Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information

Chromatographic fingerprint on serially coupled columns combined with multiple-component quantitation with a single reference standard for quality evaluation of Shen-Zhi-Ling oral liquid

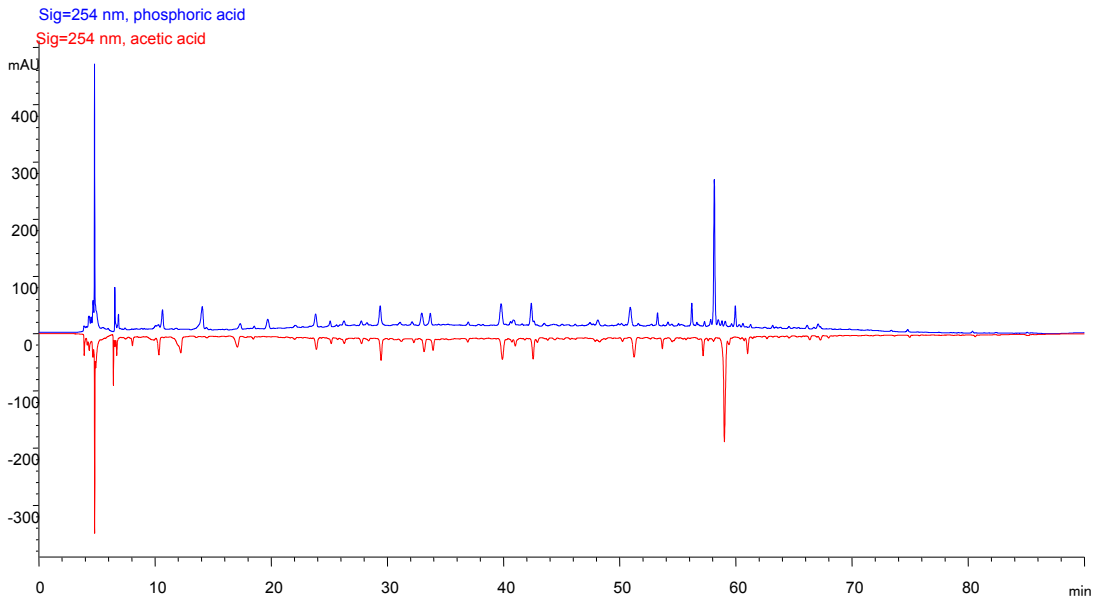

Figure. S1 Optimization diagram of different mobile phases.

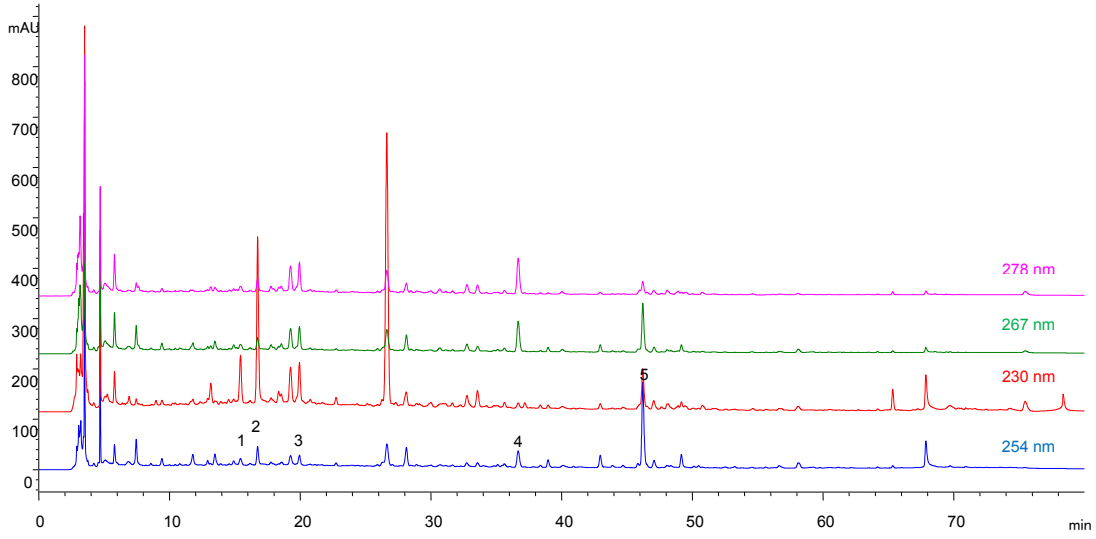

Figure. S2 Chromatograms of different wavelengths.

