 M sodium hydroxide; 200 nm detection wavelength; 4 s vacuum （16.9 kPa）injection period of sample (*ca.* 84 nl, the injection period of 1 s corresponds to a sample volume of 21 nl); and 15 kV applied voltage with the sample inlet side as the anode. Calibration graphs were prepared using synthetic

143 standards.

144

145 ## 3. Results and discussion

146

147 *3.1. Method optimization*

148

149 Rinsing the capillary with 0.1 M sodium hydroxide between each run was
150 inferred to have some effect on the reproducibility for peak area, peak height, and
151 migration time for the objective compounds. To assess that effect, a standard
152 solution containing 10 mg/l MPB was analyzed using a BGE (pH 10.5). When the
153 capillary was not rinsed, values of the relative standard deviation (RSD, n=4) of
154 peak area, peak height, and migration time were 4.4, 4.3, and 0.95%, respectively.
155 When the capillary was rinsed, the RSDs were improved to 1.8, 0.79, and 0.61%,
156 respectively. For that reason, the capillary was rinsed with 0.1 M sodium hydroxide
157 for 3 min between runs in subsequent experiments.

158 The effects of applied voltage on peak area, peak height, and migration time for
159 MPB were investigated using applied voltages of 10, 15, and 20 kV. Both the peak
160 area and migration time for MPB decreased concomitantly with increasing applied
161 voltage, whereas the peak height increased concomitantly with increasing applied
162 voltage up to 15 kV. When the applied voltage was 20 kV, the peak height was
163 almost identical as that for 15 kV. The unstable baseline with higher noise was also
164 obtained when the applied voltage was 20 kV. Considering the shorter analysis time,
165 the higher peak height, and the lower baseline noise, 15 kV was adopted as the
166 optimal voltage for determination of MPB and other compounds.

The pH of BGE, 20 mM sodium tetraborate, was varied between 8.5–11.0 using 1 M hydrochloric acid or 1 M sodium hydroxide. Standard solutions containing 5.0 mg/l PTPB, DPB, MPB, or phenol and those mixtures were analyzed to examine the effects of pH of the BGE on effective mobilities. The results are presented in Fig. 2. Effective mobility for MPB increased concomitantly with increased pH up to pH 11.0. Under a basic condition, MPB changes to an anion type because of the coordination of hydroxide ion to boron [10]. Effective mobilities for PTPB and DPB were almost constant although the former was smaller than the latter. The structures of PTPB, DPB, and MPB resemble that of boric acid (pK_a=9.23), as portrayed in Fig. 1. Boric acid, a weak monobasic acid whose boron acts as a Lewis acid, takes hydroxide ions from water [11]. Probably, the boron took hydroxide ions from water in the cases of PTPB and DPB, similarly to boric acid and MPB. As suggested by Hanada *et al.* [9], the chemical form of PTPB in water is supposed to be mainly triphenylboran after liberating pyridine, although commercial triphenylboran compounds are produced as pyridinyl complexes. On the other hand, the hydrogen of phenol (pK_a=10.0) dissociates with increased pH. The electrophoretic mobility of phenol increased because of the higher degree of dissociation. The increase of the electrophoretic mobility caused the longer migration time, because the electrophoretic migration direction of PTPB, DPB, MPB, and phenol is anodic and opposite to the EOF direction (cathodic). When the pH was 10.0, all analytes were separated completely with shorter time. Therefore, pH 10.0 was adopted as the optimal pH of the BGE.

The effects of wavelength on peak height and the signal-to-noise ratios for PTPB, DPB, MPB, and phenol were studied for wavelengths of 190–220 nm. A

191 standard solution containing 1.0 mg/l PTPB, DPB, MPB, and phenol was analyzed.
192 The peak heights for all compounds decreased with increased wavelength; the
193 signal-to-noise ratios for all compounds had maximum values at 200 nm. Therefore,
194 200 nm was adopted as the wavelength for determination of PTPB, DPB, MPB, and
195 phenol.

