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Pre-concentration of non-uniform field electrophoresis for sample introduction of capillary electrophoresis

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Abstract

A new sample introduction method of capillary electrophoresis, in which field-amplified sample injection was combined with a pre-concentration of non-uniform field electrophoresis, is presented in this paper. With an additional pre-concentration voltage applied to sample solution, a non-uniform electric field was generated, with which analytical cations or anions were pre-concentrated around an electrode adjacent to the injection end of capillary. After the pre-concentration, analytical ions were injected into the capillary and stacked at the boundary between sample and buffer solution inside capillary by field-amplified injection technique. In contrast to the conventional field-amplified injection, larger concentration factor and higher analytical sensitivity were obtained with the improved pre-concentration method. Its concentration factor was about 10~15 fold as that of field-amplified sample injection.

Keywords: Non-uniform field electrophoresis; Field-amplified sample injection; Pre-concentration; Propranolol; Metoprolol

1. Introduction

High-performance capillary electrophoresis (HPCE) has become a major analytical technique for fast and efficient separation of analytical objects in solutions [1–3]. Injecting a small volume of low concentration sample solution into a capillary leads to poor detection signal. Therefore, one of the challenges in HPCE is to improve its concentration sensitivity so as to be applicable to the analysis of low-concentration sample solutions. Several sample introduction methods were reported on on-column concentration to enhance analytical sensitivity of HPCE, such as field-amplified sample injection and isotachophoresis pre-concentration [4–10]. Concerning the stacking methods of sample introduction, sample ions either injected by hydrodynamic flow are stacked inside a capillary, viz. electrostacking, or introduced by electrokinetic flow are in the head-end of a capillary, viz, field-amplified sample injection. Although the stacking methods have been widely employed, their applications are limited to sample solutions with low conductivity. In field-amplified sample injection, an analytical sample is prepared in a low-conductivity solution and injected electrokinetically into a capillary column containing a higher conductivity buffer. Thus, high electric field strength can be distributed in a tiny sample plug at the sampling end inside the capillary because of its low conductivity. Once the sample ions inside the capil-

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lary migrate into the support buffer region, they will slow down and be stacked at the boundary between sample and buffer solution. In order to improve its analytical sensitivity further, prolonging sampling time or enhancing stacking voltage may be adopted to increase the amount of sample ions injected into capillary. However, a large injection volume will broaden its separated zones and reduce its separation efficiency obviously. Another disadvantage induced by a large sampling volume of low conductivity solution is a redistribution of electric field strength inside capillary. A major part of electric field strength will be distributed in the large sample plug and it will result in a decrease of resolution. In order to stack a large sample plug without sacrificing both separation efficiency and resolution, the water medium in sample solutions should be removed after the stacking process [11–19].

In this paper, an improved on-column concentration method to enhance analytical sensitivity is presented, in which field-amplified sample injection was combined with a non-uniform field electrophoretic pre-concentration. With the above-introduced pre-concentration method, analytical ions can be injected with higher concentration during field-amplified sample injection and it results in a notable increase of analytical sensitivity of HPCE with good reproducibility and without distinct decrease of separation efficiency. To avoid the problems caused by the large injection volume mentioned above, field-amplified sample injection was carried out under conditions of injection voltage 15 kV and sampling time 10 s [20]. The principle of non-uniform field electrostacking has been applied to an electrokinetic flow analysis system [21–24] successfully. The pre-concentration action of non-uniform field electrophoresis in low conductivity solution was also confirmed experimentally in this paper. In addition, several parameter effects of non-uniform field electrophoresis on pre-concentration factor were investigated. As an analytical example, propranolol hydrochloride and metoprolol tartrate in low-conductivity buffer or water solutions were pre-concentrated with the improved sample introduction method and separated by HPCE. The enhancement of concentration factor is evident, especially with water as the sample medium.

