Determination of Trace RA by Capillary Electrophoresis–Solid-Phase Microextraction with Direct UV Detection

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Abstract

A new method of offline solid-phase microextraction coupled with capillary electrophoresis (CE) [in an uncoated capillary using borax and boric acid in acetonitrile–water (40:60) as the running buffer] with UV detection at 254 nm is presented and used for direct determination of trace biomedically important retinoic acid (RA) without any derivation. As low as 138 ng/mL RA in aqueous solution can be determined using CE with UV detection with a concentration factor of 19.6 times.

Introduction

Retinoic acid (RA) and its metabolites have an important role in nutrition, vision, and in the treatment of various diseases in oncology and dermatology. On the other hand, it is also a toxic compound that causes mild hypervitaminosis A and, even more, tetratogeny. Therefore, the determination of RA is very necessary. RA is a monocyclic parent compound containing five carbon–carbon double bonds and a functional terminal carboxy group at the terminus of the acyclic portion, and thus it has favorable UV characteristics (1). It is sensitive to light, heat, and oxygen and is insoluble in aqueous solutions. Most commercial capillary electrophoresis (CE) instruments are equipped with UV–vis detectors, but the detection of native absorbance is not sensitive enough (2) to trace RA in very low sample volumes. The concentration detection limits of UV absorbance in CE often fall in the µmol/L range (3). Consequently, detection sensitivity has become the "bottle-neck" in CE analysis of trace components in small-volume situations (4). The capillary-modified Z-cell gives only a sixfold signal-to-noise ratio because of light attenuation, despite a 60-times longer path length (5). A double-stacking procedure based on field enhancement is described as a means to increase the concentration sensitivity in CE (6). Solid-phase extraction (SPE), because of its enrichment (preconcentration) role, can lower the concentration detection limit (7–10). The preparation of setup for offline SPE is easier and simpler than that for online SPE, but only a few methods have been reported that describe offline SPE for retinoids (1). In addition, the coated capillary may be used to suppress electro-osmatic flow, improving the separation performance (11), but commercially coated capillaries are expensive and have very short lives (12). On the other hand, a bare (uncoated) capillary also has some advantages (12–15).

The present paper is a new report concerning offline solid-phase microextraction (SPME) coupled to bare CE with native UV detection for determination of trace RA without derivitization.

Experimental

Instruments

The CE instrument used was an NT-binda 1229-HPCE analyzer made by the Beijing Institute of New Technology Application (Beijing, China).

Reagents

The RA solution was made by dissolving 1.400 mg RA (biochemical reagent) with 95% ethanol to obtain a 690-µg/mL stock solution. The solution was diluted to 13.8 µg/mL with methanol. Then, 1380 and 138 ng/mL solution were prepared by dilution with water. Borax–boric acid running buffer was prepared by dissolving 0.380 g borax and 0.310 g boric acid in 100 mL acetonitrile–water (40:60) solution.

Construction of SPME setup

A 1.5-cm long polytetrafluoroethylene (PTFE) tube with a 300-µm i.d. was cut. The inside diameter of the tube was expanded by inserting an iron thread of o.d. 360 µm. A 0.5-mm thick glass fiber was inserted, followed by a 5-cm long capillary with a 75-µm i.d. From the other end of the PTFE tube, some 40-µm Accubond ODS solid-phase packing (Agilent Technologies, Palo Alto, CA) was filled dryly and compacted up to 5-mm

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thickness. A 0.5-mm thick glass fiber and 5-cm long capillary with 75-µm i.d. were then inserted one after another.

CE–SPME procedure

The electrophoresis of RA standard solution was performed in borax–boric acid running buffer. The capillary used was 36-cm long with 375-µm o.d. and 75-µm i.d. The running voltage was 15 kV. Preconcentrated RA solution with SPME made by the authors was injected for 40 s by a 6.5-cm elevation of the capillary. The detection wavelength was 254 nm.

Results

Ordinary CE of RA standard solution

The electrophoresis of 13.8 µg/mL RA standard solution was borax–boric acid running buffer. The conditions were a running voltage of 15 kV and anodic hydrodynamic injection for 40 s by 6.5-cm elevation of the capillary. The electropherogram detected at 254 nm is shown in Figure1A. As low as 2700 ng/mL of RA cannot be detected (Figure 1B).

Highly sensitive detection of RA

A combination of CE with an SPME setup was developed to detect the more trace RA. The procedure was as follows.