196 The injection period for the sample solution was varied: 1–5 s (21–105 nl).
197 Peak heights for PTPB, DPB, MPB, and phenol increased linearly, concomitantly
198 with increasing injection periods up to 4 s; then the increasing rate of the peak
199 heights decreased with increased injection periods up to 5 s. Consequently, the
200 optimal injection period for a sample solution was inferred as 4 s. Figure 3 (B)
201 depicts an electropherogram of the standard solution containing 0.4 mg/l PTPB (b
202 in Fig. 3), DPB (c), MPB (d), and phenol (a). All compounds were well separated
203 and detected within 14 min. Figure 3 (A) is an electropherogram of 0.0012v/v%
204 pyridine in acetonitrile. The concentration of pyridine in the standard solution in
205 Fig. 3 (B) was 0.0012v/v%. No peak was observed around the area where the
206 analytes would be observed.

207

208 *3.2. Method validation*

209

210 Calibration graphs for PTPB, DPB, MPB, and phenol were linear using both
211 the peak area and peak height. Regression equations relating the area response to
212 concentration for PTPB, DPB, MPB, and phenol (x, 0–5.0 mg/l) were $y = 2.86 \times$
213 $10^4x + 2.59 \times 10^3$ (correlation coefficient, 0.9994), $y = 3.04 \times 10^4x + 7.32 \times 10^2$
214 (0.9997), $y = 1.65 \times 10^4x + 5.98 \times 10^2$ (0.9998), and $y = 2.73 \times 10^4x + 6.40 \times 10^2$

(0.9997), respectively; those relating the respective peak heights were $y = 5.09 \times 10^3 x + 1.50 \times 10^2$ (0.9998), $y = 5.70 \times 10^3 x + 1.91 \times 10^1$ (0.9999), $y = 2.96 \times 10^3 x + 4.34 \times 10^1$ (0.9999), and $y = 5.32 \times 10^3 x + 1.91 \times 10^2$ (0.9995). Table 1 presents the RSDs and limits of detection (LODs) for PTPB, DPB, MPB, and phenol using the proposed method. The RSDs (intra-day) of peak area were obtained, respectively, for PTPB, DPB, MPB, and phenol, as 3.4–4.7%, and for peak heights 1.4–3.6%, and for migration times 0.73–1.1%. The RSDs (inter-day) of peak area were obtained, respectively, as 4.4–9.1%, and for peak heights 3.7–7.2%, and for migration times 0.93–0.94%. The LOD (25 μg/l) for PTPB obtained using our method was superior to the LOD (810 μg/l) used in the HPLC method by Oda et al. [7]. It is approximately equal to the LOD (12 μg/l) in the HPLC-MS method by Hanada et al. [9]. In addition, the determination limit in the HPLC method used by Takahashi et al. [8] was 50 μg/l. The LOD for PTPB in our method is presumably similar to the LOD for their method, although the LOD was not shown in their paper.

3.3. Applications

A simple degradation experiment was conducted to verify the usefulness of the proposed method for additional PTPB degradation investigations. That is to say, an acetonitrile solution containing 3.0 mg/l PTPB in a test tube with a screw cap was put in the open air. The sample solution was analyzed using the proposed method after 8 days. Figures 4(A) and 4(B) respectively depict electropherograms of the sample solutions immediately after preparation and then after 8 days. A high peak

239 corresponding to PTPB (b in Fig. 4 (A)) and low peaks corresponding to phenol (a)
240 and DPBP (c) were observed immediately after preparation. After 8 days, 33% of
241 PTPB (b in Fig. 4 (B)) was degraded and higher peaks for phenol (a) and DPBP (c)
242 were observed; a low peak of MPB (d) was also observed. These results suggest
243 that the proposed method is a useful tool to elucidate the degradation process of
244 PTPB and its degradation products. Later we performed similar experiments for
245 much longer period with different environmental conditions [12].
246