2. Experimental

2.1. Equipment

The schematic diagram of the capillary electrophoresis (CE) setup with a non-uniform field electrophoresis pre-concentration is shown in Fig. 1. The CE instrument consists of a 1229 HPCE ANALYSER with a negative high-voltage power supply and a 9423 chromatography integrator (R) both from Application Institute of New Techniques (Beijing, China). Capillary electrophoresis was carried out in a 75 μm I.D. fused-silica capillary (Yongnian Optical Fiber Factory, Hebei, China) of 65 cm total length and 40 cm effective length. The detection wavelength was set at 214 nm. DYY-1114 electrophoretic power supply (E, from Liuyi Instrument Factory, Beijing, China) was employed to provide a pre-concentration electric field in sample solution. The high-potential loop electrode of the pre-concentration voltage was a platinum filament one with 22 mm diameter and another straight electrode, viz, the electrode on the sampling side of the HPCE system, was grounded. The electrode adjacent to the injection end of the capillary with a distance of about 1 mm was located in the middle of the loop electrode. During electrophoretic pre-concentration, the pre-concentration current was measured with a digital

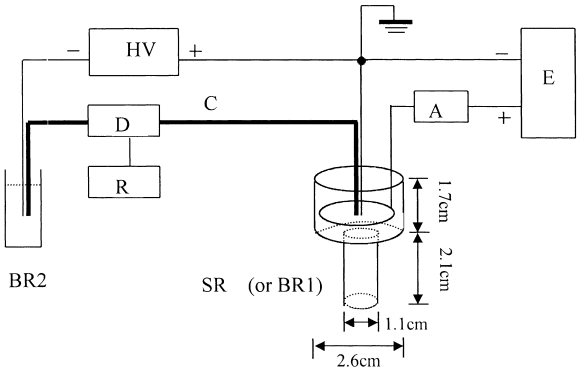

Fig. 1. Schematic diagram of field-amplified sample injection combined with non-uniform field pre-concentration system. HV, high-voltage power supply of HPCE; C, fused-silica capillary; D, ultra-violet detector; R, chromatography integrator; BR, buffer vessel; SR, sample vessel; A, digital multimeter; E, electrophoresis power supply.

multi-meter (A). Both the sample vessel (SR) and buffer vessel (BR1) set on the sampling side of HPCE were made of glass. The upside dimensions were 26 mm I.D. and 17 mm height, and the downside dimensions were 11 mm I.D. and 21 mm height. Another buffer vessel (BR2) of the HPCE system was a normal 1.5-ml centrifugal tube.

2.2. Chemicals

The experimental reagents were of analytical-reagent grade. Their solutions were prepared with deionized water, and filtered with 0.2 μm filtration membrane. A 25 m*M* buffer solution was prepared by dissolving sodium acetate (NaAc, Chemical Factory, Shanghai, China) 0.82 g into 90 ml deionized water, adjusting its pH to 4.0 with concentrated acetic acid (HAc, Chemical Factory) and diluting to 100 ml.

Two stock solutions of 200 μg ml^{-1} propranolol hydrochloride (Wujin Pharmacy Factory, Changzhou, China) and 1000 μg ml^{-1} metoprolol tartrate (ASTRA Pharmacy, Wuxi, China) were also prepared in deionized water and kept in a refrigerator.

2.3. Procedure

Before the determination processes, the capillary was washed with water, 0.1 *M* sodium hydroxide, water and 25 m*M* acetate buffer solutions for 10 min, successively. The pre-concentration and separation for propranolol and metoprolol were performed as follows: (1) non-uniform field electrophoresis pre-concentration for 5 min. The pre-concentration in sample solution was carried out with 800 V regulated, which was provided by the electrophoretic power supply (E). At the same time, an auxiliary capillary voltage (1 kV) was supplied by a HPCE high-voltage power supply (HV). (2) Field-amplified sample injection. After the pre-concentration, HV was raised up to 15 kV immediately and the field-amplified sample injection was carried out for 10 s. (3) Electrophoretic separation. With the pre-concentration power supply (E) turned off and sample vessel (SR) replaced by buffer vessel (BR1), the analytical cations introduced into the capillary were separated by HPCE and determined at 214 nm. The CE separation voltage was maintained at 15 kV throughout this work and the chromatography integrator recorded the absorption signals.