Peaks 1, 2, 3, 4 and 5 indicate albiflorin, paeoniflorin, liquiritin, cinnamic acid, and GAAS, respectively.

Table S1 Results of precision, repeatability, accuracy, and stability tests.

Analytes	Intra-day precision RSD (%) (n=6)	Inter-day precision RSD (%) (n=6)	Repeatability RSD (%) (n=6)	Recovery(%)(n=9)				Stability RSD (%) (n=6)
				ESM	RSD(%)	SSDMC	RSD (%)	
albiflorin	1.4	2.6	1.8	100.71	2.9	100.41	2.9	2.2
paeoniflorin	1.1	2.1	2.1	100.48	2.0	100.48	2.0	3.3
liquiritin	2.3	1.3	1.9	101.29	2.0	98.87	2.7	4.5
cinnamic acid	1.2	1.4	1.9	99.62	2.8	100.33	2.9	0.56
GAAS	0.48	0.94	2.1	98.56	1.9	98.68	1.9	0.28

Table S2 Robustness test of relative conversion factors and relative retention times

Equipments	Columns	RF				RT			
		Albiflorin	Liquiritin	Cinnamic acid	GAAS	Albiflorin	Liquiritin	Cinnamic acid	GAAS
Agilent 1260	①[a]	1.35	0.48	1.24	3.56	0.93	1.22	2.22	2.76
	②[b]	1.37	0.47	1.26	3.62	0.92	1.19	2.20	2.77
	③[c]	1.36	0.45	1.22	3.56	0.92	1.20	2.21	2.79
Agilent 1100	①	1.37	0.48	1.25	3.58	0.93	1.23	2.28	2.85
	②	1.39	0.46	1.28	3.62	0.92	1.20	2.24	2.84
	③	1.35	0.47	1.28	3.61	0.91	1.20	2.25	2.87
Results	Average	1.37	0.47	1.25	3.59	0.92	1.21	2.24	2.81
	RSD (%)	1.2	2.1	2.2	0.8	0.6	1.3	1.3	1.7

[a] Phenomenex Synergi Hydro-RP (4.6×250 mm, 4 μm)

[b] Phenomenex Luna C18(2) (4.6×250 mm, 5 μm)

[c] Merck Purospher STAR RP-18 (4.6×250 mm, 5 μm)