247 ## 4. Conclusions

248

249 We developed a CZE method with direct UV detection for simultaneous
250 determination of PTPB and its estimated degradation products such as
251 diphenylborinic acid (DPB), phenylboronic acid (MPB), and phenol. The proposed
252 method is a promising means to elucidate the degradation process of PTPB and its
253 degradation products. Additional improvement of the LODs is desirable for lower
254 concentrations of these compounds to make the method more useful. It is
255 worthwhile to examine other sample injection methods—e.g. electrokinetic sample
256 injection methods—to improve the LODs for these compounds.
257

258 **Acknowledgements**

259

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286 Fig. 1. Structures of pyridine-triphenylborane (PTPB), diphenylborinic acid (DPB),
287 phenylboronic acid (MPB), and phenol: (A) PTPB, (B) DPB, (C) MPB, (D) phenol.
288
289 Fig. 2. Effect of pH of background electrolyte on effective mobility for
290 pyridine-triphenylborane (PTPB), diphenylborinic acid (DPB), phenylboronic acid
291 (MPB), and phenol: ○, PTPB; △, DPB; □, MPB; ▽, phenol. The capillary was
292 pre-rinsed with 0.1 M NaOH for 3 min between runs. Electrophoretic conditions:
293 capillary, L_{tot}=72 cm, L_{det}=50 cm, 75 μm I.D. × 375 μm O.D.; BGE, 20 mM sodium
294 tetraborate adjusted to pH 8.5–11 with 1 M hydrochloric acid or 1 M sodium
295 hydroxide; voltage, 15 kV with the sample inlet side as the anode; wavelength for
296 detection, 210 nm. Sample, 5.0 mg/l PTPB, DPB, MPB, or phenol in acetonitrile;
297 vacuum injection period, 3 s (63 nl).
298
299 Fig. 3. Electropherograms of acetonitrile with pyridine and a standard solution of
300 the mixture of pyridine-triphenylborane (PTPB), diphenylborinic acid (DPB),
301 phenylboronic acid (MPB), and phenol. (A) 0.0012v/v% pyridine in acetonitrile.
302 (B) A standard solution containing 0.4 mg/l PTPB, DPB, MPB, and phenol. Peaks:
303 a=phenol, b=PTPB, c=DPB, d=MPB. Electrophoretic conditions: BGE, 20 mM
304 sodium tetraborate adjusted to pH 10.0 with 1 M sodium hydroxide; wavelength for
305 detection, 200 nm. Vacuum injection period for the sample, 4 s (84 nl). Other
306 electrophoretic conditions those presented in Fig. 2.
307
308 Fig. 4. Electropherograms obtained in the degradation experiment for
309 pyridine-triphenylborane (PTPB) in the open air. (A) Sample, 3.0 mg/l PTPB in

310 acetonitrile immediately after prepparation. (B) Sample, after 8 days.

311 Electrophoretic conditions and identification of peaks are those depicted in Fig. 3.

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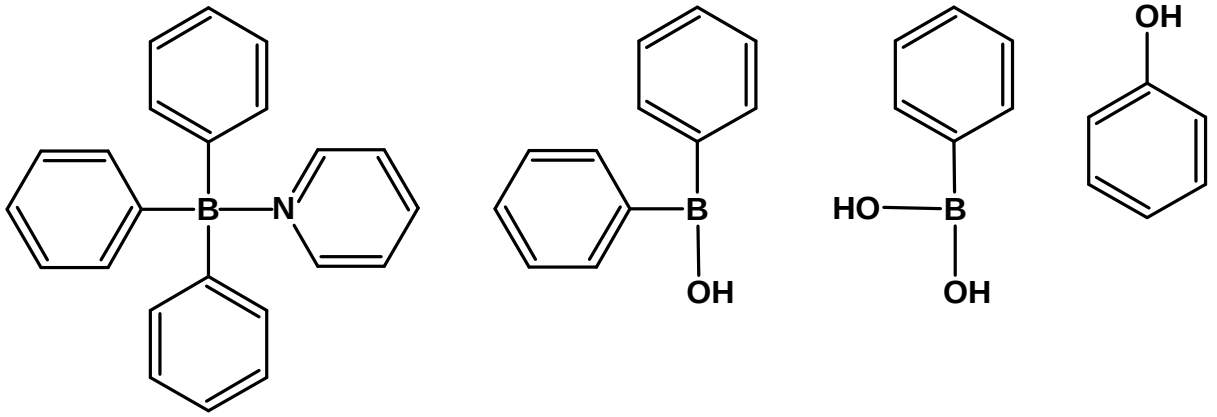

