Electronic Supplementary Information

A targeted strategy for ingredients analysis and enrichment by mass-based preparative HPLC method: application to three isomeric C_{21} steroids from *Marsdenia tenacissima*

Feng Zhang,[a] Lianna Sun,[b] Le Yang,[a] Qinghua Zeng,[a] Xia Tao,[a] Shouhong Gao,[a] Liang Sun,[a] and Wansheng Chen*[a]

CORRESPONDENCE: chenwanshengsmmu@aliyun.com

Delay volume calibration

The fraction delay volume specifies the volume between the MS detector and fraction collector needle tip. In order to take the delay time into account, delay volume calibration was performed at the flow rate of 3 mL/min. The precise determination of the delay time is of particular importance for a reliable and accurate fraction collection process with high recoveries. With the instrument used in our experiments, such a delay volume calibration could be fully automated.

Extraction and Isolation

Dried CMTE (250 g) was chromatographed on a silica gel column eluted with CH_2Cl_2/MeOH (100:1 to 50:1) to yield three main fractions (A–C). All fractions were dried under vacuum (each about 12 g). Purification of fraction A (about 3 g) by preparative LC (MeOH/H_2O, 55:45, 3 mL/min) furnished tenacigenin A (20 mg). By the same procedure, tenacigenin B (20 mg) and 17β-tenacigenin B (20 mg) were obtained from fractions B and C.

The NMR data for compounds **1–3** were presented as follows:

Compound **1**, ^{1}H NMR (600 MHz, δ ppm, CD_3OD): CH_3-18 (δ_H 0.84, s), CH_3-19 (δ_H 1.03, s), H-21 (δ_H 2.30, s), H-3 (δ_H 3.53, m), H-11β (δ_H 3.46, t, 10.0 Hz), H-17α

(δ_H 2.94, d, 6.1, 11.0 Hz); ^{13}C NMR (150 MHz, δ ppm, CD_3OD): C-1 (δ_C 39.0), C-2 (δ_C 31.8), C-3 (δ_C 71.5), C-4 (δ_C 39.5), C-5 (δ_C 46.0), C-6 (δ_C 28.2), C-7 (δ_C 33.6), C-8 (δ_C 67.8), C-9 (δ_C 55.0), C-10 (δ_C 40.3), C-11 (δ_C 69.3), C-12 (δ_C 81.6), C-13 (δ_C 46.0), C-14 (δ_C 72.9), C-15 (δ_C 28.7), C-16 (δ_C 26.1), C-17 (δ_C 64.0), C-18 (δ_C 10.9), C-19 (δ_C 13.3), C-20 (δ_C 214.0), C-21 (δ_C 32.0).

Compound **2**, ^{1}H NMR (600 MHz, δ ppm, CD_3OD): CH_3-18 (δ_H 1.03, s), CH_3-19 (δ_H 1.12, s), H-3 (δ_H 3.53, m), H-11α (δ_H 3.22, d, 10.0 Hz), H-17β (δ_H 3.10, td, 1.6, 3.0 Hz), H-21 (δ_H 2.25, s); ^{13}C NMR (150 MHz, δ ppm, CD_3OD): C-1 (δ_C 37.6), C-2 (δ_C 32.1), C-3 (δ_C 70.7), C-4 (δ_C 38.0), C-5 (δ_C 44.6), C-6 (δ_C 26.8), C-7 (δ_C 30.4), C-8 (δ_C 66.6), C-9 (δ_C 53.8), C-10 (δ_C 38.8), C-11 (δ_C 67.8), C-12 (δ_C 73.7), C-13 (δ_C 47.6), C-14 (δ_C 71.9), C-15 (δ_C 27.4), C-16 (δ_C 24.8), C-17 (δ_C 60.0), C-18 (δ_C 16.3), C-19 (δ_C 11.9), C-20 (δ_C 213.3), C-21 (δ_C 31.3).

