Supplementary data 2

In this study, we investigated two kinds of pre-treatment methods, included protein precipitation method and liquid-solid extraction method.

1. Method

2.1 protein precipitation method.

An aliquot of 200 μL rat plasma (the concentration of standards were 200ng/mL) was transferred to a 1.5 mL plastic tube for processing, 2 μL I.S. and 0.8 mL methanol (or acetonitrile) was added. The tube was vortex-mixed for 30s and centrifuged at 10,000 rpm for 10 min. The supernatant was then transferred into another 1.5 mL plastic tube and evaporated to dryness undera gentle stream of N_2 at 30℃ using a Zymark TurboVap LV drying system. The residue was reconstituted in 200 μL methanol-water (1:4, v/v) solution and centrifuged at 10000 rpm for 10 min. The supernatant was then injected into the LC-MS/MS system for analysis. As shown in Fig. 1.

2.2 Liquid-solid extraction method

An aliquot of 200 μL rat plasma (the concentration of standards were 200ng/mL) was transferred to a 1.5 mL plastic tube for processing. 2 μL I.S. was added solution. After vortexing for 30s, 101 μL treated plasma sample was added and flowed through the SPE Cartridg (including C_{18} and HLB) which was preconditioned with methanol and then water. The cartridge was washed with 1.5 mL water, sucked dry and eluted with 1.2 mL aliquots of methanol, the flow rate was 1mL/min. The eluate was evaporated to dryness undera gentle stream of N_2 at 30℃ using a Zymark TurboVap LV drying system (Westborough, MA, USA). The residue was reconstituted with 100 μL methanol-water (1:4, v/v). 10 μL of the sample was injected into the LC–MS/MS system for analysis. As shown in Fig. 2.

2. Results and discussion

From the Fi.1, we could found polygalaxanthone III (a), tenuifolin (b), tenuifoliside C (d) and (e) forsythin, each of them had a weak response, and we didn't found tenuifoliside A (c) in the appropriate retention time. In Fig.2, each of analytes had a high response in the chromatogram, even using C_{18} or HLB cartridge. Through the comparison of the two kinds of sample pre-treatment methods, and combined the economic factors, the liquid-solid extraction method used C_{18} cartridge was finally selected in this study.