(A) **(B)** **(C)** **(D)**

Fig. 1

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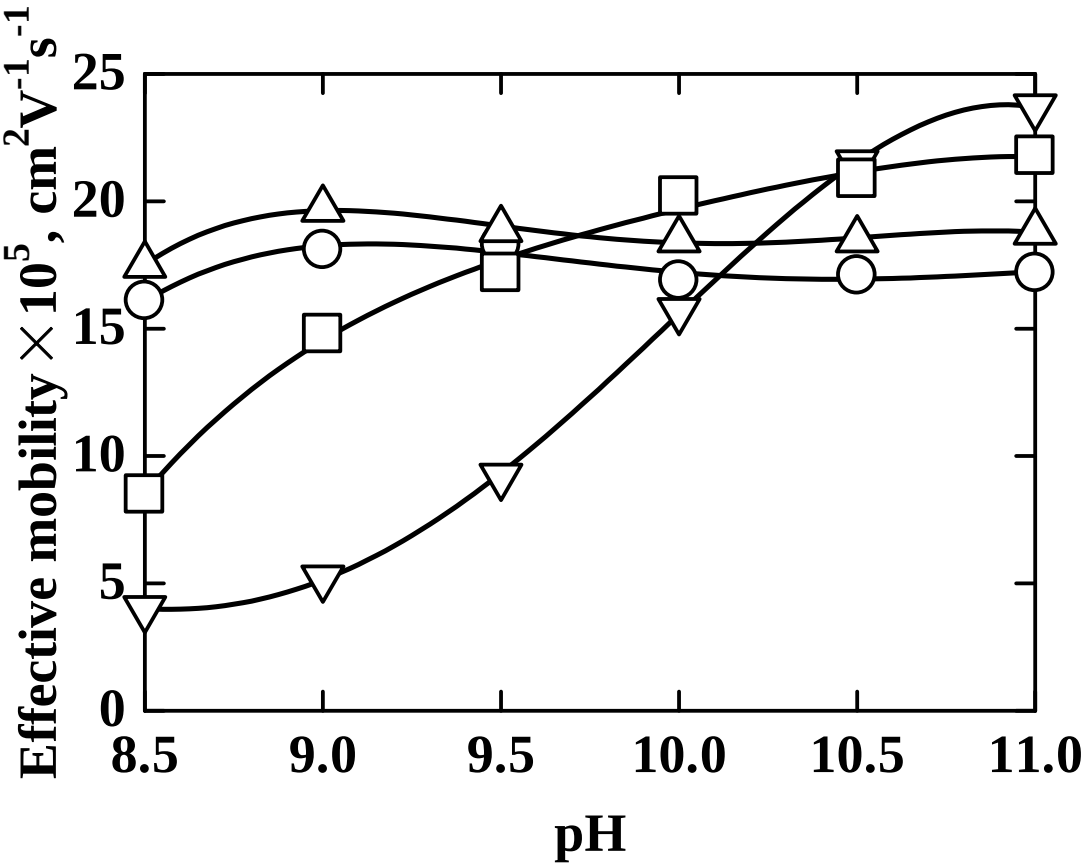
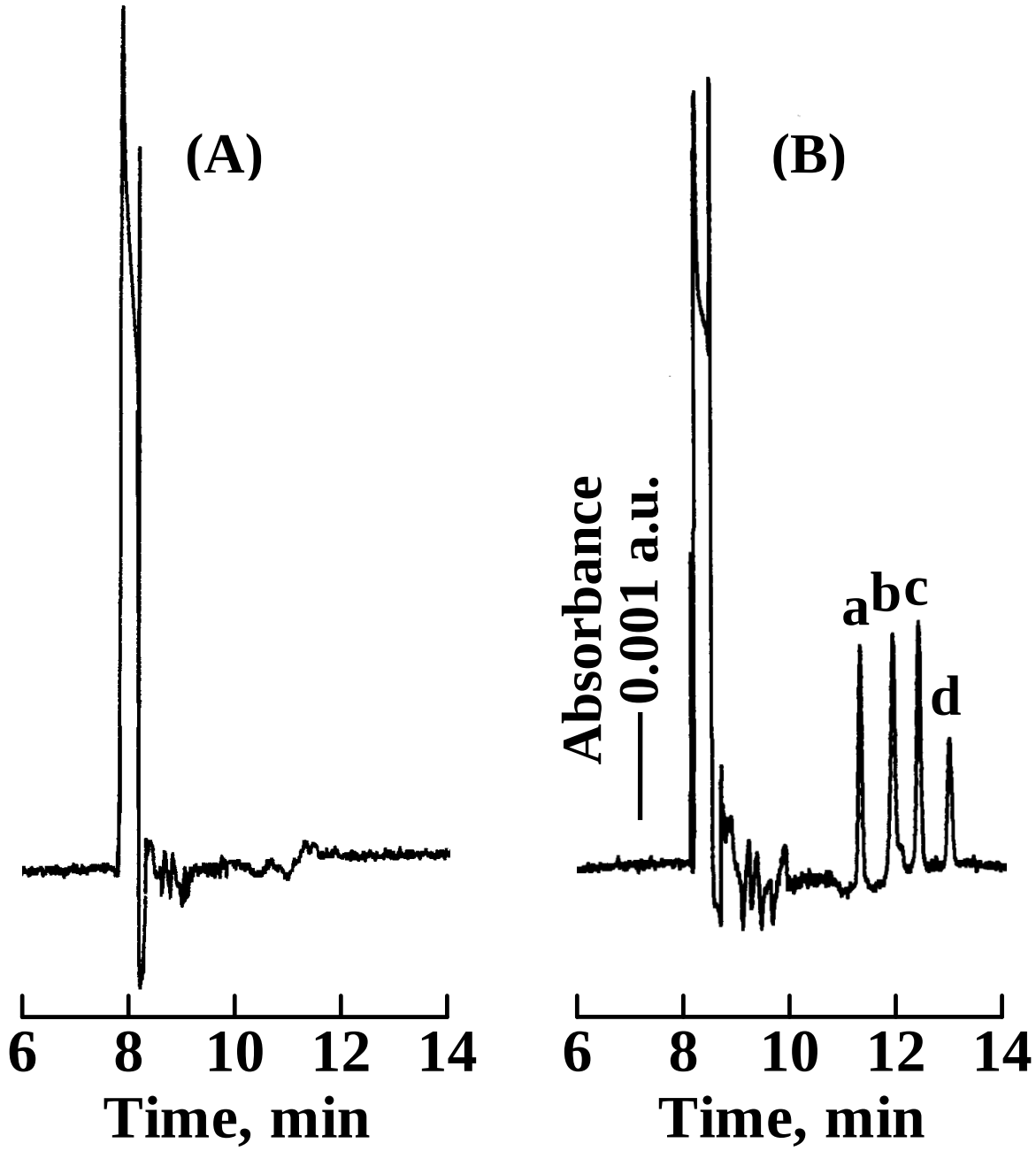


