Separation of Butachlor and its Antibody Complex by Capillary Electrophoresis

Letter to Editor

Volume 2 Issue 3 - 2015

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Received: December 19, 2014 | **Published:** July 27, 2015

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The Herbicide butachlor is one of the widely used herbicides in China, and played an important role in the agricultural production. However, it is stable in the environment, and its residues can enter the food chain and show some of the mutagenicity [1-3]. Almost all available analytical methodologies have been applied to determine butachlor, including gas chromatography (GC), high performance liquid chromatography (HPLC) coupled with different detectors [4-6]. These methods demonstrated good selectivity and reproducibility, however, the sample preparation is time consuming and the detection sensitivity is low. In addition, the above mentioned methods are time consuming, and they are usually need expensive, toxic and environmental unfriendly organic agents. The ELISA method is sensitive, efficient and low cost, but the detection process needs complicated washing and incubation [7]. In this study, the butachlor hapten (BMPA) was directly labeled with fluoresceinthiocarbamyl hexylenediamine (HDF) as the tracer (BMPA-HDF) [8], a capillary electrophoresis with laser-induced fluorescence (CE-LIF) method to separate butachlor and its antibody complex was established for the first time.

The FITC-BMPA and antiserum were diluted to the appropriate concentrations with 50 mmol/L boric acid buffer at pH 8.0. To perform competitive assay, 100 μL of 1.0 nmoL/L FITC-BMPA was mixed with 20 μL of 0 to 50.0 ng/mL butachlor according to the requirement. And then add 100 μL of 200-fold diluted antiserum to each mixed solution above. After 20 min of incubation in the dark at room temperature, the samples were analyzed by CE-LIF. To construct the standard curve, a stock solution of butachlor in MeOH (1 mg/mL) was diluted with MeOH into concentrations of 0, 2, 5, 10, 20 and 50ng/mL the competitive assay reaction formula.

$$Ag + Ag^* + Ab \rightarrow Ab\text{-}Ag^* + Ab\text{-}Ag + Ag^*$$

Ab: polyclonal antiserum anti-butachlor; Ag: butachlor; Ag*: FITC-BMPA; Ab-Ag*: immunocomplexformation.

Untreated fused-silica capillary with an inner diameter of 75 μm and total length of 60 cm, which was preconditioned by successively flushing with 0.1 mol/L HCl, 0.1 mol/L NaOH, ddH_2O and running buffer for 2 min, respectively. Electrophoresis was performed at 25 °C using borate buffer (50mmol/L, pH 8.0) as the running buffer. The samples were injected at 3447.38 Pa for 5 s. The applied voltage was 25 kV. Between runs the capillary was rinsed with 0.1 mol/L NaOH for 1 min, and then a running buffer for 2 min.

We used different separation buffer to ensure enough separation efficiency and resolution, and it was found that the 50 mmoL/L boric acid buffer at pH 8.0 was suitable for the CEIA separation. The differences in electrophoretic mobilities of the components in the immunocomplex cause them to move apart in the electrical field, resulting in dissociation of the immune complex (Figure 1).

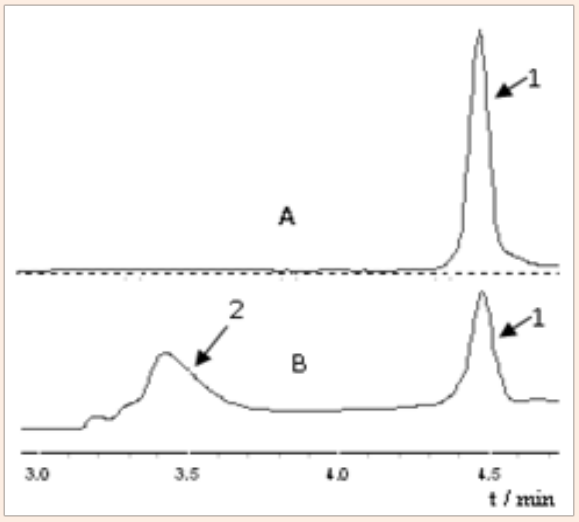

Figure 1: Electropherograms of BMPA-HDF. Samples:

A. without antiserum and
B. with antiserum. Buffer: 50 mmol/L pH 8.0 boric acid buffer; injection: 0.5p.s.i. 5s; applied voltage: 25kV; untreated fused-silica capillary: 60 cm length (50 cm effective length), 75 μm i.d., Peaks: 1=free FITC-BMPA (Ag*), 2=immunocomplex (Ab-Ag*).

Conclusion

In conclusion, a simple, sensitive CE-LIF method for the separation of free butachlor and butachlor-antibody complex was established, which will be useful for a further development of an immunoassay based on CE-LIF.

Acknowledgment

This work was supported by Natural Science Foundation

of China (U1301214), Guangdong Natural Science Foundation (S2013030013338), Guangdong Planed Program in Science and Technology (2013B051000072, 2012A020100002, 2012B090600005).

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