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SHORT COMMUNICATION

Simultaneous Determination of Pyridine-Triphenylborane Anti-Fouling Agent and Its Degradation Products in Artificial Seawater by CZE

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Abstract We developed a CZE method for simultaneous determination of pyridine-triphenylborane (PTPB) anti-fouling agent and its degradation products such as diphenylborinic acid (DPB), phenylboronic acid (MPB), and phenol in artificial seawater (ASW) with no extraction procedure. The ASW samples, in which 20% (v/v) acetonitrile (ACN) was added, were injected directly into the capillary using vacuum injection. As the background electrolyte (BGE), 60 mM sodium tetraborate adjusted to pH 9.8 was used. The LODs (S/N = 3) for PTPB, DPB, MPB, and phenol were, respectively, 55, 78, 126, and 30 μg L^{-1}. The RSDs (n = 4) for analytes listed above were in the respective ranges of 2.7–5.7, 0.68–6.1, and 0.69–1.1% for peak area, peak height, and migration time. Simple degradation experiments were conducted to verify the usefulness of the proposed method. The PTPB samples dissolved in ASW were put in the open air and rooms with and without light. The sample solutions were analyzed over time. We inferred that PTPB in ASW was more degraded by photolysis than by hydrolysis. The proposed CZE method has been demonstrated as a useful tool to elucidate the PTPB degradation process and its degradation products in ASW.

Keywords Capillary zone electrophoresis · Anti-fouling agent · Artificial seawater · Degradation of pyridine-triphenylborane

Abbreviations

ACN	Acetonitrile
ASW	Artificial seawater
BGE	Background electrolyte
DPB	Diphenylborinic acid
EKI	Electrokinetic injection
FASI	Field-amplified sample injection
HSIM	Hybrid sample injection mode
MPB	Phenylboronic acid
PTPB	Pyridine-triphenylborane

Introduction

Anti-fouling agents, which are harmful to non-target marine organisms, are usually applied to ship hulls and fishnets to prevent biofouling in seawater [1]. Such agents generally degrade to other compounds after dissolution into seawater. One anti-fouling agent, pyridine-triphenylborane (PTPB), is popularly used in some Asian countries including Japan because of its effectiveness [2]. Several reports have described the degradation process of PTPB, in addition to its degradation products and their toxicities to marine organisms [1–4]. Conventionally, analytical methods such as HPLC and HPLC-MS have been used for determination of PTPB and its degradation products diphenylborinic acid (DPB), phenylboronic acid (MPB), and phenol in acetonitrile (ACN) extracts from artificial seawater (ASW) and natural seawater [1–7]. When using such methods, PTPB and its degradation products can not be determined simultaneously. In addition, a somewhat troublesome solid phase extraction procedure must be used before the analysis. This important shortcoming is regarded as one reason why degradation products and their toxicities remain poorly understood. It is therefore worthwhile to develop a method involving no extraction procedure for simultaneous determination of these compounds in seawater.

We previously developed a CZE with direct UV detection for the simultaneous determination of PTPB, DPB, and phenol prepared in ACN [8–10]. Simple degradation experiments using ACN solutions containing PTPB were also conducted to verify the usefulness of the proposed method [8]. In this study, we modified this method to determine PTPB, DPB, MPB, and phenol dissolved in ASW directly with no extraction procedure. In this case, it is necessary to prevent interference from high matrix components such as chloride in the ASW solutions. In the previous method, voltage was applied with the sample inlet side as the anode to migrate the analytes after samples were vacuum injected into the capillary. It was expected that high concentrations of chloride in the ASW samples could be moved back to the sample inlet side during migration because of its high electrophoretic mobility. Consequently, the interference can be reduced and the separation of the analytes expected to be improved. Using the same procedures for the sample injection and voltage application, the following analytical conditions were examined: background electrolyte (BGE) concentration, ACN concentration in ASW sample solutions, and sample-injection time. In addition, degradation experiments using the ASW sample solutions containing PTPB were conducted similarly to the former experiments. This report is the first of an attempt to determine PTPB, DPB, MPB, and phenol in ASW simultaneously with no sample extraction procedure.