In order to ensure the accuracy and reproducibility of the sample determination, both the ground electrode and loop electrode on the sampling side, and the sample vessel should be cleaned with deionized water carefully before each sampling operation. The sample solution in the sample vessel was determined only once. For next determination, the capillary was washed 2 min with buffer solution by a pressurized syringe.

3. Results and discussion

3.1. Pre-concentration action of non-uniform field electrophoresis

The electric potential distribution showed that the high voltage of HPCE was almost totally distributed across the capillary in field-amplified sample injection, even if deionized water was used as sample medium, and the distance between the ground electrode and the injection end of the capillary was as large as 25 mm. It means that there is almost zero electric field strength in the sample solution during field-amplified sample injection. Thus, when field-amplified sample injection was carried out and the sample ions transported toward the capillary by electroosmotic flow, they maintained the same concentration as their original one. Meanwhile, the analytical ions injected into the capillary could migrate to the boundary between sample and buffer solution with a high speed under the action of high electric field strength. During the sample injection, the analytical ions were continuously stacked on the boundary and it resulted in the pre-concentration. Obviously, if the analytical ions in the sample solution can be collected around the injection end of capillary before field-amplified sample injection, the concentration efficiency of field-amplified injection will be improved notably. In this paper, the combination of field-amplified sample injection with non-uniform field electrophoresis pre-concentration was employed for this aim.

In the investigation of non-uniform field pre-concentration, 25 m*M* NaAc–HAc was used as buffer solution. Propranolol (PH) and metoprolol (MT) cations in sample solution were pre-concentrated by non-uniform field electrophoresis and field-amplified sample injection firstly, and then separated by HPCE.

Fig. 2 shows a comparison of the separation peaks of PH and MT using different sample introduction methods, including conventional electrokinetic sample injection (a), single field-amplified sample injection (b) and field-amplified sample injection combined with non-uniform field electrophoresis pre-concentration (c and d). The non-uniform field pre-concentration was carried out with 200 V for 1 min in (c) and 2 min in (d). In (c) and (d), the sample solution of 2 μg ml^{-1} PH and 10 μg ml^{-1} MT was prepared in 1 m*M* buffer, the same as in (b). It can be found that the concentration factor for both PH and MT were improved approximately twofold with our sample introduction method compared with single field-amplified sample injection. But the pre-concentration current was rather high, about 24 mA with 200 V. As the pre-concentration time was longer than 2 min, the sample solution was affected by thermal effect seriously. When the pre-concentration voltage was reduced to 20 V with a current 2 mA and