Pretreatment of SPME

After connecting a syringe holding solution with the SPME setup by means of a PTFE tube as the connector, 10 drops each of acetonitrile, methanol, and water were pushed out from the SPME setup column in order to wash and pre-equilibrate it.

Sampling

RA solution (1380 ng/mL) was aspired into a 5.00-mL scale injector. The solution was pushed through the SPME automatically for 11 min by means of pressure equipment. The SPME setup was taken off the syringe, one section of the capillary in it was pulled out, and the SPME setup column was back washed with water. After putting back the section of capillary to the SPME setup, the water in it was pushed dry with vacant syringe.

Elution

Methanol was aspired into the syringe and connected with the PTFE tube as the connector. After adjusting the liquid level in the syringe, the syringe with the PTFE tube connector was connected with the SPME column. By observing the scale on the syringe, a quantity of 2 µL methanol was pushed forward through the SPME setup, and the eluate was collected in a microvial.

CE detection

The mentioned methanol elution solution was electropherosized according to the previously described method.

Detection of 1380 ng/mL RA solution. A comparison of the electropherograms of 1380 ng/mL RA solution with and without using the combination of CE and SPME setup (by preconcentrating for 11 min) are shown in Figure 2. It can be seen that only if the stated combination was adopted, as low as 1380 ng/mL of RA can be detected. Otherwise, the aim could not be achieved, unless other effective methods were taken.

Detection of 138 ng/mL RA solution. A lower concentration of RA was tested. The method was the same as the previous, except that preconcentration was stopped after 60 min (to increase a further level of preconcentration). Figure 3 shows that as low as 138 ng/mL RA solution was determinable under the experimental conditions, whereas without the SPME combination, it was impossible.

Discussion

Precision of RA detection

RA (13.8 µg/mL) was electrophoresized five times repeatedly by CE–UV without SPME. The peak time of RA ranged from 3.3 to 3.7 min. The precision of detection of RA was good.

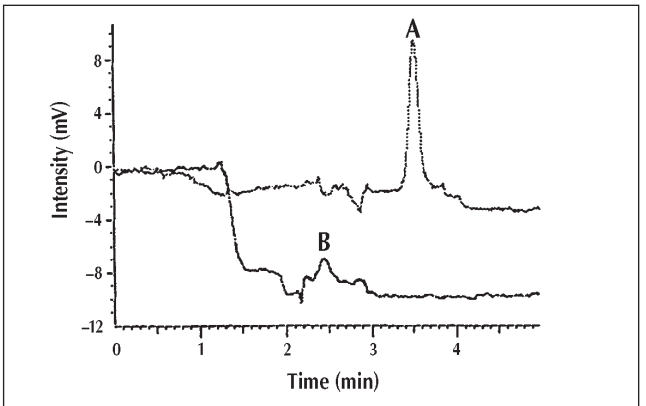

Figure 1. Electropherograms of 13.8 µg/mL (A) and 2700 ng/mL (B) of RA standard solutions by CE–UV without SPME.

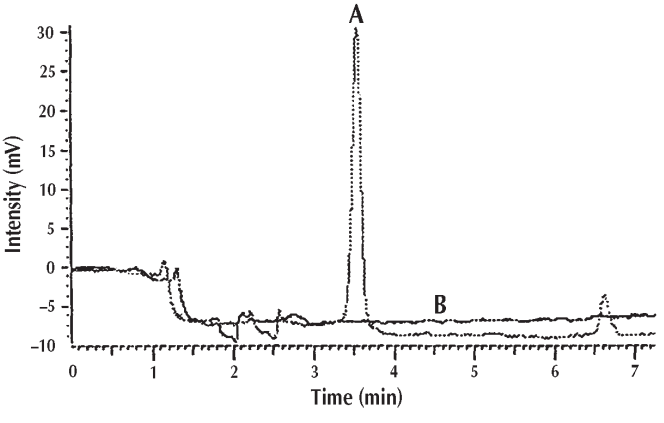

Figure 2. Comparison of electropherograms of 1380 ng/mL of RA solution obtained by CE–UV with (A) and without (B) combination of CE and SPME.

