



Silver ion chromatography for peak resolution enhancement: Application to the preparative separation of two sesquiterpenes using online heart-cutting LC-LC technique

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ARTICLE INFO

Keywords:

Positional isomers
Argentation
Peak resolution enhancement
Heart-cutting LC-LC
Preparative separation
Natural product

ABSTRACT

Silver ion chromatography, utilizing columns packed with silver ions bonded to silica gel, has proved to be an invaluable technique for the analysis of some positional isomers. In this work, silver ion chromatography by combination with online heart-cutting LC-LC technique for the preparative separation of two sesquiterpenes positional isomers from a natural product was investigated. On the basis of the evaluation that silver ion content impacts on the separation, the laboratory-made silver ion columns, utilizing silica gel impregnated with 15% silver nitrate as column packing materials, were used for peak resolution improvement of these two isomers and the preparative separation of them in heart-cutting LC-LC. The relationship among the maximal sample load, flow rate and peak resolution in the silver ion column were optimized, and the performance of the silver ion column was compared with conventional C₁₈ column and silica gel column. Based on the developed chromatographic conditions, online heart-cutting LC-LC chromatographic separation system in combination with a silica gel column and a silver ion column that was applied to preparative separation of these two isomers from a traditional Chinese medicine, *Inula racemosa* Hook.f., was established. The results showed that the online heart-cutting LC-LC technique by combination of a silica gel column and a silver ion column for the preparative separation of these two positional isomers from this natural plant was superior to the preparative separation performed on a single-column system with C₁₈ column or silica gel column.

1. Introduction

Silver ion chromatography, utilizing columns packed with silver ions bonded to a silica gel or similar substrate, has proved to be an invaluable technique for the analysis of complex triacylglycerol mixtures, fatty acids, lipids and some positional isomers [1–4]. This application based on the mechanism that the different number, or/and geometrical configuration, or/and position of unsaturated C-C bonds provide the different amount of π donors. Since silver ions act as π acceptors while unsaturated C-C bonds act as π donors, causing molecules that contain fewer π donors or do not contain π donors to be eluted firstly on silver ion chromatography [5].

Although silver ion chromatography by a single-column system is inexpensive and easy to operate for the trace separation and determination of active ingredients, or preparative separation compounds from simple sample on small-scale [6], it still restricts to the large-scale preparative separation of compounds from complex natural products.

Due to adsorption of excessive impurities, the action of silver ions becomes worse, causing the decrease of column separation efficiency, even resulting in bleeding of silver ions and affecting the UV detector. In addition, a single-column system sometimes has limited sample capacity and poor relative peak separation [7]. Notably, heart-cutting two-dimensional liquid chromatography (LC-LC) offers the probability to improve these drawbacks based on a tandem combination of two independent liquid phase separation systems [8–11].

Inula racemosa Hook.f., a medicinal plant, is widely distributed in Europe, Asia and Africa [12]. Its root as a traditional medicine has been most frequently applied for issues related to peptic disorders, phlegm, detumescence, inflammatory and vermifuge [13]. Alantolactone (AL) and isoalantolactone (IS) (Fig. 1) are two sesquiterpenes positional isomers and have abundant content in this medicinal plant. Previous studies suggested that they show many biological activities [14–25], especially in killing of cancer cells. In addition, researchers also have examined the relationship between their structural modifications and

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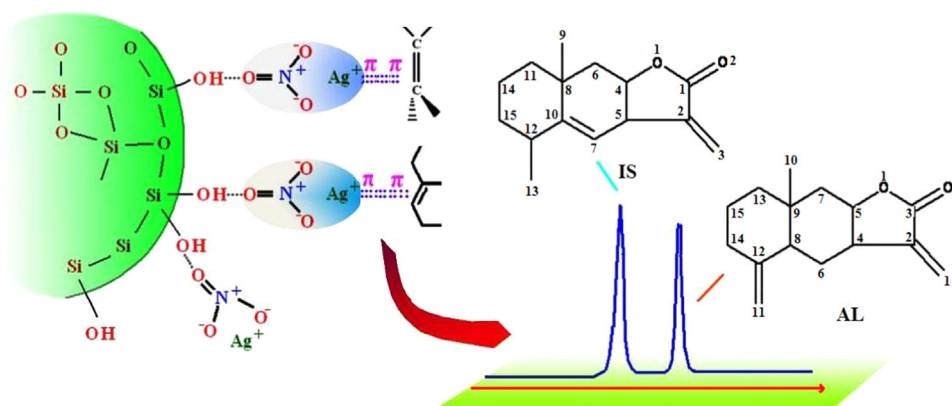


Fig. 1. Schematic illustration of silver ion chromatography for the separation of alantolactone (AL) and isovalantolactone (IS), and their chemical structures.

