Supplement

1 UPLC-MS Method

1.1 Chromatographic and mass spectrometric conditions

(1) Chromatographic conditions

Chromatographic column: Waters ACQUITY UPLC BEH-C18 (2.1 mm × 50 mm, 5 μm)

Mobile phase: 0.1 % formic acid aqueous solution : methanol = 73 : 26

Flow rate: 0.2 mL/min.

Injection volume: 5 μL

(2) Mass spectrometry conditions

ESI ion source (positive ion mode) was used, and the detection mode was multiple reaction monitoring mode (MRM). Capillary voltage 3.0KV, cone voltage 54 V, cone gas flow rate 20 L / h, desolvation gas flow rate 900 L / h, desolvation gas temperature 550 °C.

Ion quantitative analysis : 5-ASA m/z 154 → 108, collision energy 20 V ; 4-ASA m/z 154 → 119, collision energy 16 V (Fig.1).

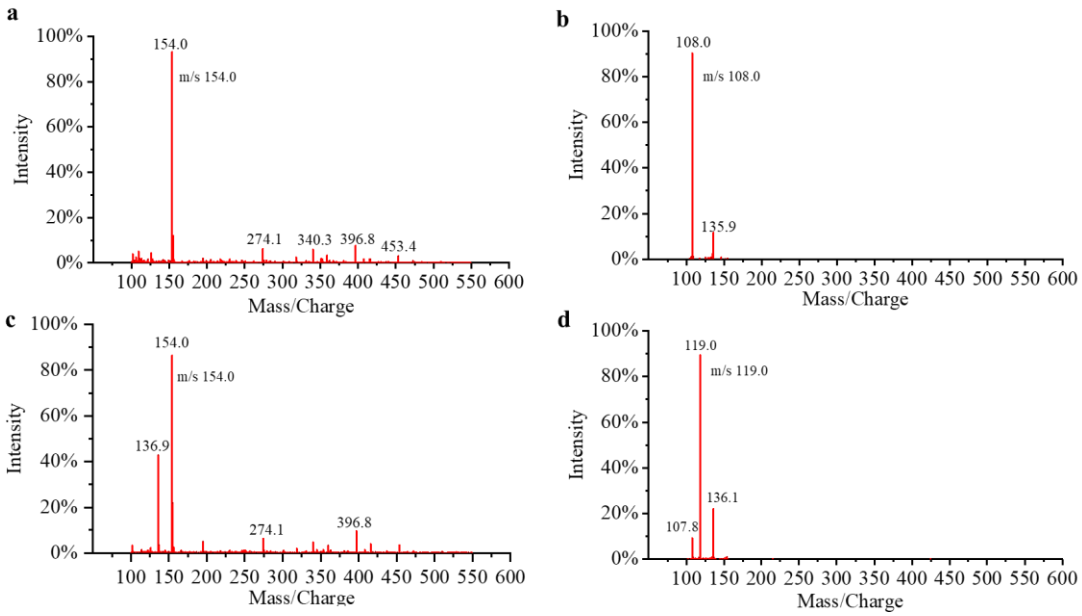

Figure 1 5-ASA primary mass spectrometry (a), 5-ASA secondary mass spectrometry (b), 4-ASA primary mass spectrometry (c) and 4-ASA secondary mass spectrometry (d).

1.2 Plasma sample treatment method

After obtaining the blood sample to be tested, it was centrifuged at 5000 rpm for 6 min, and the supernatant was taken and stored at -80 °C. Before the determination, 100 μL plasma was thawed and vortexed for 3 min with 100 μL methanol, 100 μL 4-ASA internal standard solution and 100 μL 7 % formic acid methanol solution. Then, 0.5 mL methanol was added to continue the v

ortex for 5 min. Finally, the supernatant was centrifuged at 10000 rpm for 15 min to determine the concentration of 5-ASA.

