

Methodological investigation

1. Method

1.1 Precision investigation

Xuefu Zhuyu Decoction (XFZYD) sample solution (S10) was prepared using the method under the "2.7". According to the liquid chromatography conditions under the "2.5", the chromatographic peaks were recorded for 6 consecutive times, the retention time and peak area of the main 5 peaks were determined, and the RSD value was calculated.

1.2 Repeated investigation

The XFZYD sample solution (S10) was prepared using the method under the "2.7". According to the liquid chromatography conditions under the "2.5", the chromatographic peaks were recorded, the retention time and peak area of the main 5 peaks were determined, and the RSD value was calculated.

1.3 Stability investigation

The XFZYD sample solution (S10) was prepared by the method under the "2.7", and the chromatographic peaks were recorded at 0, 3, 6, 12, 18 and 24 h according to the liquid chromatography conditions under the "2.5". The retention time and peak area of the five major peaks were determined, and the RSD value was calculated.

2. Result

2.1 Precision investigation

The precision of the instrument was investigated, and the RSD values of each peak retention time and peak area were compared, as shown in Table 1, which were all less than 2.00%, proving that the precision of the instrument was good.

Table 1 Precision inspection

No.	t_R (min)	RSD (%)	Peak area mean (n=6)	RSD (%)
1	18.38	0.37	5234624	0.65
2	21.87	0.08	3102068	0.37
3	29.88	0.10	8038911	0.05
4	33.88	0.09	4633003	0.37
5	54.81	0.00	2012573	0.13

2.2 Repeated investigation

The reproducibility of the method was investigated, and the RSD values of each

peak retention time and peak area were compared, as shown in Table 2, which were all less than 2.00%, proving that the method had good repeatability.

Table 2 Repeated investigation

No.	t_R (min)	RSD (%)	Peak area mean (n=6)	RSD (%)
1	12.27	0.46	5274339	0.55
2	21.86	0.09	3138331	1.43
3	29.94	0.13	8061978	0.67
4	33.88	0.14	4678467	1.25
5	54.75	0.02	1998572	0.75

2.3 Stability investigation

The stability of Xuefu Zhuyu Decoction within 24 h was investigated, and the RSD values of each peak retention time and peak area were compared, as shown in Table 3, which were all less than 2.00%, proving that Xuefu Zhuyu Decoction had good stability.

Table 3 Stability investigation

No.	t_R (min)	RSD (%)	Peak area mean (n=6)	RSD (%)
1	12.28	0.47	5277647	0.65
2	21.86	0.09	3126632	1.57
3	29.94	0.13	8073180	0.59
4	33.88	0.14	4678395	1.17
5	54.75	0.02	2006699	0.51