Electronic Supporting Information

Sensitive profiling of trace neurotoxin domoic acid by pressurized capillary electrochromatography with laser-induced fluorescence detection

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2. Experimental

● Instrumentation

According to the ref.[27], the HPLC-MS/MS analysis was carried out using an Accela HPLC system coupled to a TSQ Quantum Access MaxTM triple quadrupole mass spectrometer equipped with electrospray ionization (ESI) source (Thermo Fisher, USA). Chromatography separation was performed on a Hypersil GOLD aQ column (5μm particle size,150×2.1 mm). Mobile phase consisted of water (A) and acetonitrile (B) in a binary system, both containing 0.1% formic acid. Isocratic chromatography was performed using 20% B for 5 min with a flow rate 200 μL/min. The column temperature was held at room temperature, and the injection volume of analytical solution was 10 μL. MS analysis was performed using selected reaction monitoring (SRM) mode with the ion source in positive mode. Parameters such as SRM transition (DA, m/z 312 → 266), collision energy (16 eV) and tube lens (96) were obtained by auto tuning. The vaporizer temperature and capillary temperature were set at 300◦C and 350◦C, respectively. The ESI source was operated at ion spray voltage of +3000 V. Nitrogen was used as sheath gas and auxiliary gas at flow rates of 35 and 10 arbitrary unit, respectively. Helium (99.999% purity) was employed as collision gas. Data acquisition and processing were performed with LC quan 2.7 software (ThermoFisher, USA).

● Chemicals and reagents

Stock standards (1000μg/mL) of domoic acid (DA) were prepared in acetonitrile-water （10:90, v/v）. A series of concentrations standard working solutions of DA (0.05-4.00μg/mL) were prepared in acetonitrile/water (10:90, v/v) by serial dilution. The DA solutions were stored at -18ºC.

Method validation

A series of standard solutions of DA within the concentration range of 0.05–4.00μg/mL was employed as working standards for the calibration curve. Blank matrix was prepared according to the above procedures. The LOD for DA is considered to be the concentration that produced a signal-to-noise (S/N) ratio of 3 by analyzing blank matrix spiked with DA.

The recovery experiments were carried out to investigate the accuracy and precision of the method. The standard solutions of DA were spiked into three kinds of blank samples at three different levels (0.30 and 0.60 μg DA/g). Then, the samples were vortexed for 30 s and allowed to stand overnight at room temperature.

DA were extracted and purified according to the procedure mentioned in "2.3 Sample preparation". Precision of the method was evaluated by the intra-day and inter-day relative standard deviation (RSD). The intra-day RSD was obtained from analyzing five replicates of the sample spiked with 0.25 μg/mL of DA on the same day, and the inter-day RSDs were determined by analyzing the sample based on five replicates on three consecutive days.

The recovery experiments of the same samples were also detected with the authorized HPLC-MS/MS method, and the results were compared to further evaluate the feasibility of this method.