1.3 Specificity experiment

Blank plasma: According to the plasma sample treatment method under **1.2**, 100μL blank plasma was taken without adding 5-ASA solution and internal standard solution (methanol substitution).

The mixed solution in the mobile phase: The appropriate amount of 5-ASA solution and internal standard solution were added to the mobile phase to configure a mixed solution with a certain concentration.

Mixed solution in blank plasma: 5-ASA solution and internal standard solution were added to blank plasma, and plasma samples were treated according to the method in **1.2**.

The blank plasma, the mixed solution in the mobile phase and the mixed solution in the blank plasma were filtered through a 0.22 μm microporous membrane, and the filtrate was taken and the chromatogram was recorded.

1.4 Precision and accuracy experiments

A total of 100 μL of 5-ASA diluted solution (low concentration of 400 ng /mL, medium concentration of 4000 ng / mL, high concentration of 24000 ng/ mL) was prepared, and 100 μL of blank plasma was added to make the concentration of 5-ASA in the mixed solution 200,2000,12000 ng/mL. According to the steps described in **1.2**, 100 μL of 4-ASA internal standard solution and 100 μL of 7 % formic acid methanol solution were added for treatment, and the content was determined by UPLC-MS. Sampling was performed at 2 h, 6 h, and 10 h on the same day to detect the content, record the peak area, and obtain intra-day precision. At the same time, the measured concentration of 5-ASA was compared with the theoretical concentration to calculate the recovery rate.

1.5 Extraction recovery and matrix effect experiment

A total of 100 μL of 5-ASA diluted solution with concentrations of 400,4000, and 24000 ng / mL was taken, and 100 μL of blank plasma was added to make the concentration of 5-ASA in the mixed solution 200,2000, and 12000 ng / mL. According to the steps described in **1.2**, 100 μL of methanol (instead of 4-ASA internal standard solution) and 100 μL of 7 % formic acid methanol solution were added for treatment. The content was determined by UPLC-MS, and the peak area was recorded as A1.

Three 100 μL blank plasma samples were added with 100 μL 7 % formic acid methanol solution, without 4-ASA internal standard solution and 5-ASA solution. The samples were processed according to the steps described in **1.2**. The supernatant was added with 5-ASA standard solution to prepare samples with concentrations of 200, 2000 and 12000 ng/mL. UPLC-MS was used for determination, and the peak area was recorded as A2.

$$\text{the extraction recovery} = \frac{A_1}{A_2} \times 100\%$$

The mobile phase was used to dilute the 5-ASA standard solution, and it was prepared into standard samples with concentrations of 200, 2000, and 1200 0 ng/mL. The content was determined and the peak area was recorded as A3.