Fig. 2


(A)
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Absorbance
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Time, min

Fig. 3

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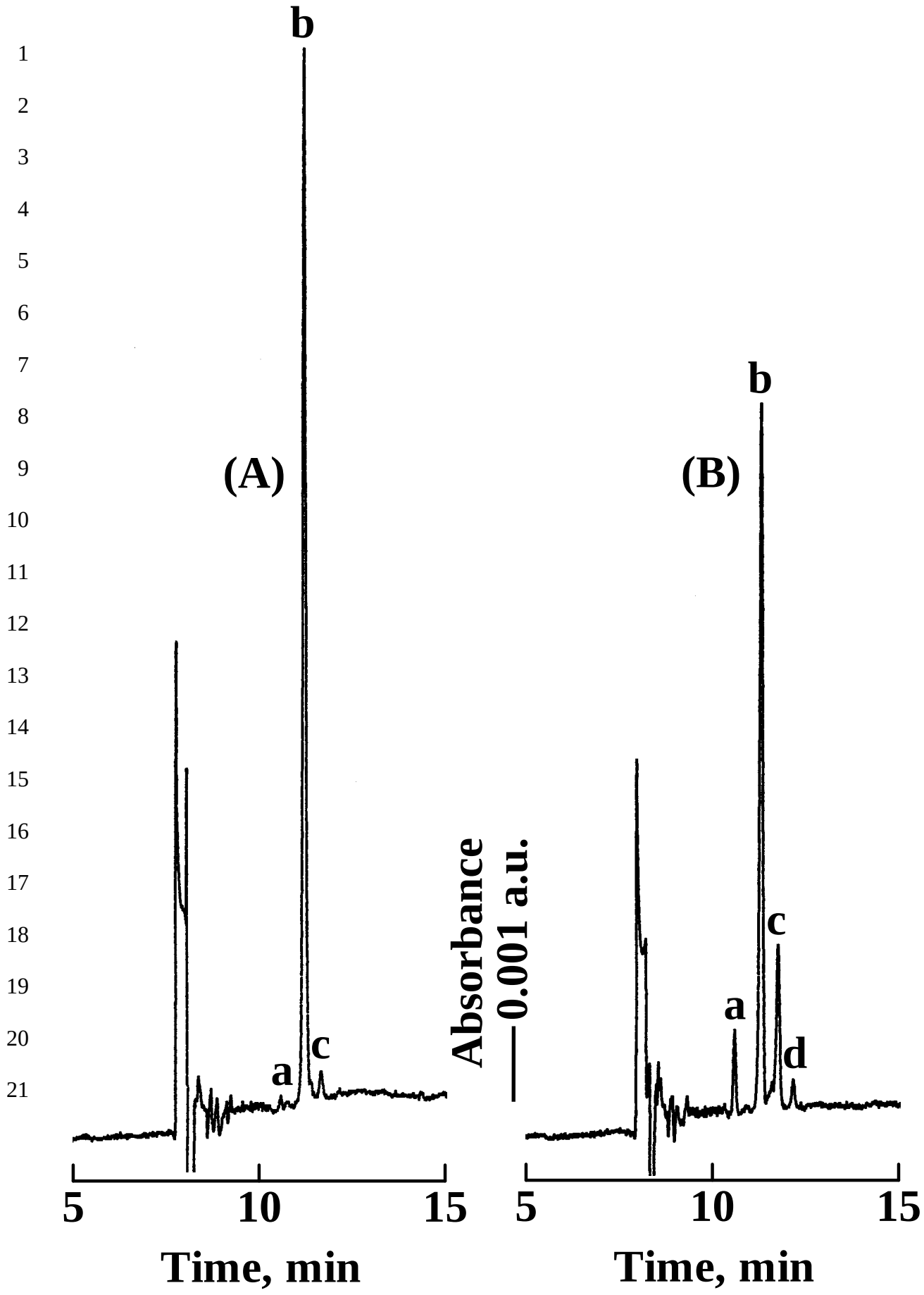

Fig. 4

Table 1

Precision and detection limits of determination of pyridine-triphenylborane (PTPB), diphenylborinic acid (DPB), phenylboronic acid (MPB), and phenol[a]

	RSD[b] (intra-day, %)			RSD[b] (inter-day, %)			LOD (S/N=3)
	Area	Height	Time	Area	Height	Time	(μg/l)
PTPB	4.1	3.6	1.1	8.5	7.2	0.94	25
DPB	4.1	3.2	1.1	4.4	5.3	0.94	30
MPB	4.7	1.7	1.0	4.9	3.7	0.93	50
Phenol	3.4	1.4	0.73	9.1	4.7	0.94	29

[a]Electrophoretic conditions are as those depicted in Fig. 3.

[b]Sample: 0.5 mg/l in acetonitrile, six determinations.