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Determination of mannitol and three sugars in *Ligustrum lucidum* Ait. by capillary electrophoresis with electrochemical detection

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Abstract

A method based on capillary electrophoresis with electrochemical detection has been developed for the separation and determination of mannitol, sucrose, glucose, and fructose in *Ligustrum lucidum* Ait. for the first time. Effects of several important factors such as the concentration of NaOH, separation voltage, injection time, and detection potential were investigated to acquire the optimum conditions. The detection electrode was a 300 μm diameter copper disc electrode at a working potential of +0.65 V (versus saturated calomel electrode (SCE)). The four analytes can be well separated within 13 min in a 40 cm length fused-silica capillary at a separation voltage of 12 kV in a 75 mM NaOH aqueous solution. The relation between peak current and analyte concentration was linear over about three orders of magnitude with detection limits ($S/N = 3$) ranging from 1 to 2 μM for all analytes. The proposed method has been successfully applied to monitor the mannitol and sugar contents in the plant samples at different growth stages with satisfactory assay results.

Keywords: *Ligustrum lucidum* Ait.; Mannitol; Sucrose; Glucose; Fructose; Capillary electrophoresis; Electrochemical detection

1. Introduction

In the present, more and more people are becoming interested in plant medicines, because of their low toxicity and good therapeutical performance. Chinese traditional medicine, *Fructus Ligustri Lucidi*, is the dried ripen fruit of *Ligustrum lucidum* Ait. As a commonly used herbal medicine, it has the therapeutical functions of nourishing liver and kidney, stimulating heart, enhancing organism immunization, diuresis, laxation, anti-inflammatory, etc. [1,2]. The leaf of *L. lucidum* Ait. has also been used as a Chinese traditional herbal drug with functions of anti-bacteria, anti-inflammation, and suppressing cough [2]. A variety of physiologically active compounds (such as ligustroside, nuzhenide, oleanolic acid, ursolic acid, betulin, mannitol, glucose, quercetin, etc.) have been found presented in *L. lucidum* Ait. Different functions of the herbal drugs come from different active constituents. [2]. It has been demonstrated that the diuresis and laxation activities of *Fructus Ligustri Lucidi* can be attributed to mannitol, a commonly used diuretic or laxative [2,3]. The mannitol content is an important parameter for evaluating the quality of *Fructus Ligustri Lucidi* [3]. As the primary metabolites, sucrose, glucose, and fructose are found widely presented in plants. Han et al. have found that higher contents of sugars can indicate the better quality of some herbal drugs [4]. Hence, it is interesting to establish some simple, economical and accurate methods for the determination of mannitol, sucrose, glucose, and fructose in *L. lucidum* Ait.

Li and Liu have determined mannitol in *Fructus Ligustri Lucidi* by spectrophotometry for quality control [3]. Recently, Liu et al. have reported an approach for the simultaneous determination of oleanolic acid and ursolic acid in this plant medicine based on micellar electrokinetic capillary chromatography [5]. In addition, thin layer chromatogra-

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phy (TLC) [6] and high performance liquid chromatography (HPLC) [7,8] have been employed for the determination of oleanolic acid, salidroside, specnuezhenide and other water-soluble active constituents in the fruits of *L. lucidum* Ait. Capillary electrophoresis (CE) has been widely used in the analysis of sugars [9–13]. The advances in separation and detection approaches of derivatized and underivatized carbohydrates are reviewed by El Rassi [14]. Nowadays, the application of CE for the separation of various active constituents in medicinal plants has become increasingly widespread because of its minimal sample volume requirement, short analysis time and high separation efficiency [15,16]. Electrochemical detection (ED) typically operated in the amperometric mode can be coupled with CE to provide high sensitivity and selectivity for the determination of electroactive substances [17–19]. Direct electrooxidation at platinum or gold electrodes has gained some popularity for the detection of underivatized carbohydrates [20,21]. To reduce the fouling of platinum and gold electrodes, the applied potential for these electrodes must be continuously pulsed based on a complicated procedure. Alternatively, the work on copper electrode by Baldwin and co-workers is of particular interest for the direct detection of carbohydrates in strongly alkaline medium where signal responses are achieved at constant potential without any electrode fouling [12,22]. This detection approach is less complicated, less expensive, but still sensitive. It can be employed to detect not only carbohydrates themselves but also their derivatives such as alditols and sugar acids [12].