Experimental

Apparatus

The CE apparatus used throughout this study was equipped with a UV–Vis absorbance detector (270A-HT; PerkinElmer Inc., Foster City, CA). The rise time for the detector was set at 0.5 s. A polyimide-coated fused-silica capillary was used (75 μm ID × 375 μm OD; GL Sciences Inc.,

Tokyo, Japan). The total capillary length was 72 cm; its effective length was 50 cm. The peak area, peak height, and migration time were measured using a Chromato-Integrator (D-2500; Hitachi Ltd., Tokyo, Japan). The pH measurements were conducted using a pH meter (F-22; Horiba Ltd., Kyoto, Japan).

Reagents and Solutions

All reagents were of analytical-reagent grade and were used as received. PTPB, DPB, and MPB were obtained from Hokko Chemical Ind. Co. Ltd. (Tokyo, Japan). Phenol was the product of Nacalai Tesque Inc. (Kyoto, Japan). Individual stock solutions (1000 mg L^{-1}) of PTPB, DPB, MPB, and phenol were prepared in ACN purchased from Nacalai Tesque Inc. To maintain their stability, 1% (v/v) pyridine (Nacalai Tesque Inc.) was added to the stock solutions except for phenol. The solutions were then covered with aluminum foil and kept at 4°C to prevent their degradation. Standard solutions used for the examination of analytical conditions and building-up of calibration graphs were prepared by mixing diluted stock solutions with ACN and ASW. The preparation of ASW was based on the Japanese Standard [11]. The pH of the BGE (a 60 mM solution of sodium tetraborate) was adjusted to 9.8 using 1 M NaOH (Nacalai Tesque Inc.). The ASW and the BGE were filtered through a 0.45-μm membrane filter (Advantec Toyo Kaisha Ltd., Tokyo, Japan) before use. Distilled, deionized water, obtained from an automatic still (WG220; Yamato Kagaku Co. Ltd.) and a Simpli Lab-UV high-purity water apparatus (Merck Millipore, Tokyo, Japan) were used throughout.

Degradation Experiments

A 1000 mg L^{-1} PTPB in ACN solution without pyridine was prepared immediately before degradation experiments. Then 500 mL of ASW solution containing 1.0 mg L^{-1} PTPB was prepared using the solution above. Into each of 30 screw-top test tubes (10.8 mm ID × 10-mm-long), 7 mL of the solution was put. Then ten test tubes each were put into three different conditions: open air (started at the beginning of August on the rooftop with 34°C average temperature), a room with light (turning on ceiling fluorescent lamps for only daytime, 25°C), and a room without light (in a plastic box with a lid, 25°C). Some test tubes were collected from the three conditions over time. Before ascertaining the concentrations of PTPB and its degradation products in the sample solutions using the proposed method, 20% (v/v) ACN and 0.003% (v/v) pyridine were added to the solutions. The concentrations were calculated using calibration graphs of the peak area.

Method Procedure

New capillaries were pretreated by flushing with 1 M NaOH 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 NaOH for 5 min, and water for 10 min. The capillary was thermostated at 30°C. The detection

wavelength was set at 200 nm. It was then filled with the BGE (a 60 mM solution of sodium tetraborate adjusted to pH 9.8 using 1 M NaOH) for 3 min. The sample solution was injected by application of a vacuum (16.9 kPa) for 4 s (corresponding to 84 nL). A positive voltage of 15 kV was applied for separation. Between runs, the capillary was flushed with 0.1 M NaOH for 3 min. Each step was run automatically. The capillary was flushed with water for 5 min to fill the capillary with water at the end of the day. Calibration graphs were prepared using synthetic standards.