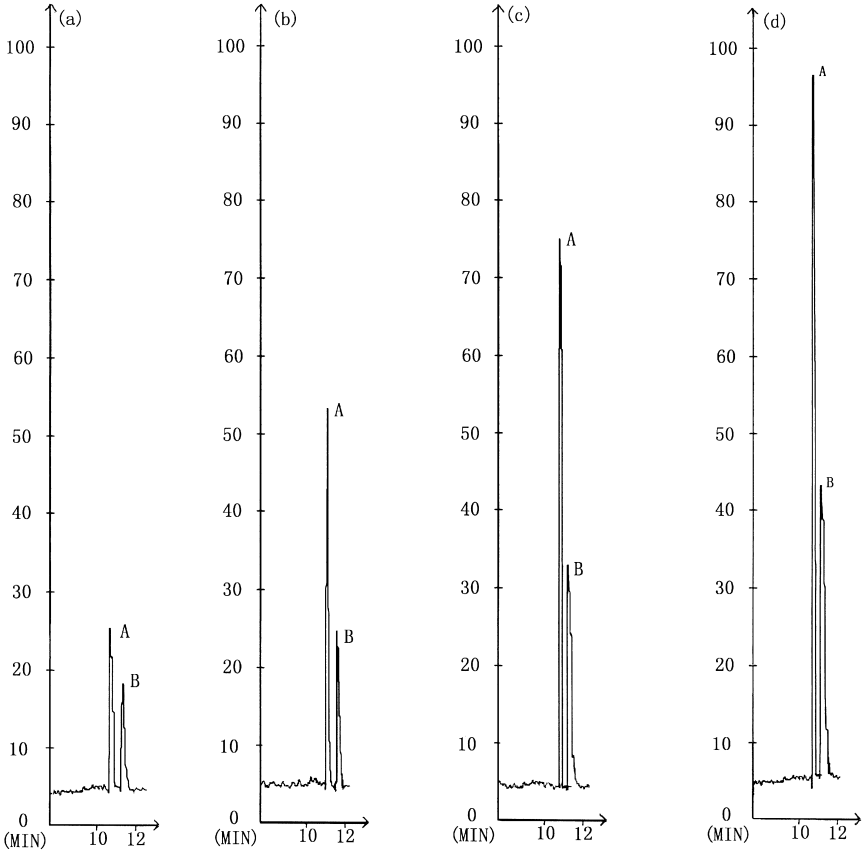

Fig. 2. Electropherogram for propranolol and metoprolol cations with three kinds of sample introduction methods. Peaks A and B correspond to propranolol (PH) and metoprolol (MT) cations, respectively. 25 m*M* NaAc–HAc (pH 4.0) was used as support buffer solution. (a) Electrokinetic injection: 25 m*M* NaAc–HAc for sample buffer, 10 μg ml^{-1} PH and 50 μg ml^{-1} MT. The electrokinetic injection voltage and time were 15 kV and 10 s, and separation voltage was 15 kV. (b) Field-amplified injection: 1 m*M* NaAc–HAc (pH 4.0) for sample buffer, 2 μg ml^{-1} PH and 10 μg ml^{-1} MT. Other conditions as in (a). (c) and (d) Field-amplified injection combined with non-uniform field pre-concentration: pre-concentration voltage, 200 V; pre-concentration for 1 min (c) and 2 min (d). Other conditions as in (b).

the pre-concentration time was prolonged to 30 min, the concentration factor was approximately four times as compared to that by single field-amplified injection. So the parameters of the non-uniform field pre-concentration should be chosen carefully in order to be appropriate to the HPCE analysis.

By decreasing sample buffer concentration, the pre-concentration current and thermal effect in sample solution was reduced. Fig. 3 shows a comparison of the electrophoretograms of PH and MT in a water medium using single field-amplified sample injection (a) and our method (b). Under the pre-concentration condition of 800 V and 5 min, the pre-concentration current was reduced to 1~2 mA. As shown in Fig. 3, the concentration factor of our method for both PH and MT were improved obviously, and the measured peak height was enhanced about eightfold for PH and fivefold for MT, respectively, compared with single field-amplified sample injection.

The purpose of the improved pre-concentration method is to increase the amount of analytical cations injected into capillary with high concentration, which is obtained by the non-uniform field electrophoresis, but without large sampling volume, which can reduce the separation efficiency and resolution. From the results of Figs. 2 and 3, it can be found that the improved introduction method can achieve the analytical purpose. During the field-amplified injection step for both methods, their injection conditions were the same, 15 kV and 10 s, and it resulted in the same sampling volume injected into capillary.