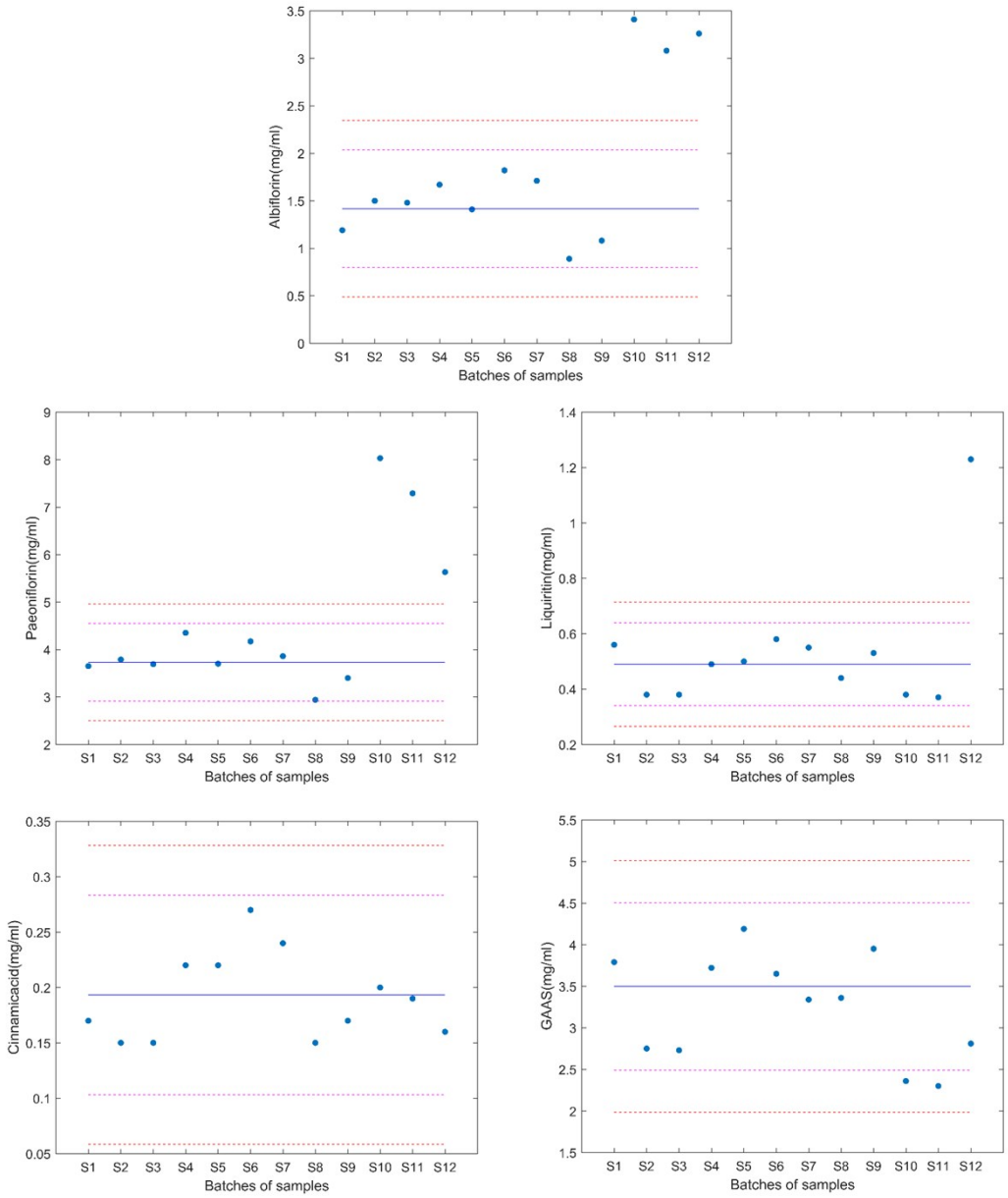

Figure. S3 Content fluctuation diagram of the five components.

The blue line refers to the average content of the component in normal samples, and the pink dashed lines were upper and lower warning lines representing average content ± 2 standard deviation (SD), respectively. The red dashed lines were upper and lower critical lines indicating average content ± 3 SD.