Results and Discussion

Optimization of CZE

A mixture of 1.0 mg L^{-1} PTPB, DPB, MPB, and phenol in ASW was analyzed to examine the effects of BGE concentration on the separation behavior. The BGE (sodium tetraborate) concentration was varied between 20 and 60 mM. The results are presented for comparison in Fig. 1. When 20 mM BGE was used, the peaks of PTPB, DPB, and MPB could not be separated, although phenol was detected separately (Fig. 1a). Separation for all analytes was improved with increasing concentration of BGE up to 60 mM. The analysis time was prolonged. This improvement can result from reduced EOF with increased buffer concentration [12]. In spite of the higher concentration of chloride in the sample solution, four analyte peaks were clearly observed (Fig. 1d). The EOF in the proposed method was 52.2×10^{-5} $cm_2V^{-1}s^{-1}$ [9] and the electrophoretic mobility of chloride was 84.1×10^{-5} $cm_2V^{-1}s^{-1}$ [13]. Simulation results obtained using Simul 5 introduced by Gaš et al. [14] suggested that chlorides, which have higher effective mobility than the EOF mobility, were moved back to the sample inlet side during migration. However, the effective mobilities of the analytes were lower than the EOF mobility [9]. Therefore, the analytes were roughly separated without being severely affected by the chloride. In addition, the sharp phenol peak was observed because of the stacking effect caused by a pH junction [15]. After application of the voltage, the sample zone pH (originally 7.8) increases because of the interaction of borate in the BGE with chloride in the sample plug. As a result, dissociation of phenol (pK_a = 10.0 [16]) is accerlated and phenol is concentrated at the boundary between the sample and the BGE zones. When higher BGE concentration (> 60 mM) was used, an unstable baseline with higher noise was observed (result not shown). Therefore, the optimum concentration of the BGE adopted in the subsequent experiments was 60 mM.

It is well known that increased conductivity difference between BGE and sample zones derives a field enhanced sample stacking. Therefore, the amount of ACN in the ASW solution containing 1.0 mg L^{-1} PTPB, DPB, MPB, and phenol, was varied between 0 and 30% (v/v). As might be apparent from Fig. 2, the peaks for PTPB, DPB, and MPB sharpened with increasing amount of ACN up to 20% (v/v). When the amount of ACN was 30% (v/v) (Fig. 2d), no marked improvement was observed, but the migration time increased. Furthermore, the addition of ACN into samples results in the dilution of the samples. The dilution of samples should be avoided for

the determination of lower concentrations of analytes. Therefore, 20% (v/v) was chosen as the optimum ACN concentration in ASW samples.

The sample-injection time was varied between 2–6 s (results are not shown). Peak heights for PTPB, DPB, and MPB increased concomitantly with increasing injection time up to 4 s; then they decreased slightly. The peak height for phenol increased with the injection time up to 5 s; then it almost leveled off. The migration time increased concomitantly with increasing injection time. The maximum peak heights were obtained for PTPB, DPB, and MPB, except for phenol when the sample-injection time was 4 s. Therefore, 4 s was used further as the optimum sample-injection time.

Method Validation

Standard solutions for PTPB, DPB, MPB, and phenol were prepared using ASW containing 20% (v/v) ACN and 0.003% (v/v) pyridine. Calibration graphs for all analytes were linear using both the peak area and peak height. Regression equations relating the area response to concentration for PTPB, DPB, MPB, and phenol (x, 0–1.5 mg L^{-1}) were $y = 3.94 \times 10^4 x + 9.20 \times 10^2$ (correlation coefficient, 0.9992), $y = 3.75 \times 10^4 x - 8.22 \times 10^2$ (0.9994), $y = 1.85 \times 10^4 x - 1.74 \times 10^2$ (0.9973), and $y = 2.84 \times 10^4 x + 9.52 \times 10^2$ (0.9983), respectively. Those relating the respective peak heights were $y = 2.83 \times 10^3 x + 8.42 \times 10^1$ (0.9967), $y = 2.64 \times 10^3 x - 2.07$ (0.9999), $y = 1.55 \times 10^3 x - 4.37$ (0.9976), and $y = 4.85 \times 10^3 x + 3.72 \times 10^2$ (0.9831). The LODs (S/N = 3) for PTPB, DPB, MPB and phenol were, respectively, 55, 78, 126, and 30 μg L^{-1}. At the level of 0.5 mg L^{-1}, the RSDs (n = 4) for the above analytes were in the respective ranges of 2.7–5.7, 0.68–6.1, and 0.69–1.1% for peak area, peak height, and migration time.

