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A feasible scaling-up separation of platycosides from Platycodi Radix: From analytical to semi-preparative high performance liquid chromatography coupling with a post-separation flash freezing treatment to obtain highly unstable components

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ABSTRACT

A method of using semi-preparative HPLC coupling with a post-HPLC flash freezing treatment to separate platycosides from Platycodi Radix is developed. Nine platycosides including unstable acetyl platycosides were separated from Platycodi Radix by semi-preparative HPLC. Separation variables of flow rate and loading amount of sample were linearly scaled up from analytical HPLC to semi-preparative HPLC. Variables of semi-preparative HPLC separation including flow rate and loading amount of sample were calculated as 29.8 mL/min and 297.7 mg, respectively. Under the optimized conditions of separation, nine platycosides were simultaneously separated on semi-preparative scale within 1 h. Separation resolutions of the peaks of platycosides were all above 0.85, of which seven platycosides were separated with resolutions above 1.00. With regards to sample pretreatment before chromatography separation, we propose a post-HPLC treatment to the unstable compounds. A post-HPLC treatment by using liquid nitrogen to flash freeze the eluent fractions from semi-preparative HPLC was used to stop the unstable platycosides transformation. By freezing treatment, the pure unstable acetyl platycosides were obtained without transformation. This was the first time that unstable acetyl platycosides were simultaneously separated by preparative HPLC. This method is fast and efficient to separate platycosides from Platycodi Radix, especially for purifying unstable platycosides.

1. Introduction

Platycodi Radix is frequently used as food and herbal medicine in East Asia. It mainly contains triterpenoid saponins, which are also well known as platycosides. Up to now, more than 50 platycosides have been isolated and identified from Platycodi Radix. It has been reported that platycosides exhibited extensive biological activities including hepatoprotective [1–3], anticancer [4–7], antioxidant [8,9], and anti-obesity effects [10,11]. Owing to its extensive benefits to human health, Platycodi Radix has attracted much interest from researchers. However, the complexity of the chemical composition of this herb makes it difficult to prepare sufficient pure platycosides to meet demand for both academic research and industrial purpose. Moreover, separation and purification of the unstable acetyl platycosides from Platycodi Radix are impeded under regular separation protocols [12].

It's been reported that high speed counter-current chromatography (HSCCC) was a useful method to separate pure platycosides, especially for acetyl platycosides, from Platycodi Radix [13,14]. However, the long run-time, relatively low resolution and the complicated procedures of solvent-system optimization made HSCCC a low efficient method for preparative separation of platycosides from raw origin [15,16]. Compared with HSCCC, the semi-preparative high pressure liquid chromatography (semi-PHPLC) could be an efficient method to rapidly separate platycosides from Platycodi Radix due to higher resolution of separation, higher purity of products and less run-time [17]. However, mobile phase of reverse phase semi-PHPLC generally consists of polar solvents such

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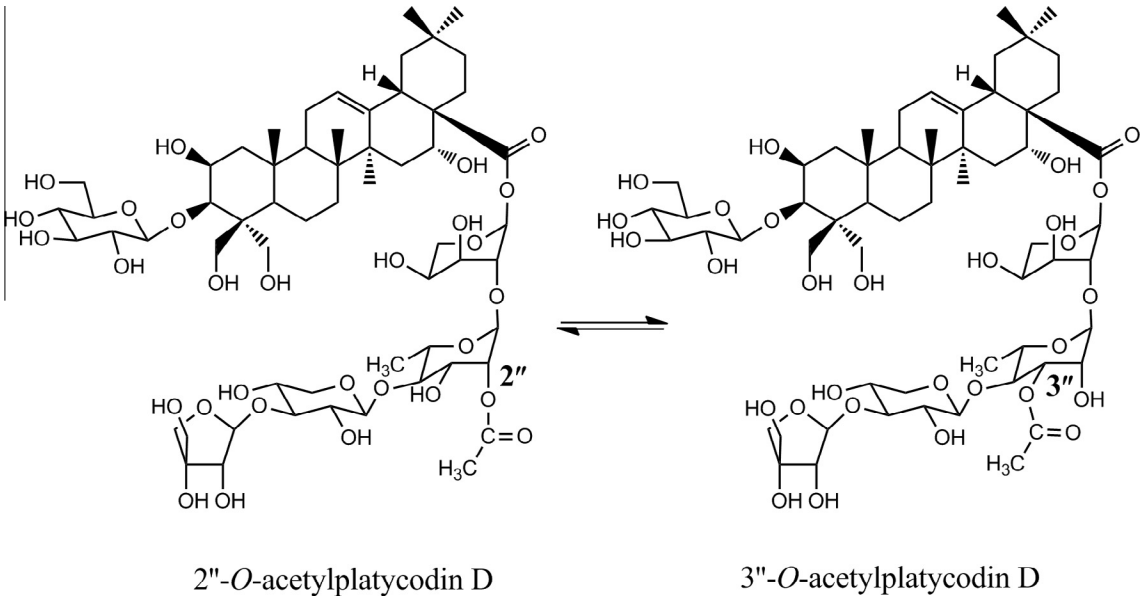

