

1 **Target screen of anti-hyperuricemia compounds from Cortex Fraxini**  
2 **in vivo based on ABCG2 and bioaffinity ultrafiltration mass**  
3 **spectrometry**

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13 **LC-HRMS conditions for compounds analysis of CS**

14 LC-MS/MS analysis was performed on an UHPLC system (Vanquish, Thermo Fisher  
15 Scientific) with a Waters UPLC BEH C18 column (1.7  $\mu\text{m}$  2.1 \*100 mm). The flow rate  
16 was set at 0.4 mL/min and the sample injection volume was set at 5  $\mu\text{L}$ . The mobile phase  
17 consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The  
18 multi-step linear elution gradient program was as follows: 0 - 3.5 min, 95 - 85% A; 3.5 - 6  
19 min, 85 - 70% A; 6 - 6.5 min, 70 - 70% A; 6.5 - 12 min, 70 - 30% A; 12 - 12.5 min, 30 -  
20 30% A; 12.5 - 18 min, 30 - 0% A; 18 - 25 min, 0 - 0 % A; 25 - 26 min, 0 - 95% A; 26 - 30  
21 min, 95 - 95% A.

22 An Orbitrap Exploris 120 mass spectrometer coupled with an Xcalibur software was  
23 employed to obtain the MS and MS/MS data based on the IDA acquisition mode. During  
24 each acquisition cycle, the mass range was from 100 to 1500, and the top four of every  
25 cycle were screened and the corresponding MS/MS data were further acquired. Sheath gas  
26 flow rate: 30 Arb, Aux gas flow rate: 10 Arb, Ion Transfer Tube Temp: 350  $^{\circ}\text{C}$ , Vaporizer  
27 Temp: 350  $^{\circ}\text{C}$ , Full ms resolution: 60000, MS/MS resolution: 15000, Collision energy:  
28 16/38/42 in NCE mode, Spray Voltage: 5.5 kV (positive) or -4 kV (negative) .