Analytical Application

Results for degradation experiments are depicted in Fig. 3. In the open air, PTPB was degraded rapidly with 34% of the degradation ratio after one day, and disappeared after 15 days (Fig. 3a). Our results resemble those obtained by Zhou et al. [3]: 51% of PTPB was degraded under UV-A irradiation (50°C) after one day. Concentration of phenol increased to 0.57 mg L^{-1} after 30 days with decreasing concentration of PTPB. After 7 days, MPB peak appeared (0.16 mg L^{-1}), then decreased, and disappeared after 20 days. Figures 4a, 4b, and 4c respectively depict electropherograms of the sample solutions immediately after preparation, after 8, and 30 days. In a room with light, 25% of PTPB was degraded after one day. Then it was degraded slightly until 10 days. The concentration was almost constant until 30 days (Fig. 3b); 34% of PTPB was degraded after 30 days. Phenol was observed after 7 days. Its concentration increased gradually until 30 days (0.068 mg L^{-1}). In a room without light, 18% of PTPB was degraded after one day, then it was degraded slightly until 7 days, and the concentration was almost constant until 30 days (Fig. 3c); 25% of PTPB was degraded after 30 days, which was similar to results obtained by Zhou et al. [3]: 23% of PTPB degraded in ASW by hydrolysis. Phenol was observed after 10 days. Its concentration increased gradually until 30 days (0.037 mg L^{-1}). These results suggest that the

effect of photolysis on the degradation of PTPB in ASW was stronger than that of hydrolysis. In no conditions was DPB observed.

For the degradation experiments using higher concentrations of PTPB, the LODs for the above analytes are enough. However, the LODs must be improved and some extraction procedure is necessary for similar experiments using lowere concentrations of PTPB or real seawater analysis. According to the analytical results of PTPB in coastal seawater samples, PTPB concentrations were 4.8-21 pg L^{-1} [7].

Conclusion

We developed a CZE method for the simultaneous determination of anti-fouling agent PTPB and its degradation products, DPB, MPB, and phenol in ASW with no extraction procedure. The proposed simple method provides sufficient detection power and precision to be useful for degradation experiments in ASW. Additional improvements of the LODs and examination of the effects of coexisting constituents in natural seawater are expected to make the method more useful.

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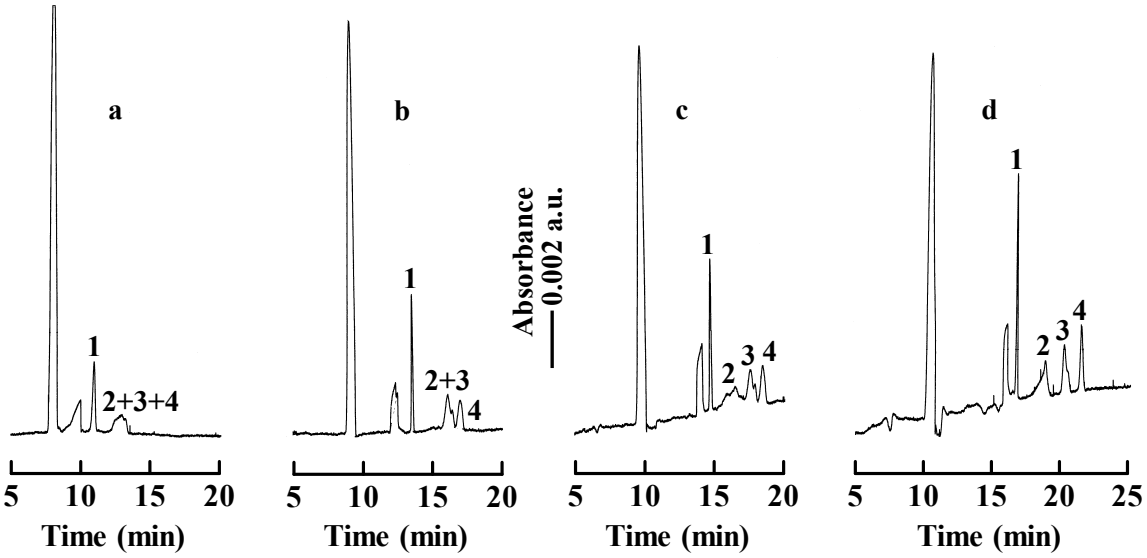
Figure Captions