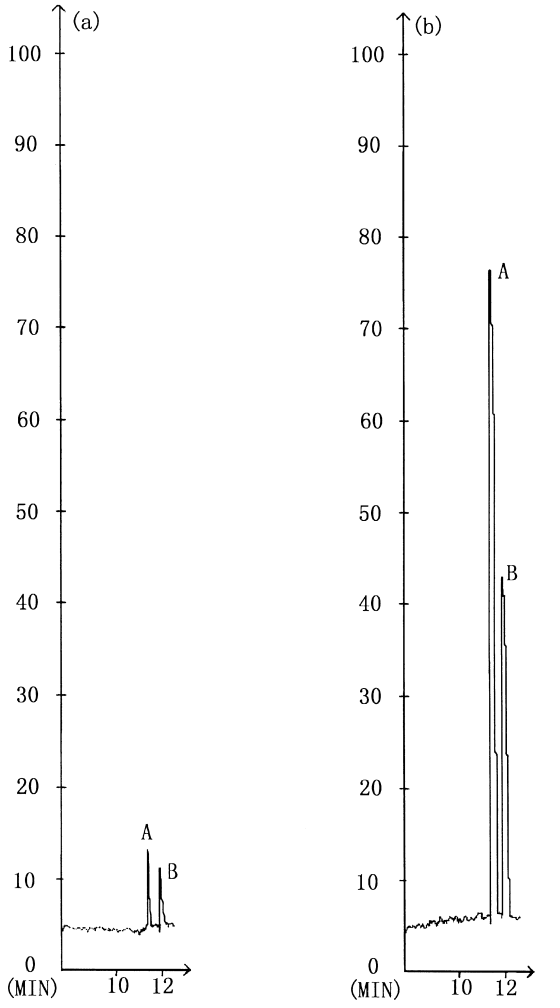

Fig. 3. Electropherogram for propranolol and metoprolol cations using field-amplified sample injection (a) and field-amplified sample injection combined with non-uniform field pre-concentration (b). Sample solution was 0.025 μg ml^{-1} PH and 0.125 μg ml^{-1} MT in water. The pre-concentration voltage and time was 800 V and 5 min, the field-amplified sample injection voltage and time was 15 kV and 10 s, and the HPCE separation voltage was 15 kV, respectively.

3.2. Effects of pre-concentration voltage, time and auxiliary capillary voltage

As mentioned above, the pre-concentration action of non-uniform field electrophoresis was confirmed experimentally. Here, the influence factors on non-uniform field pre-concentration are elucidated. Fig. 4 illustrates the effects of pre-concentration voltage (a) and pre-concentration time (b) on the relative concentration factor. The relative concentration factor was calculated from the ratio of the corresponding peak height of analytical ions measured by our method to that of single field-amplified sample injection. High pre-concentration voltage and long pre-concentration time can improve the concentration factor and the analytical ions can be concentrated more efficiently. However, it will also result in an increase of thermal effect in sample solution. Thus, the improvement of concentration efficiency was limited by the thermal convection and concentration diffusion. As shown in Fig. 4a, the relative concentration factors for both PH and MT were increased with the enhancement of pre-concentration

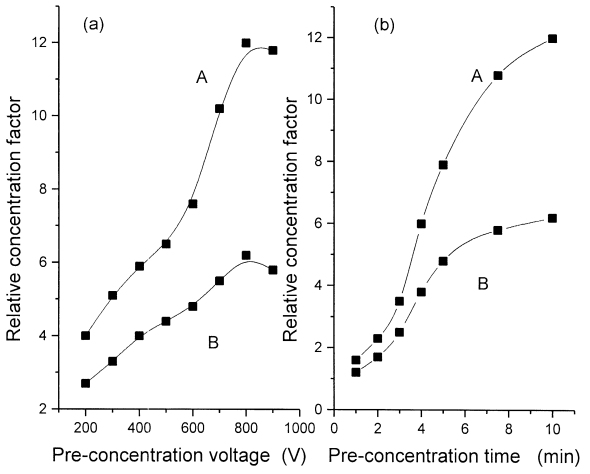

Fig. 4. Effect of pre-concentration voltage (a) and pre-concentration time (b) on pre-concentration efficiency. A and B correspond to propranolol (PH) and metoprolol (MT) cations, respectively. The relative concentration factor was calculated from the ratio of the corresponding peak height of analytical ions measured by using the improved sample introduction method to the conventional field-amplified sample injection. Sample solution was 0.025 μg ml^{-1} PH and 0.125 μg ml^{-1} MT in water. (a) Pre-concentration time was 10 min; (b) pre-concentration voltage was 800 V. Other conditions as in Fig. 3.

