Electronic Supplementary Information

1 Validation for the macromolecule impurity detection method

1.1 validation for instrument precision

Table 1 The RSDs (%) of the instrument precision (peak area) for macromolecule impurity detection method

Compounds	Peak area							SD	RSD%
	1	2	3	4	5	6	average		
Rib A	626433	634316	641612	632069	646831	652750	639001.833	9850.053	1.54
Ins	1268392	1265826	1257418	1250354	1262129	1250638	1259126.167	7637.777	0.61
Thy	643692	641814	638811	627471	650589	629518	638649.167	8790.469	1.38
Som	2184669	2180650	2182355	2178473	2214299	2184213	2187443.167	13355.645	0.61

Table 2 The RSDs (%) of the instrument precision (retention time) for macromolecule impurity detection method

Compounds	Retention time							SD	RSD%
	1	2	3	4	5	6	average		
Rib A	14.761	14.759	14.76	14.758	14.757	14.754	14.758	0.002	0.02
Ins	16.484	16.483	16.484	16.483	16.481	16.475	16.482	0.003	0.02
Thy	17.623	17.621	17.622	17.621	17.619	17.612	17.620	0.004	0.02
Som	18.846	18.843	18.844	18.843	18.84	18.834	18.842	0.004	0.02

1.2 validation for intra-day precision

Table 3 The RSDs (%) of the intra-day precision (peak area) for macromolecule impurity detection method

Compounds	Peak area							SD	RSD%
	sample 1	sample 2	sample 3	sample 4	sample 5	sample 6	average		
Rib A	592963	581754	563670	590844	580016	570257	579917.083	11404.086	1.97
Ins	1139586	1123627	1107834	1158299	1128763	1140170	1133046.083	17169.265	1.52
Thy	611452	612367	597658	616705	600637	604118	607156.083	7466.443	1.23
Som	1297562	1295533	1304992	1232347	1136663	1221154	1248041.417	65307.703	5.23

1.3 validation for stability

Table 4 The RSDs (%) of the stability (peak area) for macromolecule impurity detection method

Compounds	Peak area									RSD%
	0	2	4	6	8	10	12	24	average	
Rib A	609675	613703	634707	627933	610830	604690	605380	607265	614273	1.80
Ins	1130888	1142663	1128343	1124823	1125387	1128458	1122131	1117717	1127551	0.65
Thy	626385	628915	616301	637353	616908	632270	627036	631746	627114	1.17
Som	1247151	1241927	1234656	1250992	1230013	1234184	1228862	1237707	1238187	0.64

1.4 validation for accuracy

Table 5 Accuracy for macromolecule impurity detection method

Compounds	Peak area							average recovery (%)
	sample	sample	sample	sample	sample	sample	area of standard	

	1	2	3	4	5	6		
Rib A	592963	581754	563670	590844	580016	570257	639383	90.70
Ins	1139586	1123627	1107834	1158299	1128763	1140170	1168675	96.95
Thy	611452	612367	597658	616705	600637	604118	632304	96.02

2 validation for phenol sulfuric acid assay

2.1 validation for instrument precision

Table 6 The RSDs (%) of the instrument precision (absorbance) for phenol sulfuric acid assay

Samples	Absorbance (sample solution)	Absorbance (standard solution)
1	0.6082	0.5927
2	0.6077	0.5927
3	0.6079	0.5925
4	0.6077	0.5934
5	0.6078	0.5924
6	0.6082	0.5925
average	0.6079	0.5927
RSD (%)	0.04	0.06

2.2 validation for intra-day precision

Table 7 The RSDs (%) of the intra-day precision (content) for phenol sulfuric acid assay

Samples	Absorbance	Content（%）	Average（%）	RSD (%)
1	0.5864	0.3152		
2	0.5799	0.3143		
3	0.5935	0.3207	0.3170	1.55
4	0.5771	0.3107		
5	0.6137	0.3246		