Supporting Scheme and Figures

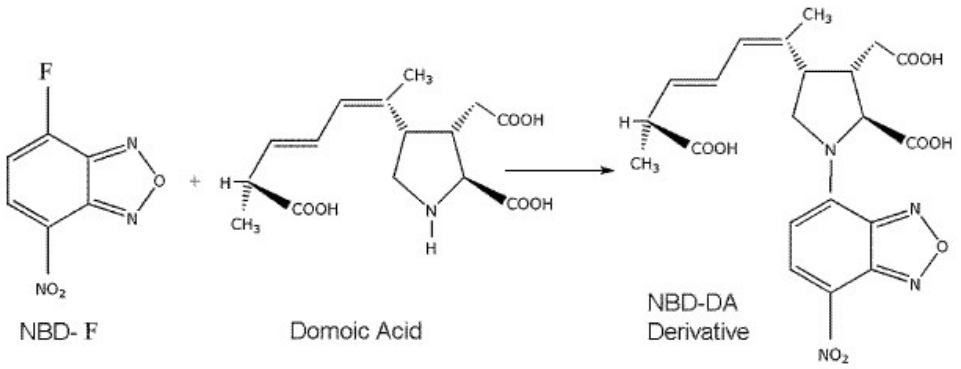

Fig.S1 Derivation scheme of DA for LIF detection

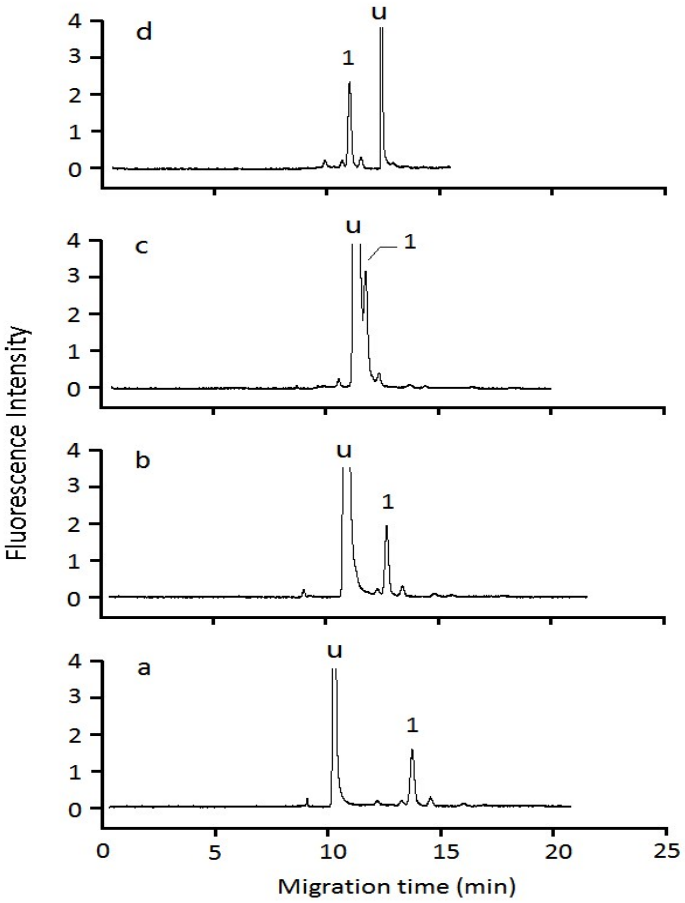

Fig.S2 pCEC and capillary chromatography (cLC) migration behavior of DA

Applied voltage (a) 0 kV, (b) +2 kV, (c) +4kV, (d) +6kV. Mobile phase, 60% (v/v) acetonitrile, 40% (v/v) of 5 mmol/L phosphate buffer, supplementary pressure 1000 psi, flow rate 0.05 mL/min; DA standard solution: 0.50 μg/mL.

Peak 1: DA, peak U: unknown NBD-F hydrolysis products.

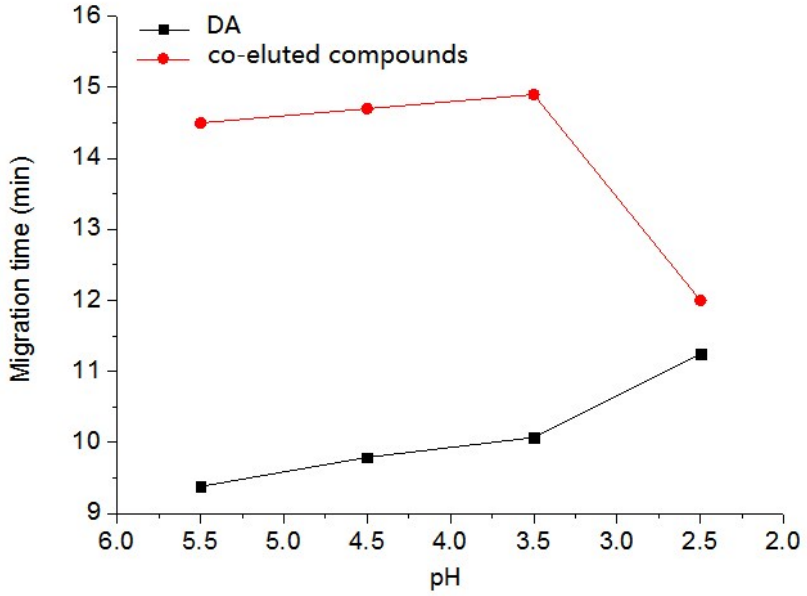

Fig.S3 Migration time of DA in pCEC method under different pH conditions

Mobile phase, 60% (v/v) acetonitrile, 40% (v/v) of 5 mmol/L phosphate buffer, supplementary pressure 1000 psi, flow rate 0.05 mL/min; DA standard solution: 0.50 μg/mL. Applied voltage: +6kV.

