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# A simple and sensitive method of nonaqueous capillary electrophoresis with laser-induced native fluorescence detection for the analysis of chelerythrine and sanguinarine in Chinese herbal medicines

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**Abstract**

Laser-induced fluorescence (LIF) is a highly sensitive detection method for capillary electrophoresis (CE). However, it usually requires analyte to be derivatized, unless the wavelength of native fluorescence of analyte matches the laser's. That limits its application in drug analysis. In this work, we introduced a rapid, simple and sensitive method of nonaqueous capillary electrophoresis with laser-induced native fluorescence (NACE–LIF) detection for the analysis of chelerythrine and sanguinarine for the first time. As these two alkaloids have some native fluorescence, they were directly detected using a commercially available $Ar^+$ laser without troublesome fluorescent derivatization. The fluorescence was enhanced by nonaqueous media. Compared with previously reported UV detection method, lower limit of detection (LOD) is achieved thanks to the high sensitivity of LIF detection (2.0 ng/mL for chelerythrine and 6.3 ng/mL for sanguinarine). Moreover, with NACE, the baseline separation of these alkaloids is finished within 3.5 min. This method is successfully applied to determine the contents of chelerythrine and sanguinarine in *Macleaya cordata* (Willd.) R. Br. and *Chelidonium majus* L.


*Keywords:* Chelerythrine; Sanguinarine; Chinese herbal medicine; Nonaqueous capillary electrophoresis; Laser-induced native fluorescence

## 1. Introduction

Laser-induced fluorescence (LIF) is one of the most sensitive detection methods of capillary electrophoresis (CE), whose limit of detection (LOD) can easily reach attomole to zeptomole levels and even single molecule [1]. Generally, to reach maximum sensitivity, the fluorescence wavelength of analyte is required to match that of the laser. However, there are only a few wavelengths provided by commercially available lasers, such as He–Ne laser (544, 593 and 633 nm), He–Cd laser (320 and 442 nm) and Ar ion laser (275 and 488 nm) [2]. Most drugs lack native fluorescence, or lack a suitable laser to induce their native fluorescence. Therefore, it becomes a common trend to chemically derivatize the drugs with a fluorescent label to make them detectable. But fluorescent derivatization is usually time-consuming due to the slow kinetics of the derivatization reaction [3]. And lots of drugs including most alkaloids can still not be derivatized well due to the lack of a reaction group or proper fluorescent label. Especially for drug analysis in plants, derivatization is not a wise choice due to the complicated matrix of ingredients. These drawbacks greatly limit the application of LIF detection. However, in our work, we found that although the native fluorescence of some drugs does not perfectly match the laser's wavelength, very low LOD can still be achieved due to the high sensitivity of LIF detection, for instance, chelerythrine and sanguinarine. Properly utilizing the native fluorescence of the analyte can avoid the troublesome fluorescent derivatization.

Chelerythrine and sanguinarine (Fig. 1), the most important members of quarternary benzo[*c*]phenanthridinium alkaloids, always coexist in plants such as Chinese herbal medicine *Macleaya cordata* (Willd.) R. Br. and *Chelidonium majus* L. They exhibit various bioactivities such as anti-inflammatory [4], antitumor [5], SH-enzymes inhibition [6] and antiplaque effect [7]. While they also show enormous toxicity when being used at relatively high concentration [8]. To better exploit and safely

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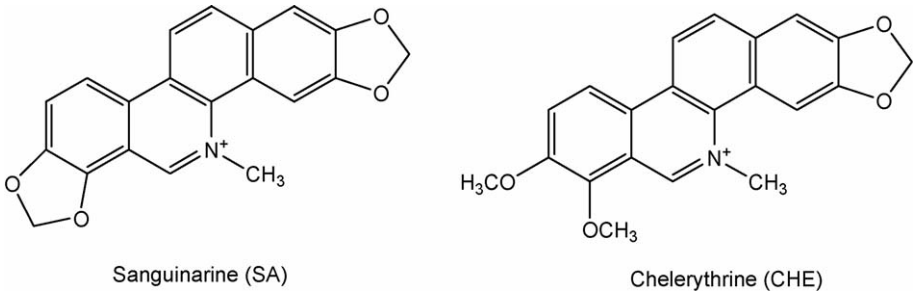