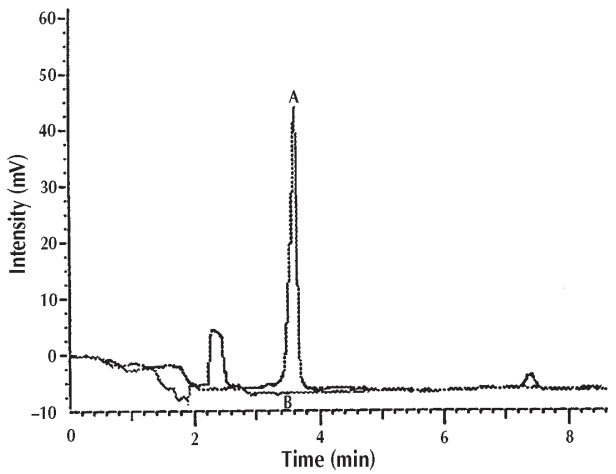

Figure 3. Detection of 138 ng/mL RA solutions by CE–UV after pushing them through the SPME for 60 min (A) and without SPME (B).

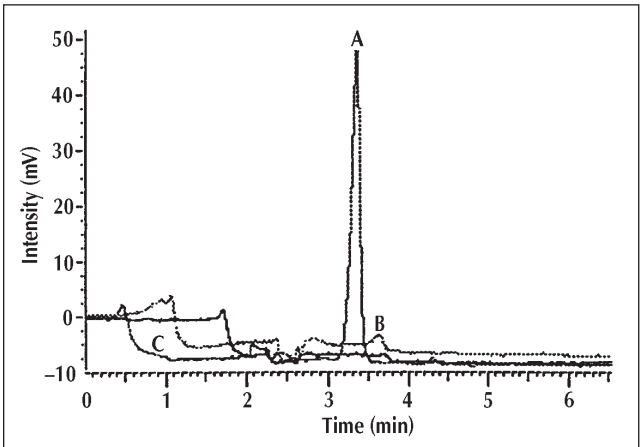

Figure 4. The effect of eluate volumes of RA standard on electropherograms obtained by CE–UV–SPME (A, B, and C represent electropherograms of first, second, and third consecutive eluates, respectively).

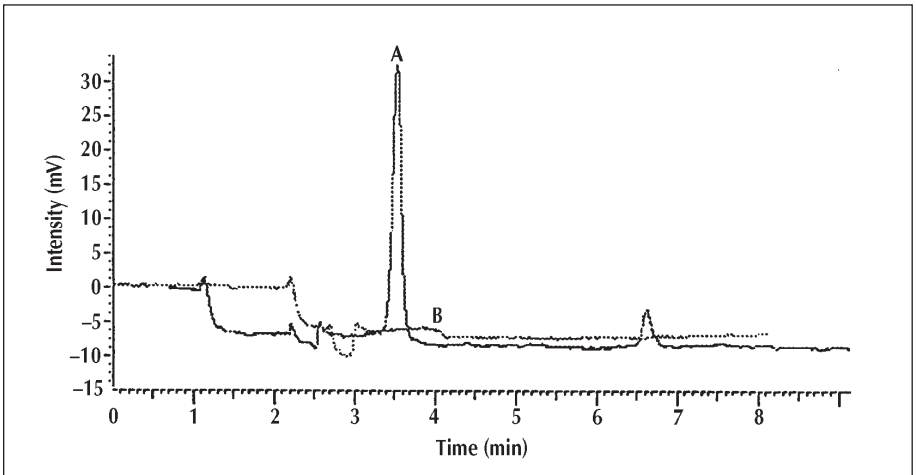

Figure 5. The effect of eluate volumes of RA standard on electropherograms obtained by CE–UV–SPME (A and B represent eluates of the first 2.0 µL and consecutive second 2.0 µL, respectively).

Effect of elution volume on detection

RA (1380 ng/mL) was electrophoresized and detected after having been absorbed by SPME and eluted. The effect of the eluate volume on detection was investigated by collecting certain volumes of eluates. In the case of Figure 4, 1.40, 1.50, and 1.50 µL (curves A, B, and C, respectively) of RA eluates were collected one after another and electrophoresized. From Figure 4, it may be seen that first eluate (1.4 µL) already contained all of the separated RA.

It was anticipated that the first collection of 2.0 µL of eluate would be more suitable for detecting RA with less reading error of the volume and higher repeatability than the 1.4-µL eluate, and with enough sensitivity. Another 1380 ng/mL of standard solution was sampled for 15 min, and two 2.0 µL of RA eluates were sequentially collected and then electrophoresized. The experimental results shown in Figure 5 confirm that the first 2.0-µL eluate volume was suitable (it contained all RA separated).