Compound **3**, ^{1}H NMR (600 MHz, δ ppm, CD_3OD): CH_3-19 (δ_H 1.11, s), CH_3-21 (δ_H 1.18, s), CH_3-18 (δ_H 1.18, s), H-3 (δ_H 3.55, m), H-12α (δ_H 3.89, d, 4.2 Hz), H-11β (δ_H 4.18, d, 2.0, 4.3 Hz); ^{13}C NMR (150 MHz, δ ppm, CD_3OD): C-1 (δ_C 38.0), C-2 (δ_C 29.9), C-3 (δ_C 71.6), C-4 (δ_C 36.6), C-5 (δ_C 46.2), C-6 (δ_C 27.4), C-7 (δ_C 32.5), C-8 (δ_C 78.5), C-9 (δ_C 58.4), C-10 (δ_C 35.4), C-11 (δ_C 70.6), C-12 (δ_C 70.4), C-13 (δ_C 44.3), C-14 (δ_C 80.7), C-15 (δ_C 33.8), C-16 (δ_C 22.7), C-17 (δ_C 53.9), C-18 (δ_C 16.3), C-19 (δ_C 14.8), C-20 (δ_C 99.5), C-21 (δ_C 23.1).

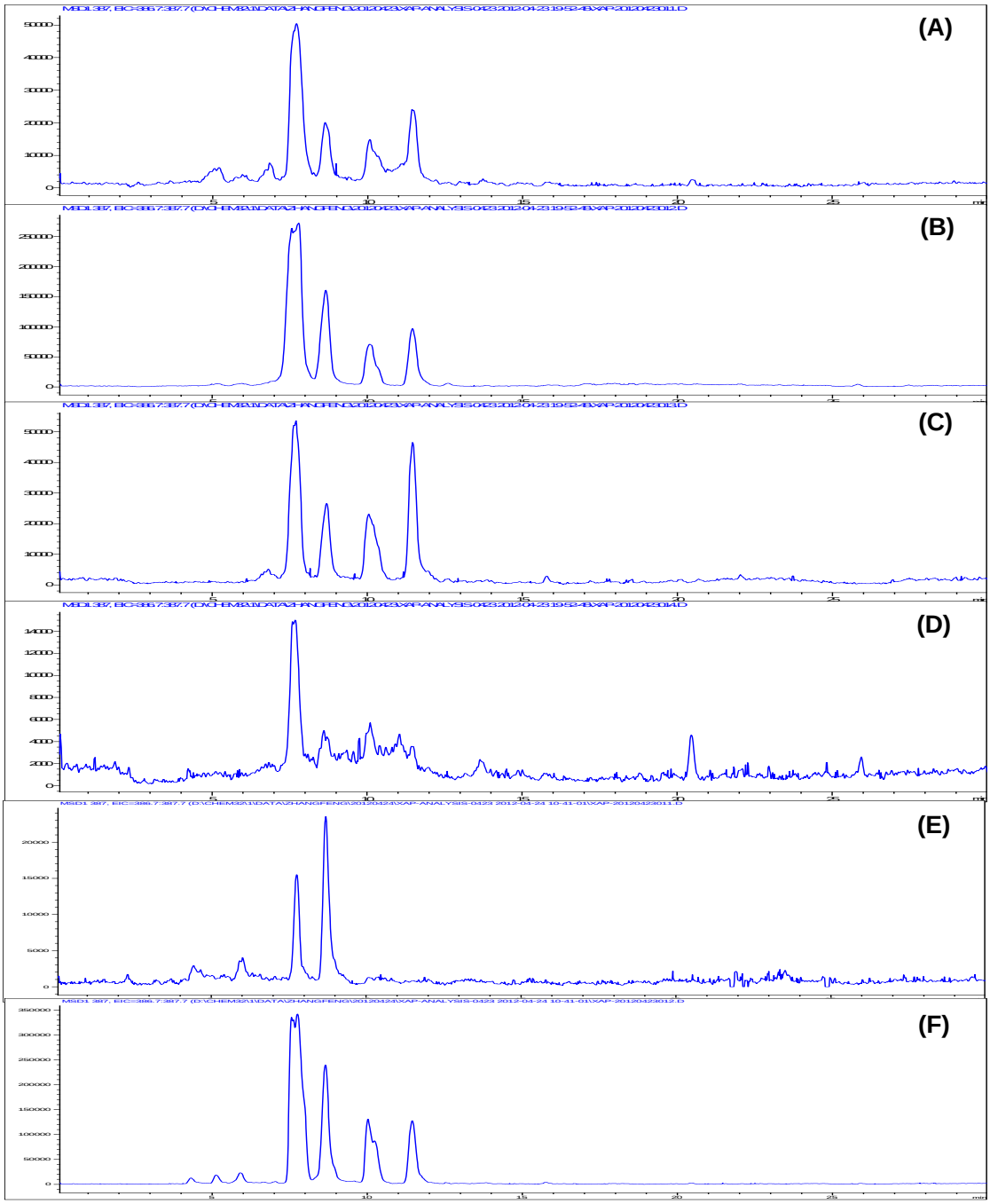

Fig. S1 EICs at *m/z* 387 for CMTE **(A)**, MAR1 **(B)**, MAR2 **(C)**, MAR3 **(D)**, S1 **(E)**, and S2 **(F)**.

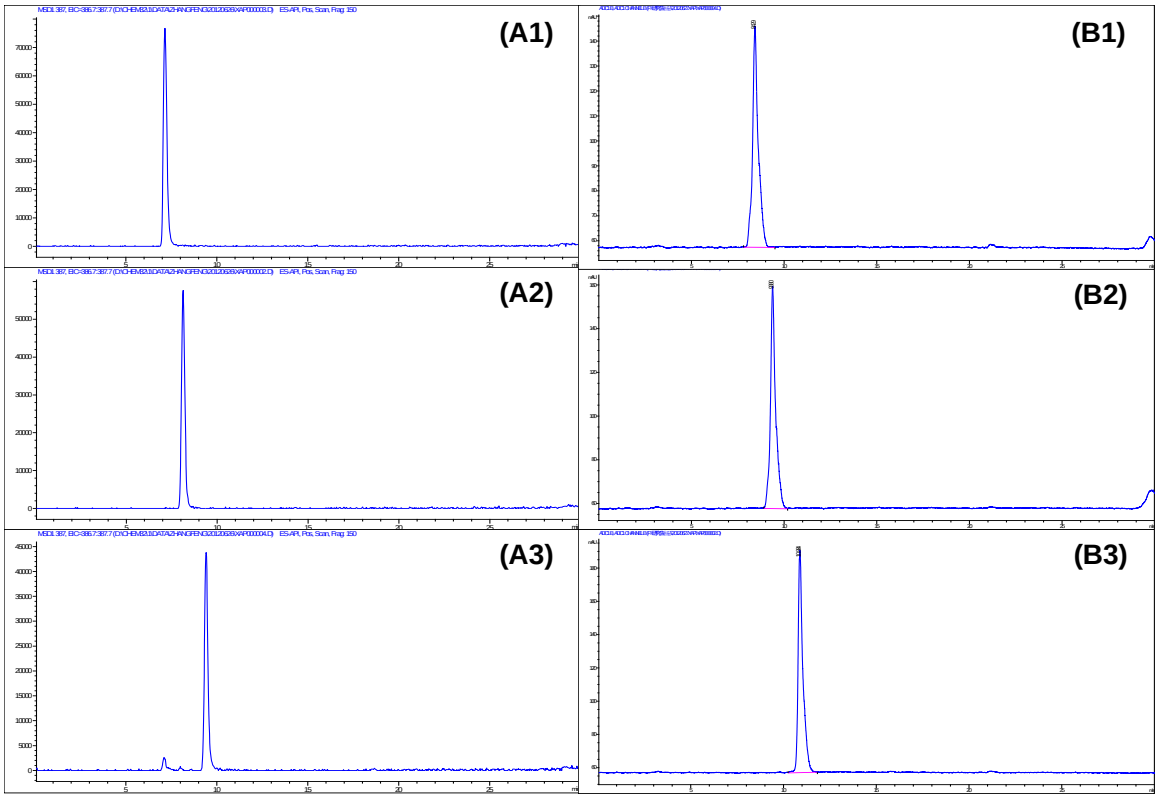

Fig. S2 **(A1–A3)** EICs at *m/z* 387 of compounds **1**, **2**, and **3**, respectively. **(B1–B3)** HPLC-ELSD spectra for purities study of compounds **1**, **2**, and **3**, respectively.