Available online at www.sciencedirect.com
ScienceDirect
Analytica Chimica Acta 581 (2007) 298–302
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

An effective method for fast determination of artemisinin in *Artemisia annua* L. by high performance liquid chromatography with evaporative light scattering detection

Chun-Zhao Liu [a,b,*], Hua-Ying Zhou [a,b], Yan Zhao [a,b]

[a] *National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100080, PR China*
[b] *Graduate School of the Chinese Academy of Sciences, Beijing 100049, PR China*

Received 16 May 2006; received in revised form 16 July 2006; accepted 21 August 2006
Available online 26 August 2006

Abstract

Artemisinin isolated from the aerial parts of *Artemisia annua* L., is a promising and potent antimalarial drug, which meets the dual challenge posed by drug-resistant parasites and rapid progression of malarial illness. The aim of the current study was to develop a reliable and fast analytical procedure for the determination of artemisinin in *A. annua* using high performance liquid chromatography (HPLC) with evaporative light scattering detection (ELSD) in couple with microwave-assisted extraction (MAE) as an efficient sample preparation technique. The HPLC conditions were Agilent C18 column using water:acetonitrile (40:60 v/v) mixture as mobile phase at a flow rate of 1 mL min^{-1}. ELSD conditions were optimized at nebulizer-gas flow rate of 2.0 L min^{-1} and drift tube temperature of 70 °C under the impactor off-mode, and the gain was set at 2. Afterwards, method validation system for HPLC–ELSD analysis was developed. Calibration range was 0.2–1.0 mg mL^{-1} and correlation coefficient r was above 0.9990. Precision experiments showed relative standard deviation (R.S.D.) of retention time was less than 0.5% and R.S.D. of peak area was less than 1.30%. Inter-day and intra-day variabilities showed that R.S.D. was ranged from 1.01% to 4.66%. Limit of detection was less than 40 μg mL^{-1} and limit of quantification was less than 100 μg mL^{-1}. Accuracy validation showed that average recovery was between 98.23% and 104.97%. The developed analytical procedure was successfully applied to determine the contents of artemisinin in the different parts of *A. annua* plants.

Keywords: Evaporative light scattering detection; High performance liquid chromatography; Microwave-assisted extraction; Artemisinin; *Artemisia annua* L

1. Introduction

Artemisinin, a sesquiterpene lactone with an endoperoxide oxygen bridge across the seven member rings (Fig. 1), is derived from *Artemisia annua* L. [1]. The artemisinin class of drugs is considered to be the most promising compounds in the search for treatment of drug-resistant malaria. They have become increasingly popular in Asia over the last 10 years [2]. Nowadays, artemisinin and its derivatives are considered as part of the ideal strategy for malaria in Africa by WHO [3].

Various methods have been proposed and assessed to detect and quantify artemisinin. Thin layer chromatography is not a reliable technique to quantify artemisinin due to the poor staining characteristics of the intact molecule and interference with other constituents of the plants [4]. Gas chromatography (GC) and GC combined with mass spectrometry (MS) have also been applied for the analysis of artemisinin, however, artemisinin is not stable under high column temperature (higher than 200 °C) because artemisinin usually degrades into number of products when heated at 180–200 °C [5,6]. Due to the absence of appropriate UV absorption, an alkaline hydrolyzed derivation is needed to convert artemisinin into a UV active compound before HPLC–UV analysis [7]. Therefore, UV detection process of artemisinin is time consuming, and hampers the results by deriving the other compounds present in the crude extract. HPLC with diode array detection (DAD) was proposed to analyze an alkaline hydrolyzed derivative of artemisinin [8], but DAD is sensitive to the variation of mobile phase and the temperature of the column, so its sensitivity and reproducibility remain a problem.

* Corresponding author. Tel.: +86 10 82622280; fax: +86 10 82622280.
E-mail address: czliu@home.ipe.ac.cn (C.-Z. Liu).

0003-2670/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2006.08.038

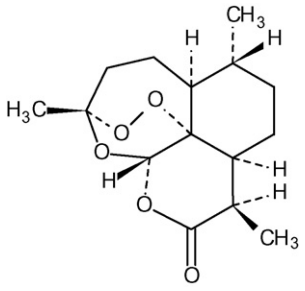

Fig. 1. The chemical structure of artemisinin.

Evaporative light scattering detection (ELSD) is increasingly being used coupled with HPLC during the last decade. The principle of its operation involves nebulizations of the column effluent to form an aerosol, followed by solvent evaporation in a heated drift tube and then the detection of the remaining non-volatile solute particles in the light scattering cell [9]. ELSD is a kind of mass and non-selective detector, it can analyze all samples which have low volatility than mobile phase, especially for non-UV-absorbing phytochemicals [10].