Fig. 1. Inter-conversion of 2″-*O*-acetylplatycodin D (2aPD) and 3″-*O*-acetylplatycodin D (3aPD).

as methanol and water, which might lead to the acetyl platycosides unstable (Fig. 1 shows inter-conversion of typical acetyl platycosides).

According to our previous investigation [18], the pure acetyl platycosides readily undergoes the acetyl transfer reaction in polar solvents, and the activation energy and enthalpy of the reaction were respectively 63.01 and 7.48 kJ mol^{-1}, which indicated that acetyl platycoside readily transformed to its acetyl isomer (Fig. 2). Naturally occurring acetyl esterification is common substitution in sugar chain [19,20], especially in cell wall matrix polysaccharides. The acetyl substituent on sugar chain plays an important role in protection of cellular polymers against enzymatic degradation and as a potent inhibitor in microbial fermentations [21]. The acetyl migration is a common phenomenon in natural products [22–24], but seldom research focuses on investigation of the mechanism of the acetyl transfer reaction or the separation of the acetyl isomers from biological matrix.

As well known, suitable sample pretreatment techniques are quite important for proper separation and enrichment of target components from complicated sample matrices. While seldom research focuses on post chromatographic separation treatment of the elution fractions which contain unstable compounds.

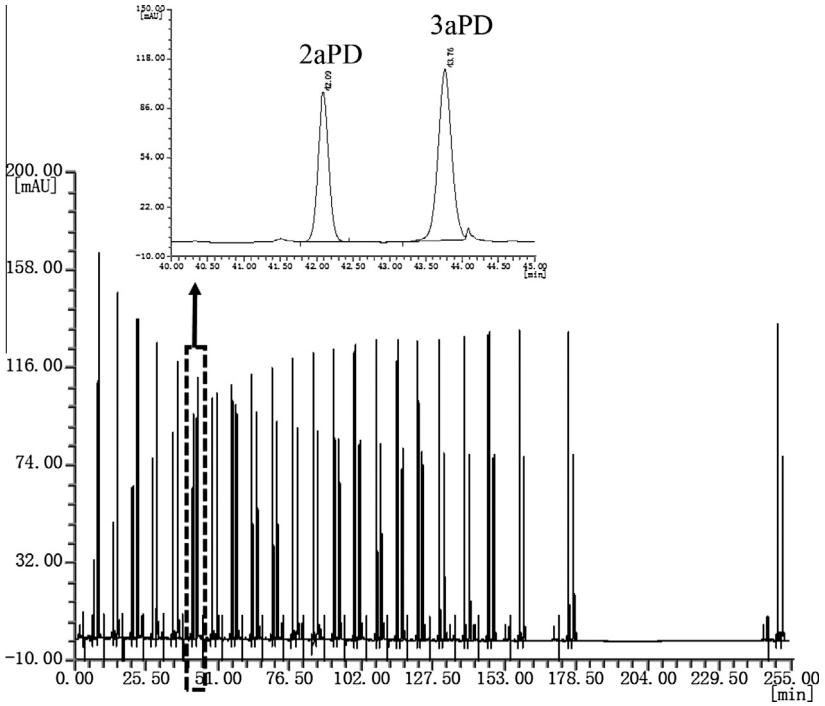

Fig. 2. HPLC-UV chromatogram of 2″-*O*-acetylplatycodin D gradually transformed to 3″-*O*-acetylplatycodin D (0.7 mg/mL 2″-*O*-acetylplatycodin D in 30 °C water was continuously injected into an Agilent Zorbax SB-Aq RP-C_{18} column (250 mm × 4.6 mm, 5μ) at a flow rate of 1 mL/min under 210 nm).