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Analysis of *Strychnos* alkaloids in traditional Chinese medicines with improved sensitivity by sweeping micellar electrokinetic chromatography

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Abstract

The application of an on-line sweeping preconcentration method in micellar electrokinetic chromatography (MEKC) for the determination of *Strychnos* alkaloids, namely strychnine and brucine, has been investigated in this work. After experimental optimizations, the best separation was achieved in 50 mmol l^{-1} H_3PO_4 (pH 2.0) containing 100 mmol l^{-1} SDS and acetonitrile in a ratio of 4:1 (v/v), with an applied voltage of −20 kV at 20 °C. The sample matrix consisted of 100 mmol l^{-1} H_3PO_4 (pH 2.0), and sample introduction was performed at 0.5 psi for 270 s, with photodiode array detection at 203 nm. Compared with the conventional MEKC injection method, up to 100-fold improvement in concentration sensitivity was achieved in terms of peak height by using this sweeping injection technique. In the method, the compound berberine was used as the internal standard for the improvement of the experimental reproducibility. The calibration curve was linear over a range of 0.5–15 μg ml^{-1} for both strychnine and brucine, with a correlation coefficient of 0.998 and 0.997, respectively. The detection limits (S/N = 3:1) for strychnine and brucine were 0.05 and 0.07 μg ml^{-1}, respectively. The sweeping-MEKC method has been successfully applied to the analysis of strychnine and brucine in *Strychnos nux-vomica* L. and its Chinese medicinal preparations.

Keywords: Micellar electrokinetic chromatography; Sweeping; Strychnine; Brucine

1. Introduction

Strychnine and brucine (their chemical structures are shown in Fig. 1) are extremely toxic alkaloids that exist in the seed of *Strychnos nux-vomica* L. and other species in genus *Strychnos*, which is frequently used as an important ingredient in traditional Chinese herbal medicines to treat nervous diseases, vomiting, arthritic and traumatic pains [1]. At low doses, *Strychnos* alkaioids exhibit high pharmacological activities. However, high doses of strychnine are known to be deadly poisonous, and sometimes can cause violent muscular convulsions [2]. Moreover, the content of the active ingredients in Chinese medicine varies to a considerable extent owing to the differences in the species, region of growth, season of harvest, and processing procedures. Often, *S. nux-vomica* L. needs to be properly processed so as to reduce its toxicity before being used in clinics. To better exploit the *Strychnos* plant and to ensure its safe use, it is necessary to establish a simple and economical approach for the determination of strychnine and brucine in *S. nux-vomica* L. and its Chinese medicinal preparations.

Several methods have been documented for the determination of strychnine and brucine, including high-performance liquid chromatography (HPLC) [3–5], liquid chromatography–mass spectrometry (LC–MS) [6], gas chromatography (GC) [7], GC–MS [8], H-1 nuclear magnetic resonance spectroscopy (^{1}H NMR) [9], thin-layer chromatography (TLC) [10], etc. However, some shortcomings exist in these methods, such as incomplete separation, long analysis time, expensive operation cost and so on. Meanwhile, in HPLC methods [3,4], unsatisfactory peak shapes were encountered for the alkaloids because they could be adsorbed strongly onto the packing materials of HPLC column, resulting in a fast column degradation or even an irreversible damage.

Nowadays, capillary electrophoresis (CE) is increasingly recognized as an important analytical tool and as a method of choice for the determination of macromolecules, drugs, natural compounds and various active components in medicinal plants, because of its high separation power, short analysis time, small

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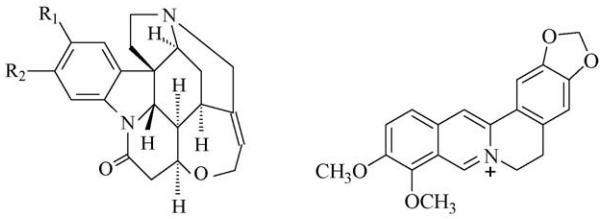

Fig. 1. Molecular structures of strychnine, brucine and berberine.

sample requirements and low operation cost. However, the main drawback of CE is the poor concentration sensitivity due to the small injection volumes and a short optical path length in the most commonly used UV detection. In general, the concentration detection limit of UV in CE is about two orders magnitude higher than that in HPLC [11].

To improve the concentration sensitivity of CE, several major on-line sample preconcentration methods have been explored, such as stacking [12,13], transient isotachophoresis (t-ITP) [14], dynamic pH junction [15,16], and sweeping [17,18]. These on-line concentration techniques have advantages of simplicity and economy because of no requirements of modification in CE instrumentation. However, t-ITP and dynamic pH junction methods are unsuitable for concentrating neutral compounds [19].