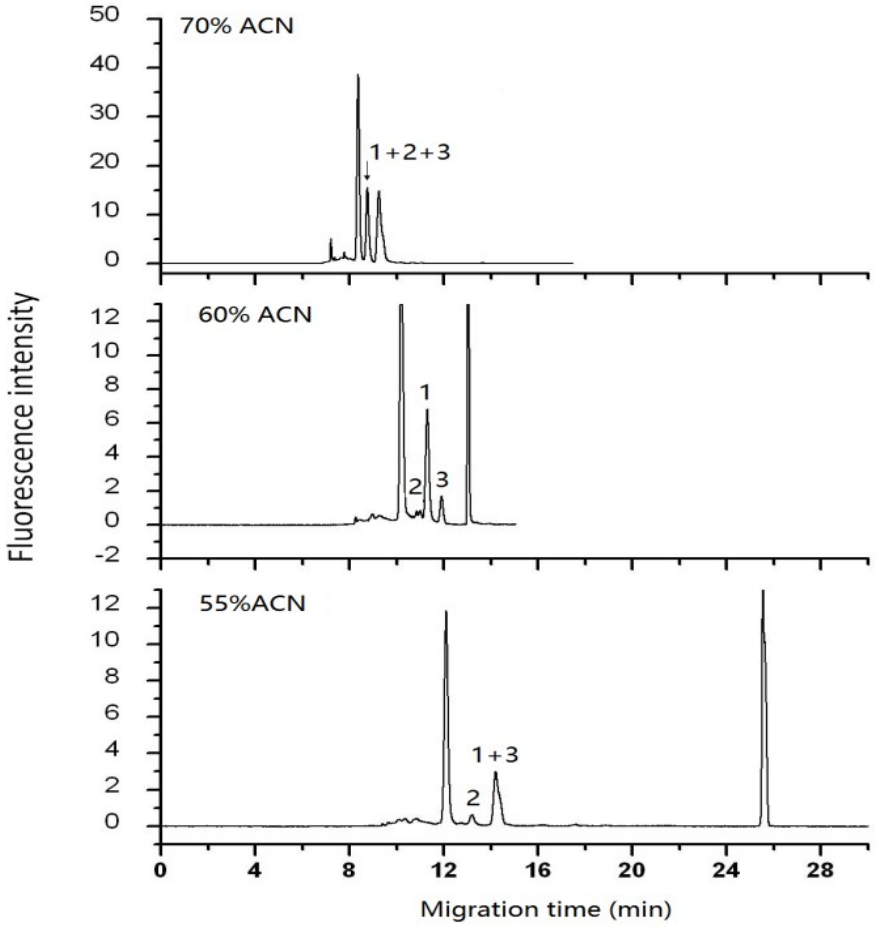

Fig.S4 Effect of the content of acetonitrile (ACN) on the separation of DA

Mobile phase, acetonitrile-5 mmol/L phosphate buffer (pH2.5), other conditions as Fig.S3. DA: 1.00 μg/mL.

Peak: 1: DA-NBD; 2 and 3: co-eluted compounds.

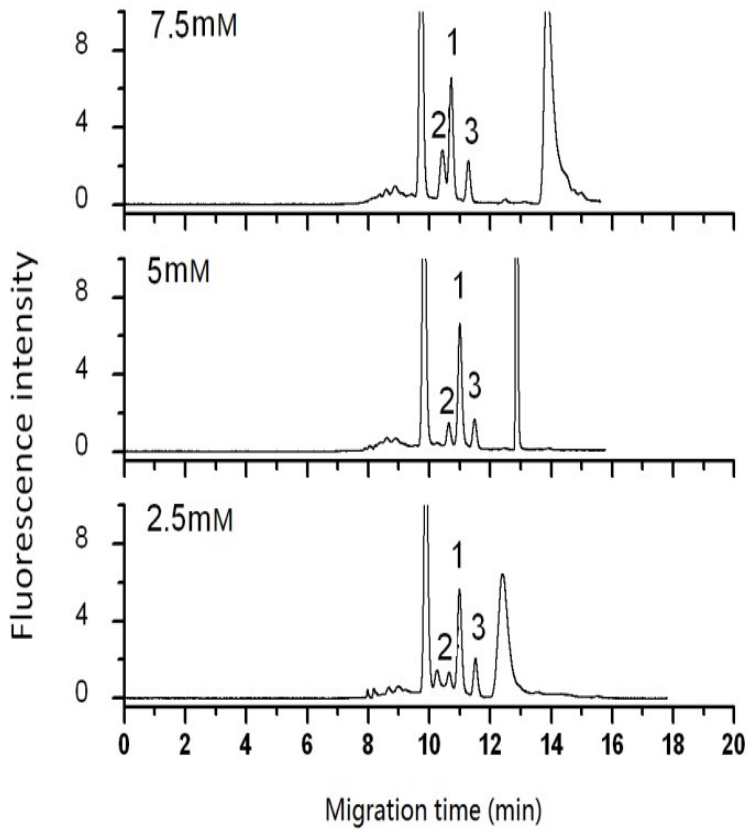

Fig.S5 Effect of buffer concentration on the separation of DA in tissue extracts

Mobile phase, 60% (v/v) acetonitrile, 40% (v/v) of phosphate buffer solution (pH2.5); other as in Fig.S3.

Peak 1: DA-NBD , 2,3: co-eluted compounds.