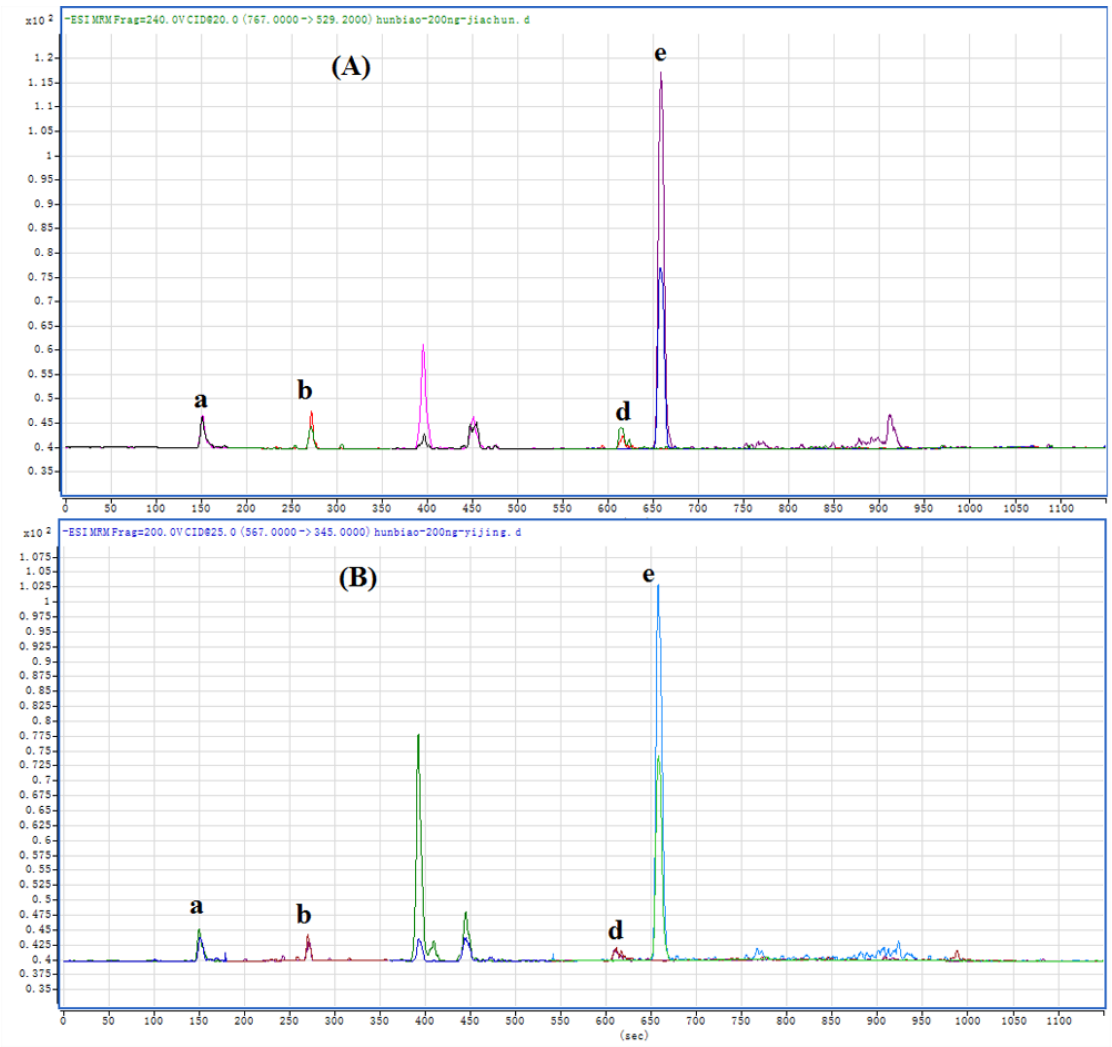

Fig.1. Representative MRM chromatograms of polygalaxanthone III (a), tenuifolin (b), tenuifoliside C (d) and IS (e) in rat plasma by protein precipitation method. (A) Methanol; (B) Acetonitrile.

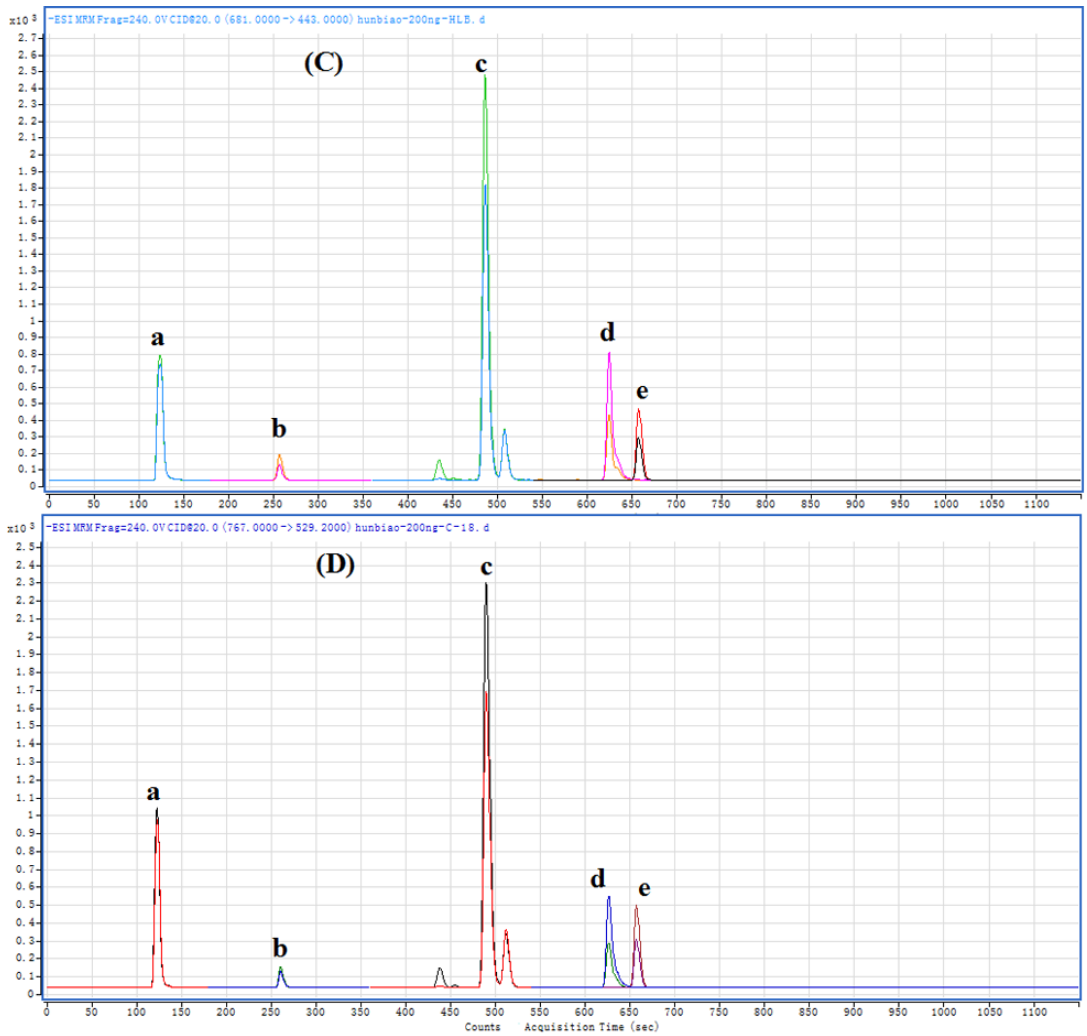

Fig.2. Representative MRM chromatograms of polygalaxanthone III (a), tenuifolin (b), tenuifoliside A (c), tenuifoliside C (d) and IS (e) in rat plasma by 2.2 Liquid-solid extraction method. (C) HLB cartridge; (D) C_{18} cartridge.