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Simultaneous electrochemiluminescence determination of galanthamine, homolycorine, lycorenine, and tazettine in Lycoris radiata by capillary electrophoresis with ultrasonic-assisted extraction

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ABSTRACT

After ultrasonic-assisted extraction, four lycoris radiata alkaloids: galanthamine, homolycorine, lycorenine, and tazettine were determined by capillary electrophoresis electrochemiluminescence. Polyvinylpyrrolidone was added to the running buffer (RB) to obtain better resolution. Experimental conditions influencing the determination were examined, including the additives, detection potential, separation voltage, injection voltage and time, and RB pH and concentration. Under optimal experimental conditions, the baseline separation of the four alkaloids occurred within 16 min. The proposed method displayed the following linear ranges (in ng/mL): galanthamine [60–5000], homolycorine [40–5000], lycorenine [5.0–1500], and tazettine [8.0–2500]. The detection limits in ng/mL, (S/N = 3), were galanthamine [14], homolycorine [11], lycorenine [1.8], and tazettine [3.1]. Intra-day and inter-day RSDs for the four alkaloids of the six replicates were less than 2.7% and 3.1%, respectively. The recoveries in% were: tazettine [102.5], lycorenine [98.20], galanthamine [97.30], and homolycorine [98.33].

1. Introduction

Lycoris radiata, the red spider lily, is used in traditional Chinese medicine. It contains several alkaloids, including galanthamine, homolycorine, lycorenine, tazettine, and pseudolycorine [1]. These physiologically, and biologically, beneficial alkaloids have several anti-cancer, anti-viral, anti-bacterial, analgesic and acetylcholinesterase (AchE) inhibitory effects, Lycoris radiata has become a focus of drug development research [2–6]. A lycoris radiata extract, galanthamine (Fig. S1a), has been shown effective in treating polio sequelae, enteroparalysis, and myasthenia gravis. It can also selectively inhibit cholinesterase activity, regulate brain nicotinic receptors, and improve class choline efficiency. It is effective in Alzheimer treatment and is superior to another AChE inhibitor [7,8]. Homolycorine (Fig. S1b) strongly suppresses human immunodeficiency virus-1 (HIV-1) replication in MT4 cells and has a marked antiproliferative effect on cancer cells. It may induce myelogenous leukemia-derived K562, acute lymphoblastic leukemia, and malignant melanoma apoptosis [9]. Lycorenine (Fig. S1c) lowers blood pressure and reduces heart rate [10,11]. Tazettine (Fig. S1d) inhibits mouse lymphoma cell growth and has been used to clinically treat cough, traumatic injuries, and toothache [12].

Various technologies have been used to extract lycorisi radiata alkloids. Ultrasonic extraction technology is quick, highly efficient, and energy-saving. It improves extraction rates through ultrasonic mechanical action and cavitation to increase speed, frequency and solvent penetration [13]. Research indicates that, HPLC [14,15], GC–MS [16] and ESI-IT-MS [17] can be used to analyze amaryllidaceous alkaloids. These methods have drawbacks, such as costly instrumentation, time-consuming procedures and large reagent use. Electrochemiluminescence (ECL), which provides electrochemical and chemiluminescent analysis, has become an important and powerful analytical application tool [18–20]. Compared with other reported methods, ECL detection coupled with capillary electrophoresis (CE) used to analyze amaryllidaceous alkaloids possesses several advantages including higher separation efficiency, shorter analysis time, less reagent consumption, easier operation, higher sensitivity, and wider linear range [21–27]. The method has been widely applied for pharmaceutical analysis [28–32]. The simultaneous determination of these four lycoris radiata alkaloids has not been reported. In this paper, ultrasonic-assisted extraction was used to obtain galanthamine, homolycorine, lycorenine, and tazettine in lycoris radiata. A CE-ECL method for simultaneously determining galanthamine, homolycorine, lycorenine, and tazettine in lycoris radia-

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ta has been developed. Conditions influencing separation and detection were examined in detail.

2. Experimental

2.1. Apparatus and reagents

The CE–ECL system (MPI-B) was produced by Xi'an Remex Electronic Science-Tech Co., Ltd. (Xi'an, China). It includes a digital readout, capillary electrophoresis high-voltage power supply, a multi-function chemiluminescence detector, a multichannel data collection analyzer, and a numerical control flow injection sample injector. The end-column ECL cell is a three-electrode system: a Pt disk as the working electrode; a Pt wire as the auxiliary electrode; and, Ag/AgCl (saturated KCl) as the reference electrode. A 50 cm long, uncoated fused-silica capillary (75 μm I.D) was obtained from Yongnian Optical Conductive Fiber Plant (Hebei, China). A HSJ-4A model pH meter (Shanghai Precision and Scientific Instrument Corporation, Shanghai, China), a SK3200H ultrasonic cleaner (Shanghai Kudos Ultrasonic Instrument Co., Ltd., Shanghai, China), a DZF-300 vacuum drying oven (Zhengzhou Greatwall Scientific Industrial and Trade Co., Ltd, Zhengzhou, China), and a TG16-W high-speed, refrigerated centrifuge (Hunan Xiangyi Instrument Centrifuge Instrument Co., Ltd., Changsha, China) were also used.

