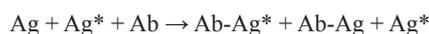


# Separation of butachlor and its antibody complex by capillary electrophoresis

## Letter to editor

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.<sup>1-3</sup> Almost all available analytical methodologies have been applied to determine butachlor, including gas chromatography (GC), high performance liquid chromatography (HPLC) coupled with different detectors.<sup>4-6</sup> 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.<sup>7</sup> In this study, the butachlor hapten (BMPA) was directly labeled with fluoresceinthiocarbamyl hexylenediamine (HDF) as the tracer (BMPA-HDF),<sup>8</sup> 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 50mmol/L boric acid buffer at pH 8.0. To perform competitive assay, 100 $\mu$ L of 1.0nmol/L FITC-BMPA was mixed with 20 $\mu$ L of 0 to 50.0ng/mL butachlor according to the requirement. And then add 100 $\mu$ L of 200-fold diluted antiserum to each mixed solution above. After 20min 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 (1mg/mL) was diluted with MeOH into concentrations of 0, 2, 5, 10, 20 and 50ng/mL the competitive assay reaction formula.



Ab, polyclonal antiserum anti-butachlor; Ag, butachlor; Ag\*, FITC-BMPA; Ab-Ag\*, immuno complex formation

Untreated fused-silica capillary with an inner diameter of 75 $\mu$ m and total length of 60cm, which was preconditioned by successively flushing with 0.1mol/L HCl, 0.1mol/L NaOH, dd H<sub>2</sub>O and running buffer for 2min, 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 5s. The applied voltage was 2 kV. Between runs the capillary was rinsed with 0.1mol/L NaOH for 1min, and then a running buffer for 2min.

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

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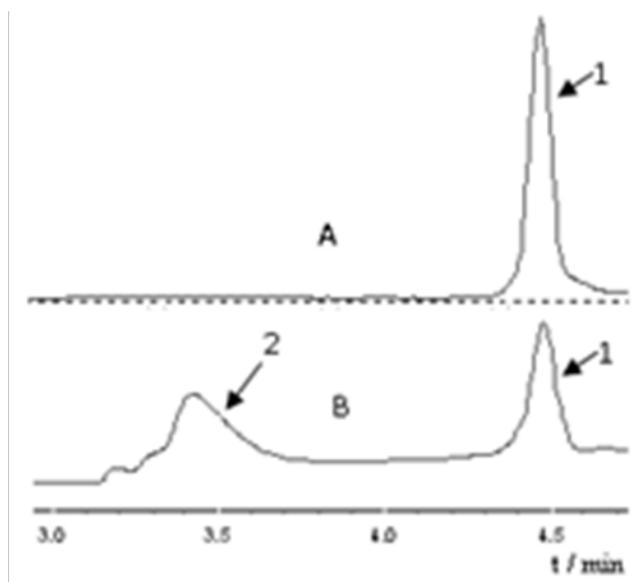
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**Figure 1** Electropherograms of BMPA-HDF.

### Samples

A) without antiserum and

B) With antiserum. Buffer: 50mmol/L pH 8.0 boric acid buffer; injection: 0.5p.s.i. 5s; applied voltage: 25kV; untreated fused-silica capillary: 60cm length (50cm effective length), 75 $\mu$ m 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.

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## Conflict of interest

Author declares that there is no conflict of interest.

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