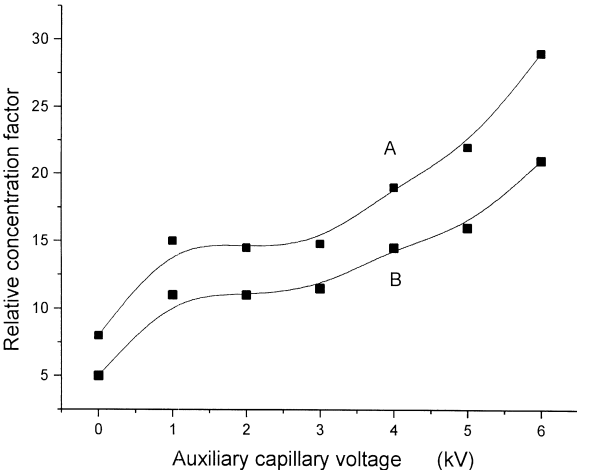

Fig. 5. Effect of auxiliary capillary voltage on non-uniform field pre-concentration. Pre-concentration parameters were 800 V and 5 min. Sample solution was 0.0125 μg ml^{-1} PH and 0.0625 μg ml^{-1} MT in water. Other conditions as in Fig. 4.

voltage in the range from 200 V to 800 V, and decreased slightly at 900 V. Similarly, it can be seen that the relative concentration factors were also increased with prolonging pre-concentration time in Fig. 4b. However, the increase tendency was slow down when the pre-concentration time was longer than 5 min. The influence of thermal effect and concentration diffusion was considerable, as the pre-concentration voltage and time was larger than 800 V and 10 min in water medium. By selecting appropriate pre-concentration voltage and time, a higher pre-concentration factor can be achieved. In this paper, 800 V and 5 min were chosen as the optimized performance parameters for our sample introduction method.

Although the analytical cations were collected around the ground electrode, nearby the sampling end of capillary, in the non-uniform field pre-concentration, the pre-concentration action was also limited by the thermal convection and concentration diffusion in the sample solution inevitably. In order to improve pre-concentration factor further, an auxiliary capillary voltage was introduced during the non-uniform field electrophoresis pre-concentration.

Fig. 5 shows the effect of auxiliary capillary voltage on the pre-concentration factor, in which the pre-concentration voltage and time were 800 V and 5 min, respectively. It can be seen that a selected auxiliary capillary voltage can increase the amount of analytical ions injected into capillary and enhance the pre-concentration factor. For example, with 1 kV as the auxiliary capillary voltage, the relative concentration factor was increased about 15-fold for PH and 11-fold for MT, respectively, compared to that of single field-amplified sample injection, which had a concentration factor of about 400-fold compared to the conventional electrokinetic sample injection. Thus, the actual concentration factors of about 6000-fold for PH and 4400-fold for MT, respectively, were obtained with 1 kV auxiliary capillary voltage.

Though the relative concentration factors increased rapidly with the increase of auxiliary capillary voltage in the range from 0 to 1 kV, they retained constant in the range from 1 to 3.0 kV. Thus, in this range of auxiliary capillary voltage, it cannot be satisfactorily explained by the action of electroosmotic flow and field-amplified stacking at the capillary end, otherwise the relative concentration

factor should increase with the increase of auxiliary voltage. The auxiliary capillary voltage can further assemble the analytical cations collected around ground electrode, but the detailed mechanism about the assembling effect need a further investigation. However, in the range higher than 3.0 kV, an effect of field-amplified sample injection appeared during the non-uniform field electrophoresis pre-concentration and resulted in an increase of the amount of analytical cations introduced into capillary, so that the concentration factor was enhanced. At the same time, the resolution of HPCE was reduced because of the large sampling volume. When the auxiliary capillary voltage was increased to 6 kV, the peaks of PH and MT cannot be separated from each other entirely, although the relative concentration factors were enhanced to 29-fold for PH and 22-fold for MT.