biological activities [26–28]. These studies suggested that AL and IS may be good potential lead compounds for future anticancer agent development or act as chemical templates for the design, synthesis, and semisynthesis of new substances. Nevertheless, further studies are required in preclinical and clinical applications and to explain their potential role. For these studies, it certainly will need a large number of materials. In the current ways, acquisition of AL and IS from natural plants is a short-cut. Therefore, a robust method for the preparative separation of AL and IS from this medicinal plant may be necessary. Unfortunately, the structural similarity of AL and IS increases the preparative separation difficulty of them from this natural plant. Although the traditional separation methods used in the trace separation and determination of these two compounds have been performed with capillary electrophoresis (CE) [29], gas chromatography (GC) [30] and RP-HPLC [31,32], when these methods are used to isolate AL and IS from *Inula racemosa* Hook.f. on a larger scale, it becomes difficult due to the limitation of the resolution between their peaks. Hence, the establishment of an effective method for increase of the peak resolution and its application to their preparative separation from this plant becomes a key problem. Since AL and IS constitute a pair of positional isomers related to C=C bond, silver ion chromatography provides the probability to improve the separation of them (Fig. 1). Therefore, the aim of this study was to improve the separation of these two isomers by silver ion chromatography in preparative scale and obtain them from a crude extract of *Inula racemosa* Hook.f. by preparative separation with silver ion chromatography technique in combination with online heart-cutting LC-LC technique.

2. Experimental

2.1. Materials and reagents

The dried roots of *Inula racemosa* Hook.f. were purchased from Bai Ding (Tibet, China). Alantolactone and isovalantolactone were

purchased from National Institute of Food and Drug Control for the quantification and qualitative study (Beijing, China). Silver nitrate was of chemical grade (Zhengzhou Kaidi Chemical Products Co., Ltd, Henan, China). Silica gel (5 ~ 10 μm particles and 10 ~ 40 μm particles) was purchased from Qingdao Yida Silica Reagent Factory (Fujian, China). Ethyl acetate, n-hexane, 95% ethanol and acetonitrile reagents were of analytical grade and purchased from Xi'an Chemical Reagent Factory (Shaanxi, China).

2.2. Sample preparation

The dried roots of *Inula racemosa* Hook.f. were pulverized and sieved through a screen (100 ~ 200 mesh). 100 g of this powder was extracted with 1000 mL of 95% ethanol solution by ultrasonic treatment for 30 min at 45 °C and 100 kHz/450 W (Bandelin Sonorex, Germany) [33] and repeated three times. The extracts were concentrated on a rotavapor at 45 °C (REC32E, Shanghai Yarong Instrument, China). The residues were dissolved in n-hexane/ethyl acetate (100 mL: 70/30, v/v) solution to get sample solutions for the preparative separation, and the contents of AL and IS in this sample solution were 25.62 mg/mL and 31.32 mg/mL, respectively.

The stock solution for the analysis of AL and IS was prepared by dissolving AL and IS into n-hexane/ethyl acetate (70/30, v/v) and mixing at concentration containing AL of 25.86 mg/mL and IS of 30.26 mg/mL.

2.3. Column packing

The laboratory-made silver ion column, utilizing silica gel impregnated with silver nitrate as column packing materials, was prepared for the preparative separation in heart-cutting LC-LC. The silica gel impregnated with silver nitrate was prepared by the method reported previously with slightly modification [34] (Fig. 2). To avoid waste and the risk of blindness, the silver ion content in silica gel

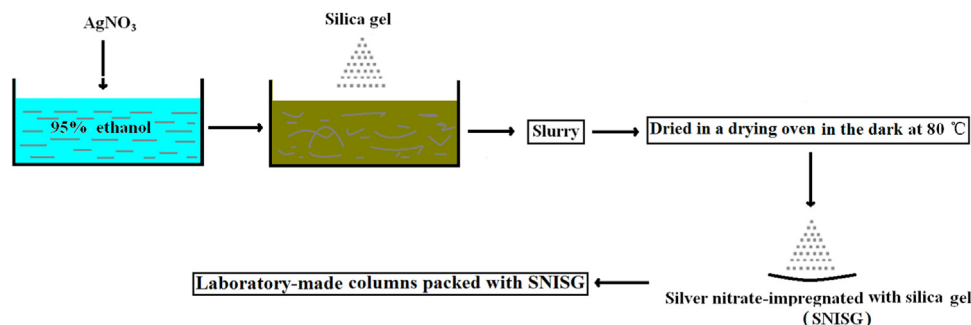


Fig. 2. Schematic illustration of the preparation of silver ion columns packed with silica gel impregnated with silver nitrate (SNISG).

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