Fig. 1. Structures of chelerythrine (CHE) and sanguinarine (SA).

utilize these plants, several methods have been developed to analyze chelerythrine and sanguinarine in plants [9–12]. Fluorescence detection [13] and electrospray mass spectrometry (ESI-MS) [14] have been coupled to high-performance liquid chromatography (HPLC) to determine sanguinarine and chelerythrine, which were sensitive but uneconomical and rather time-consuming. CE with UV detection has also been applied to analyze these alkaloids [15,16], and study their acid–base properties [17] and interactions with molecules containing mercapto group [18,19]. However, UV detection cannot offer a satisfactory sensitivity without preconcentration. To our best knowledge, there have been no reports about the analysis of these alkaloids using CE–LIF due to the lack of a proper fluorescent label or detector. In addition, owing to its high resolving power and short analysis time, nonaqueous capillary electrophoresis (NACE) has been widely used as an attractive method for the analysis of active ingredient in plants. Therefore, in this work, we introduced a rapid, simple and sensitive method of NACE with LIF detection for the analysis of chelerythrine and sanguinarine in Chinese herbal medicine skillfully using the native fluorescence of these alkaloids for the first time.

## 2. Materials and methods

### 2.1. Apparatus

CE was performed on a Beckman P/ACE MDQ capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA) with 488 nm $Ar^+$ laser module controlled by 32 Karat software. A fused-silica capillary (Yongnian Photoconductive Fiber Factory, Hebei, China) of 30 cm (20 cm to the detector) × 75 μm i.d. was used for separation. The virgin capillary was conditioned by rinsing with 0.1 M NaOH for 10 min, water for 2 min, and then buffer for 5 min. Before each run, the capillary was rinsed with buffer for 3 min. The buffer was renewed after every five runs to ensure good reproducibility. Samples were injected from the anode end of the capillary with a pressure of 0.5 psi for 5 s. The capillary was thermostated at 25 °C. All solutions were filtered through a 0.22 μm pore size membrane filter before use.

The fluorescence spectra were recorded using a Hitachi module F-4500 fluorescence spectrophotometer. The excitation wavelength was fixed at 488 nm, and the emission wavelength scanned from 500 to 700 nm.

### 2.2. Reagents

Chelerythrine and sanguinarine were obtained from Chemistry and Chemical Engineering College, Hunan Normal University (Changsha, China). Ammonium acetate and acetic acid were purchased from Antai Ltd. (Changsha, China). Methanol was from Merck Co. (Darmstadt, Germany) and acetonitrile (ACN) was from Tedia Co. (Fairfield, OH, USA). *M. cordata* (Willd.) R. Br. and *C. majus* L. were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All reagents were of analytical grade.

### 2.3. Sample preparation

Stock solutions of chelerythrine and sanguinarine (1000 mg/L) were prepared in methanol. Solutions of lower concentration were prepared by dilution of the stock solutions with the proper running buffer. The running buffer solutions were prepared by mixing 2.0 mL of 200 mM ammonium acetate, 0.5 mL acetic acid and 5.0 mL ACN in a 10 mL volumetric flask and then being diluted to volume with methanol.

The powdered total grass (1.0 g) of *M. cordata* (Willd.) R. Br. and *C. majus* L. were extracted with 10.0 mL methanol for 30 min in an ultrasonic bath. After centrifugation, the extract was collected and the residues were extracted for another two times. The extracts were combined together and then diluted to 50 mL with methanol as stock solution. When injected into the capillary, 0.1 mL stock solutions were diluted to 1.0 mL with the proper running buffer as sample solutions.

## 3. Results and discussion

### 3.1. Fluorescence emission spectra of chelerythrine and sanguinarine

The fluorescence emission spectra of chelerythrine and sanguinarine were firstly investigated to estimate the feasibility of this method. The $\lambda_{ex}/\lambda_{em}$ (maximum excitation and emission wavelength) of chelerythrine and sanguinarine are 294/407 and 299/408 nm, respectively. To reach maximum sensitivity, the $\lambda_{ex}/\lambda_{em}$ of analyte should match that of detector [20]. However, there are no commercially available lasers that can offer such wavelengths, and these alkaloids are difficult to derivatized