The conventional sample preparation methods used for HPLC analysis of artemisinin are room temperature extraction, heat-reflux extraction and Soxhlet extraction. Microwave-assisted extraction (MAE) has been used for extraction of interested components from a wide variety of sample matrices and has been used as an alternative sample preparation technique for a number of applications [11–14]. Compared to conventional methods, MAE can considerably reduce both extraction time and solvent consumption [15,16].

The aim of this work is to develop a valuable method for rapid determination of artemisinin content in *A. annua* plant by HPLC–ELSD in coupled of rapid sample preparation process with automatic microwave-assisted extraction.

2. Experimental

2.1. Materials and reagents

A. annua L. plant was kindly provided by local company in Sichuan province. Artemisinin standard was purchased from Sigma (USA). Acetonitrile and methanol (HPLC grade) were purchased from Cakedib Laboratories Ltd. (Georgetown Ont., Canada). Ultra-pure water was produced by Milli-Q system (Millipore, 18.2 Ω, Bedford, MA, USA). Other reagents used were analytical grade purchased from local chemical company.

Artemisinin stock solutions were prepared by dissolving 10 mg artemisinin in 10 mL methanol and stored at −20 °C. The standard working solutions at the concentration of the calibration range were prepared by serial dilutions of stock solutions with methanol.

2.2. HPLC conditions

The Agilent 1100 HPLC system was equipped with a quaternary pump (G1311A), an on-line solvent vacuum degasser (G1379A) and an auto-sampler with 20 μL injection loop (G1313A). The detector was 2000ES (Alltech, USA). The data were required and processed by means of HP chemstation for LC software. The proposed HPLC–ELSD method was performed on an Agilent C18 column (150 mm × 4.6 mm i.d., 5 μm) fitted with an Alltech C18 guard cartridge (8 mm × 4.6 mm i.d., 5 μm). The column temperature was set at 30 °C. The mobile phase was water:acetonitrile (40:60 v/v) at a flow rate of 1 mL min^{-1}. ELSD conditions were optimized in order to achieve maximum sensitivity under impactor off-mode, nebulizer-gas flow rate 2.0 L min^{-1}, drift tube temperature 70 °C and gain 2.

2.3. Sample preparation for HPLC analysis

Leaves, flower buds, stems and roots were collected from *A. annua* plant separately. All the samples were milled to 60 mesh powder before extraction. The extraction solvent was petroleum ether–acetone (4:1 v/v). For room temperature extraction, 10 g *A. annua* sample was extracted in a sealed flask (250 mL) containing 100 mL extraction solvent under room temperature for a given time. Heat-reflux extraction using a water-bath was performed with 10 g *A. annua* sample and 100 mL extraction solvent in a flask (250 mL) with mechanical stirrer. The temperature was kept at 50 °C. For Soxhlet extraction, 10 g *A. annua* sample was put into 200 mL Soxhlet thimble which was fitted with 250 mL round bottom flask containing 100 mL extraction solvent. The temperature was kept at 50 °C. A household microwave oven was modified in our laboratory with the addition of a magnetic stirrer, water condensor, temperature measurement and time controlling for automatic microwave-assisted extraction [17]. Ten grams of *A. annua* sample was mixed with 100 mL extraction solvent, and the suspensions were irradiated automatically with microwave in a pre-setting procedure for 40 min under 700 W. Microwave power was switched on to reach the desired temperature of about 50 °C and then for heated for 2 s and the power was switched off for cooling for 6 s.

After extraction using various methods, all supernatants were centrifuged at 4250 × *g* for 5 min, then filtered through 0.45 μm membrane before HPLC analysis. In the present work, the percentage extraction of artemisinin was defined as follows: percentage extraction of artemisinin (w/w) = mass of artemisinin extracted/mass of material (*A. annua* sample) × 100%.

3. Results and discussion

3.1. Optimization of the chromatographic system

To effectively separate artemisinin from other compounds in the *A. annua* sample, water:acetonitrile mixture was used as HPLC mobile phase. Different ratios (from 80:20 to 30:70) were assayed at a flow rate of 1 mL min^{-1} for peak separation. The increase of solvent volatility reduces the droplets condensation of the wall of nebulization chamber, which enhances the ELSD response [18]. The best resolution and sensitivity was obtained by using water:acetonitrile (40:60 v/v) mixture. The injection volume was set at 10 μL. Under proposed chromatographic conditions, the total time between injections was 12 min (Fig. 2).