The standard samples of galanthamine and lycorenine were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) and the Shanghai Annaide Chemical Technology Center (Shanghai, China), respectively. The standard samples of homolycorine and tazettine were obtained from the Xi'an Baichun Biotech Co., Ltd. (Xi'an, China). Tris(2,2′-bipyridyl) ruthenium(II) chloride hexahydrate came from Alfa Aesar (Johnson Matthey, Ward Hill, MA, USA). Na_2HPO_4, Na_3PO_4, NaH_2PO_4, NaOH, anhydrous alcohol, Tween 80 (TW-80) and propyl alcohol were purchased from Xilong Chemical Co., Ltd. (Guangdong China). Polyvinylpyrrolidone (PVP) and sodium dodecyl sulfate(SDS) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The four alkaloid stock solutions were prepared using double-distilled water (DDW) with a concentration of 0.5 mg/mL and stored at 4 °C. A series of standard solutions with suitable concentrations were prepared by diluting the solution with DDW. The lycoris radiata was obtained from the Guangxi Institute of Botany (Guilin, Guangxi, China). All reagents used were analytical grade and DDW was used throughout. Prior to CE analysis, the sample solutions and the phosphate buffer solution (PBS) were filtered through 0.45 μm membrane filters (Shanghai Xinya Purification Material Factory, Shanghai, China).

2.2. Procedures

New capillaries were activated by filling them with 0.1 mol/L NaOH for 12 h. An activated capillary was then flushed with 0.1 mol/L NaOH for 10 min. This was followed by DDW for 10 min, and a corresponding RB for 10 min before initiating the analyses. Prior to use, working electrode surface was polished using 0.3 μm alumina powder and cleaned with DDW water in an ultrasonic cleaner. A Ru $(bpy)_3^{2+}$–phosphate solution was replaced every 2 h during the experiments in order to maintain good ECL measurement reproducibility. In all experiments, samples were introduced into the capillary by an electrokinetic injection at 13 kV for 10 s and then separated in the capillary at 10 kV. Detection potential was fixed at 1.20 V. The 10 mmol/L phosphates containing 0.2% PVP (pH 8.0) were used as a RB. The potential of the photomultiplier tube (PMT) was operated at 800 V with magnification set at 3.

2.3. Sample preparation

An ultrasonic-assisted extraction method, with some modifications, was used to extract lycoris radiata alkaloids [22]. A pulverized lycoris radiata sample was screened through a 100 mesh. A 0.10 g sample was then added to 10 mL ethanol. Extraction was performed in an ultrasonic cleaner at 140 W for 10 min. This solution was centrifuged at 3500 rpm for 10 min. The extraction was repeated twice. The extracts were combined and evaporated to dry under a dry nitrogen stream at 60 °C. The residue was dissolved in DDW and diluted to 25 mL. The sample solutions were filtered through 0.45 μm membrane filters and then analyzed.

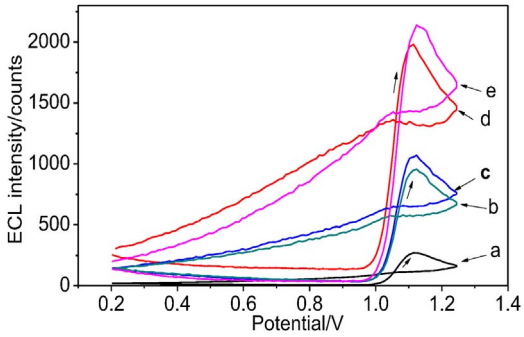

Fig. 1. Profile of ECL.Detection conditions: scan rate 100 mv/s; (a) 50 mmol/L pH 8.5 PBS containing 5 mmol/L $Ru(bpy)_3^{2+}$; (b) a + 1000 ng/mL galanthamine; (c) a + 1000 ng/mL homolycorine; (d) a + 1000 ng/mL tazettine; and, (e) a + 1000 ng/mL lycorenine.

3. Results and discussion

3.1. Electrochemical behavior of $Ru(bpy)_3^{2+}$ and four alkaloids

ECL intensity depended on chemical reaction light emission rates. ECL response to galanthamine, homolycorine, lycorenine, and tazettine in a $Ru(bpy)_3^{2+}$–PBS was examined (Fig. 1). ECL intensity was weak when only $Ru(bpy)_3^{2+}$–PBS was present in a ECL detection cell (Fig. 1a). ECL intensity increased when either tazettine, lycorenine, galanthamine, or homolycorine was added to the ECL detection cell containing $Ru(bpy)_3^{2+}$-PBS. These observations indicate that the alkaloids could be measured with an ECL assay.

3.2. Medium effects on ECL intensity

When using electrokinetic injection, capillary injection port resistance can be significantly greater than intra-capillary resistance if the sample medium is either water, or a low-concentration buffer solution. Injection amounts also increase with electric-field intensity increase at the injection port which improves detection sensitivity [33]. To investigate the effects of a standard solution medium on ECL intensity, DDW and 5 mmol/L PBS were used to prepare the four alkaloids standard solutions, respectively. Experimental results showed that ECL intensities for the four alkaloids solution prepared, at the same concentration, with DDW were ten times greater than solutions prepared with a 5 mmol/L phosphate buffer solution at pH 8.0.

3.3. Effect of additives

Galanthamine, homolycorine, lycorenine, and tazettine have similar structures which complicates separation. The effects of running buffers at pH 6.0, 7.0, 8.0, 9.0 on separation were performed while maintaining a 10 mmol/L buffer concentration. The four analytes could not be separated by Changing RB pH (Fig. 2). Adding 0.1% TW-80, 0.1% SDS, 0.1% PVP, and 20% propyl alcohol to the RB solution (pH 8.0) was tried. Adding either 0.1% SDS, or 20% propyl alcohol, did not result in analyte separation. Adding 0.1% PVP, or 0.1% TW-80 as an additive, it would be done (Fig. 3). The effects of 0.2% PVP, 0.2% TW-80, 0.3% PVP, and 0.3% TW-80 on resolution (R) and ECL intensity were also