Fig. 1 Electropherograms of a standard solution of phenol, PTPB, DPB, and MPB obtained with different BGE concentrations: (**a**) 20 mM, (**b**) 40 mM, (**c**) 50 mM, (**d**) 60 mM. CZE conditions: capillary, 50/72 cm × 75 μm ID; BGE, sodium tetraborate adjusted to pH 9.8 with 1 M NaOH; voltage, 15 kV; wavelength for detection, 200 nm. Sample, 1.0 mg L^{-1} of each analyte in ASW; vacuum injection period, 4 s (84 nL). Peak identification: 1, phenol; 2, PTPB; 3, DPB; 4, MPB

Fig. 2 Electropherograms of a standard solution of phenol, PTPB, DPB, and MPB obtained with different ACN concentrations in the samples: (**a**) 0% (v/v), (**b**) 10% (v/v), (**c**) 20% (v/v), (**d**) 30% (v/v). BGE, 60 mM sodium tetraborate adjusted to pH 9.8 with 1 M NaOH. Analyte concentrations, other CZE conditions, and identification of peaks are as in Fig. 1

Fig. 3 Degradation of PTPB and the formation of MPB and phenol in (**a**) the open air (on the rooftop), (**b**) a room with light (25°C), and (**c**) a room without light (25°C). Sample: original sample, 1.0 mg L^{-1} PTPB in ASW; samples used for analysis, 20% (v/v) ACN and 0.003% (v/v) pyridine were added. Other CZE conditions are as shown in Fig. 1. ○: PTPB; □: MPB; ▽: phenol

Fig. 4 Electropherograms obtained in the degradation experiment for PTPB in the open air (on the rooftop): (**a**) sample, 1.0 mg L^{-1} PTPB in ASW immediately after preparation containing 20% (v/v) ACN and 0.003% (v/v) pyridine; (**b**) sample, collected after 7 days containing 20% (v/v) ACN and 0.003% (v/v) pyridine; (**c**) sample, collected after 30 days containing 20% (v/v) ACN and 0.003% (v/v) pyridine. Other CZE conditions and identification of peaks are as in Fig. 1



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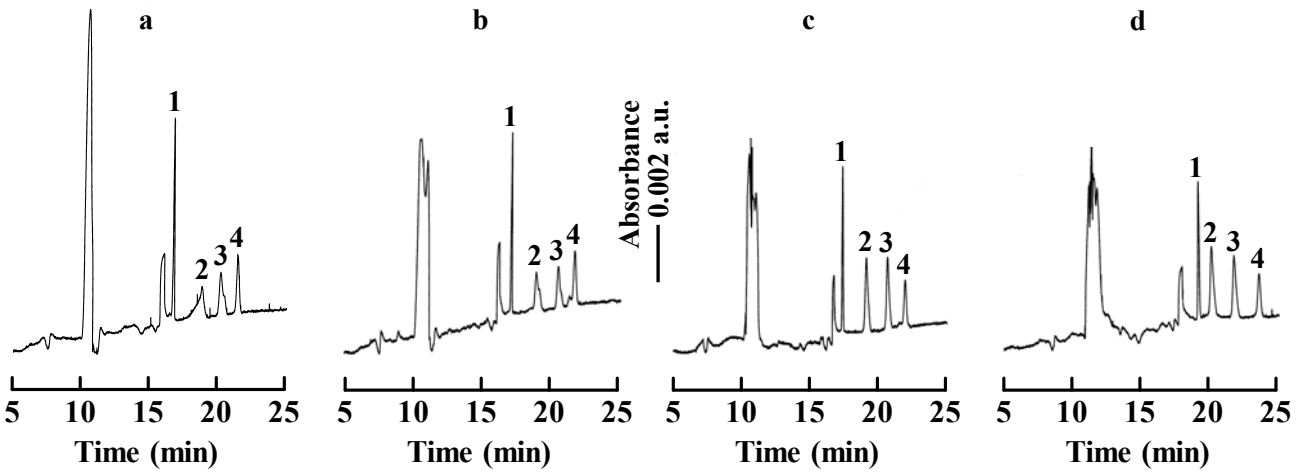