Sweeping, an on-line sample concentration technique, is defined as a phenomenon where analytes are picked up and concentrated by the pseudostationary phase that penetrates the sample zone containing no pseudostationary phase in micellar electrokinetic chromatography (MEKC). The sweeping phenomenon was firstly observed and described with anionic micelles and suppressed electro-osmosis in a low pH background electrolyte (BGE) [17]. With negative polarity applied, the anionic micelles present in cathodic vial enter the sample zone through its rear side and sweep neutral or weakly basic analytes that are slowly pushed against them due to the reduced electro-osmotic flow (EOF). A thin zone is formed as the result of the extraction of an analyte into the micellar phase [17,18].

In sweeping, both charged and neutral analytes can be preconcentrated, making it a versatile enrichment technique. In theory, it can provide almost unlimited detection sensitivity for analytes that have high affinities toward the pseudostationary phase. Up to 5000-fold sensitivity enhancement has been reported [17].

To date, a few of on-line sweeping methods have been developed for the on-line focusing of drugs or metabolites present in biological samples [20–24], but the studies concerning the determination of active ingredients in herbal plants by sweeping are still very few in the documents [25–27]. Capillary zone electrophoresis (CZE), the most commonly used CE mode, with either UV or MS detection has been employed for the determination of strychnine and brucine in Chinese medicines and in biological samples [28–31], but the sensitivities were relatively low. So it is necessary to develop an on-line concentration method to enhance the sensitivity of the detection. Recently, Hu and coworkers applied field-amplified sample stacking techniques for the detection of the *Strychnos* alkaloids in *S. nux-vomica* L., and a stacking efficiency of about 30-fold improvement with respect to the conventional sample injection was obtained [32]. Here, we report for the first time an on-line sweeping-MEKC method for the sensitive determination of strychnine and brucine in *S. nux-vomica* L. and its Chinese medicinal preparations.

2. Experimental

2.1. Apparatus

All CE experiments were performed on a Beckman P/ACE MDQ Capillary Electrophoresis System (Beckman Coulter, Fullerton, CA, USA), equipped with an auto sampler and a diode array detector (DAD). The temperature of the capillary tube during electrophoresis was maintained at 20 °C by a liquid coolant in the capillary cartridge. An uncoated fused-silica capillary (Yongnian Ruifeng Optical Fiber Factory, Yongnian, Hebei, China) of 50 cm (effective length, 40 cm) × 75 μm i.d. was used throughout the experiments. The DAD was set at 203 nm for detection. All of the operations were computer-controlled using Beckman P/ACE MDQ software. The pH of the solution was measured with a PHS-3C digital pH meter (Hangzhou Dongxing Instrument Factory, Zhejiang, China). The conductivities were measured using a Delta 326 conductivity meter (Mettler Toledo, Shanghai, China).

2.2. Chemicals and materials

Strychnine (98%), brucine (98%), and berberine hydrochloride (99%) were purchased from the National Institute for the Control of Pharmaceuticals and Biological Products of China, Beijing, China, and used without further purification. *S. nux-vomica* L. was obtained from Hebei Medical College for Continuing Education. *Shen jin huo luo wan*, which contains processed seeds of *S. nux-vomica* L. and other seven Chinese herbs, was purchased from Wanbaotang Medicine Store, Baoding, China. All other reagents used in the experiment were of analytical grade.

About 0.5 mol l^{-1} H_3PO_4 was first prepared in water and was diluted to different concentrations with an appropriate amount of water as needed. The pH of H_3PO_4 solutions was adjusted with 1.0 mol l^{-1} NaOH. The stock solution of 0.5 mol l^{-1} sodium dodecyl sulfate (SDS) was prepared every week in water. The water used throughout the work was double-distilled on a SZ-93 automatic double-distiller (Shanghai Yarong Biochemistry Instrumental Factory, Shanghai, China). All solutions were sonicated and filtered through 0.45 μm filter (Tianjin Automatic Science Instrument Co., Ltd., Tianjin, China) prior to use. The background solution (BGS) was 50 mmol l^{-1} H_3PO_4 (pH 2.0) containing 100 mmol l^{-1} SDS and acetonitrile in a ratio of 4:1 (v/v). The conductivity of the BGS was 9.32 mS cm^{-1}.

Berberine hydrochloride was used as the internal standard. The stock solution of berberine hydrochloride at 1.0 mg ml^{-1} was first prepared in 95% ethanol, which was diluted to 0.1 mg ml^{-1} with 95% ethanol prior to use. A mixture stock solution containing each of strychnine and brucine at 1.0 mg ml^{-1} was first prepared in 95% ethanol, which was then diluted