$$\text{matrix effect} = \frac{A_2}{A_3} \times 100\%$$

2 Result

2.1 Specific experimental results

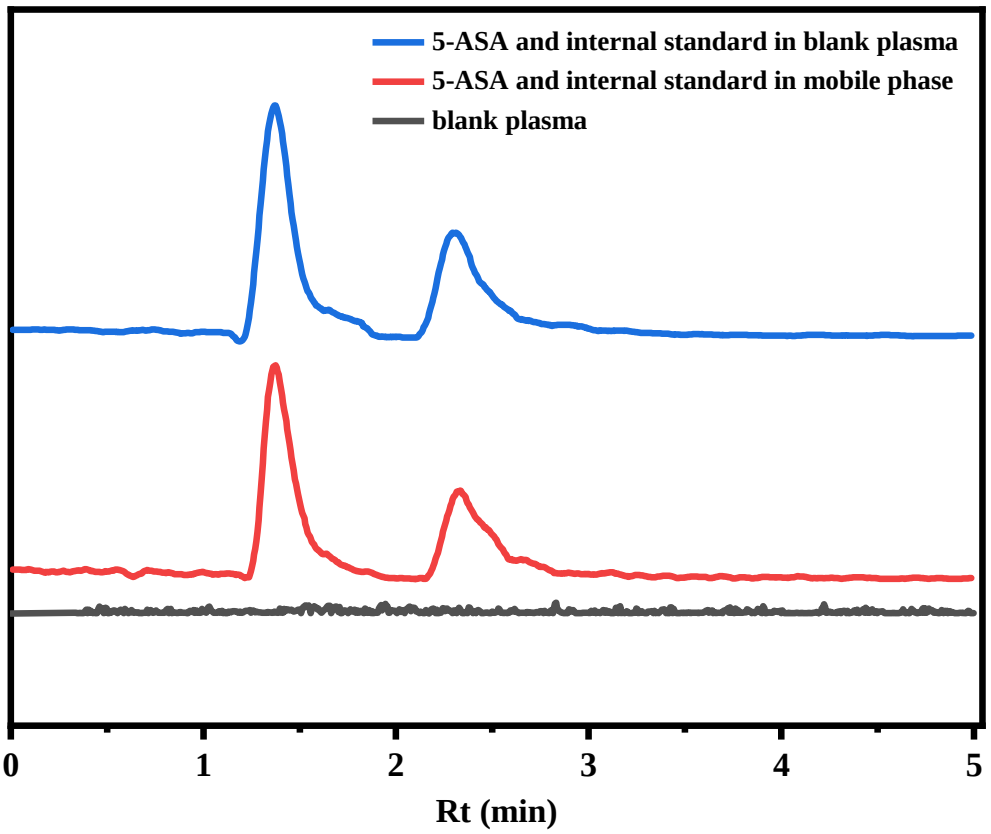

Figure 2 Blank plasma, mobile phase plus 5-ASA and internal standard and blank plasma plus 5-ASA and internal standard chromatogram

As shown in Figure 2, the retention time of 5-ASA was about 1.2 min, t he peak time was short, the resolution was high, and the peak shape was goo d. The retention time of internal standard 4-ASA was about 2.2 min. There wa s no peak in blank plasma within 5 min, indicating that endogenous substances in biological samples did not interfere with the determination of compounds, and the method had good specificity.

2.2 Precision and accuracy experimental results

Table 1 Intraday precision data（n=3）

concentration（ng/mL）	measured concentration（ng/mL）			mean value±SD（%）	RSD（%）
	2 h	6 h	10 h		
200	220.12	199.54	233.31	217.66±17.02	7.82
2000	2045.46	2085.67	1920.62	2017.04±86.07	4.27
12000	11835.57	12455.64	11990.09	12093.33±322.77	2.67

Table 2 Method recovery results（n=3）

concentration（ng/mL）	measured concentration（ng/mL）	method recoveries（%）	mean value±SD（%）	RSD（%）
	186.61	93.31%		
200	194.97	97.49%	97.79±4.65	4.75
	205.17	102.59%		
	1957.49	97.87%		
2000	2086.16	104.31%	99.04±4.80	4.84
	1898.57	94.93%		
	12123.39	101.03%		
12000	11582.18	96.52%	99.92±3.01	3.01
	12267.36	102.23%		

The data in Table 1 show that the method has good precision. The data i n Table 2 showed that the recovery rates of low, medium and high concentrati ons in plasma were in the range of 95 % -100 %, and the RSD was small, in

dicating that the method had good accuracy.

2.3 Extraction recovery and matrix effect experimental results

Table 3 Extraction recovery and matrix effect results（n=3）

concentration （ng/mL）	the extraction recovery（%）	matrix effect（%）
200	82.31±6.24	90.98±4.84
2000	83.56±3.75	92.54±3.75
12000	81.15±2.54	93.38±2.78

According to the experimental results in Table 3, it can be seen that the extraction recovery rates of the three concentrations of drugs in plasma are hig her than 80 %, and the matrix effect is also higher than 90 %. Therefore, the method meets the requirements of extraction recovery and matrix effect in biol ogical sample analysis.