3.3. Reproducibility and linearity

As shown in Fig. 6, which represents the analytical reproducibility of our sample introduction method, a sample solution was repeatedly pre-concentrated and separated four times under the conditions of pre-concentration voltage 800 V, pre-concentration time 5 min and auxiliary capillary voltage 1 kV. The relative standard deviation (RSD) of peak height was 1.5% for PH and 2.3% for MT ($n=4$). But there was no considerable difference in separation efficiency between our sampling method and single field-amplified injection. Since the pre-concentration action exists in the whole sample solution, the solution can be determined only one time. Furthermore, the pre-concentration factor is affected by the conductivity of sample solution, both electrodes on the sampling side and sample vessel should be washed with deionized water carefully and the buffer contamination should be avoided in the analysis. It is one of the keys to improve the reproducibility with our sample introduction method.

In accordance with the separation results, the peak height was proportional to the analytical concentration in the linear range of PH from 0.6 to 12.5 ng ml^{-1} and MP from 3.0 to 62.5 ng ml^{-1}, respectively, under the same determination conditions mentioned above.

4. Conclusions

The experimental results in this paper indicated

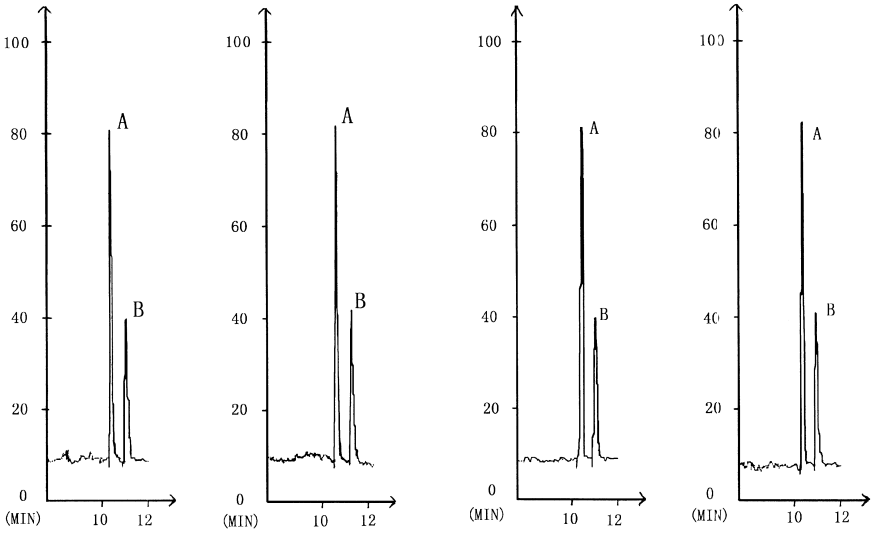

Fig. 6. Reproducibility of the improved sample introduction method. Pre-concentration parameters were 800 V and 5 min, and auxiliary capillary voltage was 1 kV. Sample solution was 0.0125 μg ml^{-1} PH and 0.0625 μg ml^{-1} MT in water. Other conditions as in Fig. 5.

that the combination of field-amplified sample injection with non-uniform field electrophoresis pre-concentration could improve concentration factor significantly and make the method with good reproducibility. This sample introduction method can become one of the effective on-column concentration techniques to enhance the analytical sensitivity of HPCE. However, the non-uniform field electrophoresis pre-concentration is only adequate to ions with the same charges and in low-conductivity sample solutions. The application of non-uniform field pre-concentration in high-conductivity sample solutions and simultaneous pre-concentration for both cations and anions is the topic of our future work.

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