In 2000, U.S. Food and Drug Administration (FDA) published a draft of Guidance for Industry Botanical Drug Products. Before a plant drug becomes logically marketed, its spectroscopic or chromatographic fingerprints and chemical assay of characteristic markers are required. CE should find more applications in this area. In this study, CE-ED was employed for the determination of mannitol, sucrose, glucose, and fructose in *L. lucidum* Ait. without derivatization. It has been found that the mannitol and sugar contents changed at different growth stages. This method is simple, sensitive, reliable, and efficient providing not only a way for evaluating the quality of plant medicines made from *L. lucidum* Ait. in marketplaces, but also an excellent method for quality control in medicinal factories and constituent investigation of other plants. To our best knowledge, there are no reports published on the determination of mannitol and sugars in *L. lucidum* Ait. by CE. The optimization, detailed characterization, and advantages of CE-ED approach are reported in the following sections in connection to the measurement of mannitol and three sugars.

2. Experimental

2.1. Reagent and solutions

Mannitol, sucrose, glucose, and fructose were all purchased from Sigma (St. Louis, MO, USA) and were used as received without further purification. The fruits and leaves of *L. lucidum* Ait. (Glossy Privet) were collected from the east campus of Medical Center of Fudan University (Shanghai, China) and were kindly identified by Professor D. Chen (Department of Pharmacognosy, Medical Center of Fudan University, Shanghai, China). All aqueous solutions were made up in doubly distilled water. Other chemicals were of analytical grade.

Stock solutions of mannitol, sucrose, glucose, and fructose (50 mM) were prepared in doubly distilled water and were kept in a 4 °C refrigerator. They were stable for at least 1 month. The electrophoretic separation medium was 75 mM NaOH aqueous solution unless mentioned otherwise. The stock solutions were diluted to desired concentration with the separation medium just prior to use.

2.2. Apparatus

The CE-ED system used has been described previously [23]. A ±30 kV high-voltage dc power supply (Shanghai Institute of Nuclear Research, China) provided a separation voltage between the ends of the capillary. The inlet of the capillary was held at a positive potential and the outlet of capillary was maintained at ground. The separations were proceeded in a 40 cm length of 25 μm i.d. and 360 μm o.d. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA).

A three-electrode electrochemical cell consisting of a laboratory-made 300 μm diameter copper disc working electrode, a platinum auxiliary electrode and a saturated calomel electrode (SCE) as the reference electrode, was used in combination with a BAS LC-4C amperometric detector (Bioanalytical Systems Inc., West Lafayette, IN, USA). The filter of the detector was set at 0.1 Hz. The working electrode was positioned carefully opposite the outlet of the capillary with the aid of a micromanipulator (CORRECT, Tokyo, Japan) and arranged in a wall-jet configuration. The distance between the tip of the working electrode and the capillary outlet was adjusted to ∼25 μm by comparison with the bore (25 μm) in the capillary while being viewed under a microscope. The electropherograms were recorded using a LKB·REC 1 chart record (Pharmacia, Sweden). A YS 38-1000 220 V alternate constant-voltage power supply (Shanghai Instrumental Transformer Factory, Shanghai, China) was employed to suppress the voltage fluctuation of the power line. The whole system was assembled in a 10 m^2 Faraday room that was air-conditioned at 20 °C to minimize the effects of external noise sources.

2.3. Sample preparation

Three batches of fruits and leaves of *L. lucidum* Ait. were collected from the same tree on different days and dried in the air. The batch numbers (031110, 031130, and 031220) were given based on the collection dates. All plant samples were dried at 60 °C for 3 h and then were pulverized. An accurate