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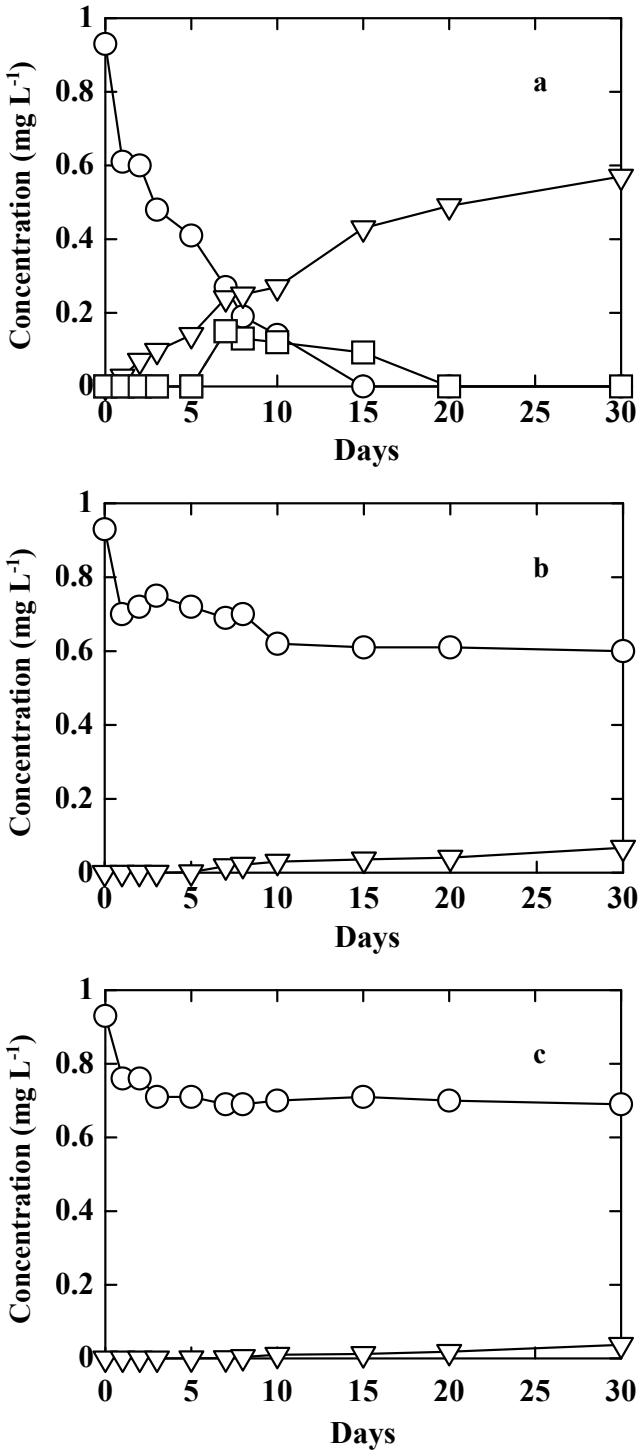

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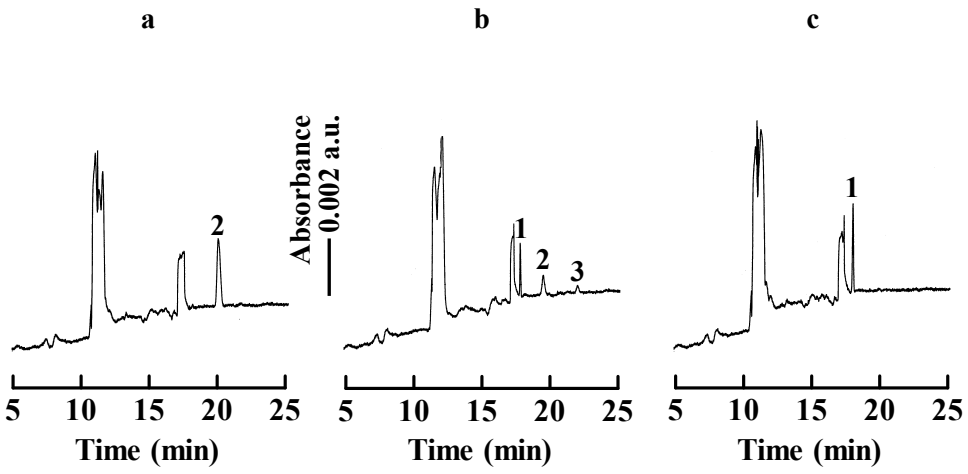

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