Table S3

Results of precision, accuracy, and stability in fingerprint method validation

Peak	Intra-day precision (n = 6) RSD (%)		Inter-day precision (n = 6) RSD (%)		Repeatability (n = 6) RSD (%)		Stability (24h, n = 6) RSD (%)	
	RS[a]	RT[b]	RS	RT	RS	RT	RS	RT
1	0.87	0.02	3.8	0.04	2.9	0.16	4.1	0.05
2	0.57	0.05	2.6	0.11	2.4	0.11	3.5	0.12
3	1.6	0.06	1.9	0.57	2.8	0.31	0.95	0.09
4	1.6	0.04	1.5	0.12	3.8	0.39	1.3	0.07
5	0.94	0.02	1.3	0.15	2.9	0.25	1.2	0.06
6	1.8	0.02	2.6	0.11	3.8	0.35	3.1	0.05
7	2.1	0.02	4.1	0.06	4.3	0.23	3.9	0.04
8	0.65	0.01	1.8	0.16	4.3	0.19	1.2	0.03
9	0.68	0.02	1.8	0.08	3.0	0.28	3.9	0.03
10	2.2	0.01	2.9	0.03	4.6	0.13	3.7	0.02
11	1.5	0.02	3.1	0.04	2.9	0.04	2.1	0.03
12	0.81	0.01	1.6	0.03	2.3	0.04	1.2	0.04
13	0.90	0.02	2.1	0.02	3.9	0.04	1.9	0.03
14	0.90	0.02	2.1	0.02	4.0	0.05	2.0	0.03
15	0.96	0.02	1.9	0.05	2.3	0.12	0.56	0.04
16	0.63	0.01	1.9	0.02	3.1	0.07	1.2	0.02
17	0.85	0.02	1.9	0.06	4.6	0.11	1.4	0.04
18	2.5	0.02	2.1	0.04	2.1	0.08	1.1	0.03
19	3.5	0.01	3.4	0.02	2.5	0.03	3.5	0.03
20	1.6	0.01	2.6	0.02	3.1	0.04	2.2	0.01
21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22	0.54	0.02	1.4	0.05	1.6	0.22	1.2	0.01
23	0.54	0.01	1.1	0.04	2.1	0.23	0.92	0.01
24	1.5	0.01	1.9	0.04	1.6	0.25	1.6	0.01
25	0.86	0.01	1.8	0.05	2.5	0.24	1.9	0.01
26	0.58	0.01	1.1	0.04	2.3	0.24	0.89	0.01
27	0.61	0.01	0.64	0.05	4.8	0.25	0.93	0.01
28	0.77	0.01	0.62	0.05	4.0	0.23	0.86	0.01
29	0.89	0.01	2.8	0.02	2.5	0.13	1.5	0.02

[a] RSD values of the relative peak areas

[b] RSD values of the relative retention times

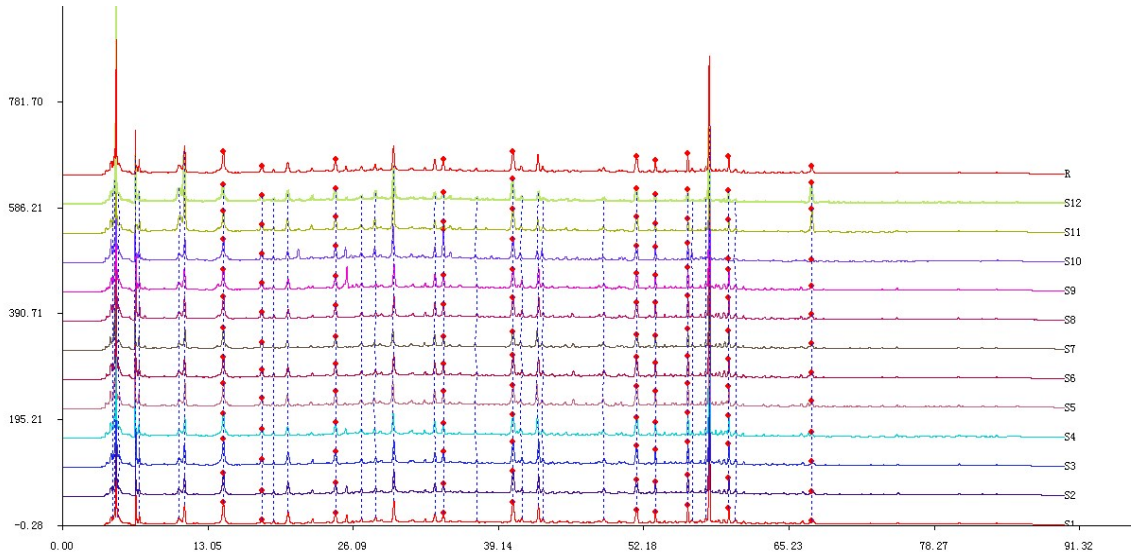

Figure. S4 HPLC chromatographic fingerprints of 12 batches of SZL samples and representative fingerprint (R) based on two coupled columns.