Reproducibility of detecting low concentrations (1380 ng/mL) of RA

After a 1380-ng/mL RA standard solution was sampled and eluted from the SPME column by methanol (4.0 µL in all), for which the electropherogram is shown in Figure 5, another 1380-ng/mL RA solution was directly pushed through the previously described SPME column at once, without any pre-equilibration with water. The resulting electropherogram is shown in Figure 6. Comparing Figures 5 and 6, the detection of the low concentration (1380 ng/mL) of RA was confirmed with good reproducibility using this SPME column.

Effect of methanol elution on the CE detection of RA

Usually the organic solvent (i.e., methanol) contained in a sample can improve the detection sensitivity of the sample because of the stacking effect of CE. However, in the case of Figure 7, a comparison of the electropherograms of 1380 ng/mL RA diluted with water and detected by CE–UV (curve A), diluted with methanol and detected by CE–UV (curve B), and diluted with water and detected by CE–SPME–UV (curve C) was made. It can be seen from Figure 7 that combining CE with SPME was key for improving the detection sensitivity of common UV spectrophotometry, whereas the addition of methanol alone and without the cooperation of SPE could not, unless another effective supplementary method was taken.

Test of blank solution

A blank solution without RA was tested by the CE–SPME–UV method. The experimental results showed that there was no peak in the electropherogram obtained from the water blank. The result proved that there was no interference from the packing, tube, etc. for the determination of RA in samples.

Conclusion

Compared with online SPME, the offline SPME is relatively easy and convenient to make, and its effect is also satisfactory in the preconcentration of trace components. Specifically, the sampling is unimpeded because the offline SPME column is not blocked from troublesome bubbles and high pressure caused by sampling into the very thin and long capillary. Furthermore, the combination of uncoated CE and offline SPME with ordinary UV detection carries respective advantages and may make up each other's deficiencies. The combination procedure has been applied to enrich and measure native absorbance of trace (as low as 138 ng/mL) RA without any chemical derivation. The whole operation requires less than 1 h every time, and this one column may be used 3 times. Because of the facility of make-and-adjustable-length characteristics of the SPME setup column, the method is practical and robust.

In short, this is an example of a method using offline SPME to enhance the concentration sensitivity in CE, and it is recommended.

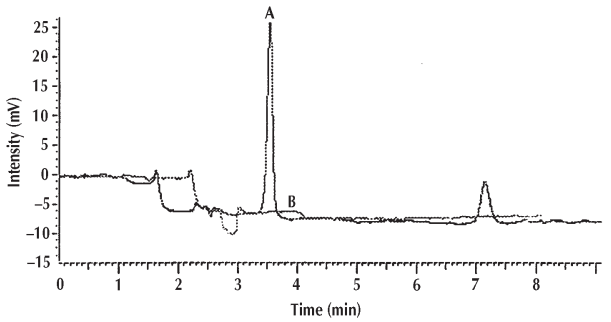

Figure 6. Electropherogram detected by the combination of CE–SPME setup. The electropherogram of 2 µL elution solution of 1380 ng/mL RA without pre-equilibration (A) and that of the methanol elution as a starting contrast (B).

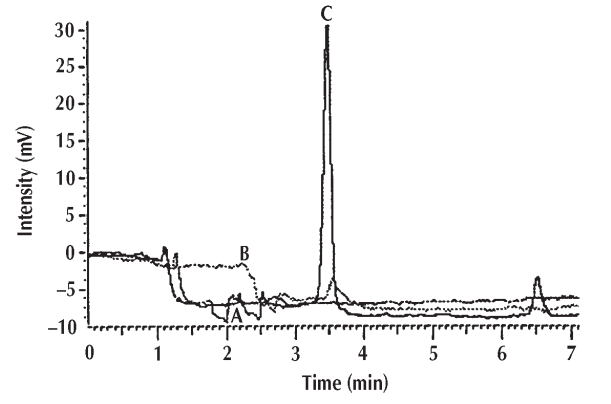

Figure 7. Comparison of electropherograms of 1380 ng/mL RA solution obtained under different conditions. A, B, and C represent RA diluted with water and detected by CE–UV, RA diluted with methanol and detected by CE–UV, and RA diluted with water and detected by CE–SPME–UV, respectively.

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