6	0.5892	0.3168

2.3 validation for stability

Table 8 The RSDs (%) of the stability (absorbance) for phenol sulfuric acid assay

Time (min)	0	20	40	60	80	100	120	240	360	Average	RSD (%)
absorbance	0.6058	0.605	0.6038	0.6028	0.6018	0.6007	0.6000	0.5968	0.5943	0.6012	0.59

2.4 validation for accuracy

Table 9 Accuracy for phenol sulfuric acid assay

Samples	m_1（mg）	m_2（mg）	m_3（mg）	Recovery rate（%）	Average recovery rate（%）	RSD（%）
1	0.03214	0.03105	0.06252	97.86	101.89	3.73
2	0.03214	0.03105	0.06320	100.03		
3	0.03214	0.03105	0.06542	107.20		
4	0.03214	0.03105	0.06387	102.19		
5	0.03214	0.03105	0.06490	105.52		
6	0.03214	0.03105	0.06274	98.55		

m_1: the content contained in the sample. m_2: the content added in the sample. m_3: the content detected in the sample.

3 validation for HPLC-ELSD method

3.1 validation for instrument precision

Table 10 The RSDs (%) of the instrument precision (the logarithm of the peak area) for HPLC-ELSD method

Samples	The logarithm of the peak area			
	Fru	Glu	Mel	Man
1	8.0819	6.9306	5.5049	6.2938
2	8.0819	6.9306	5.5049	6.2938

3	8.0912	6.9197	5.5385	6.3208
4	8.0717	6.9454	5.5936	6.3637
5	8.0766	6.9353	5.5428	6.3197
6	8.1004	6.9224	5.5722	6.3916
average	8.0840	6.9307	5.5428	6.3306
RSD (%)	0.13	0.13	0.64	0.62

Table 11 The RSDs (%) of the instrument precision (retention time) for HPLC-ELSD method

Samples	Retention time			
	Fru	Glu	Mel	Man
1	21.062	26.019	30.502	33.362
2	21.065	26.021	30.488	33.355
3	21.058	26.022	30.495	33.358
4	21.096	26.031	30.507	33.366
5	21.057	26.038	30.539	33.39
6	21.061	26.009	30.506	33.367
average	21.067	26.023	30.506	33.366
RSD%	0.070	0.039	0.058	0.037

3.2 validation for intra-day precision

Table 12 The RSDs (%) of the intra-day precision (concentration %) for HPLC-ELSD method

Samples	Concentration (%)			
	Fru	Glu	Mel	Man
1	0.1505	0.0464	0.0231	0.0849
2	0.1528	0.0475	0.0236	0.0872
3	0.1511	0.0478	0.0243	0.0878
4	0.1520	0.0476	0.0234	0.0875
5	0.1535	0.0474	0.0235	0.0866
6	0.1527	0.0476	0.0238	0.0893
average	0.1505	0.0464	0.0231	0.0849

RSD (%)	0.75	1.03	1.79	1.66

3.3 validation for inter-day precision

Table 13 The RSDs (%) of the inter-day precision (concentration %) for HPLC-ELSD method

Time (day)	Concentration (%)			
	Fru	Glu	Mel	Man
1	0.1508	0.0473	0.0223	0.0783
2	0.1464	0.0458	0.0217	0.0763
3	0.1509	0.0477	0.0231	0.0802
4	0.1516	0.0480	0.0227	0.0796
5	0.1523	0.0473	0.0214	0.0760
6	0.1490	0.0473	0.0227	0.0776
7	0.1510	0.0487	0.0232	0.0796
average	0.1503	0.0474	0.0225	0.0782
RSD (%)	1.32	1.88	3.02	2.13

3.4 validation for stability

Table 14 The RSDs (%) of sample solution stability (the logarithm of the peak area) for HPLC-ELSD method

Time (h)	Fru	Glu	Mel	Man
0	9.5419	7.0124	5.1596	6.4224
3	9.5482	7.0353	5.2062	6.4921
6	9.5650	7.0329	5.2100	6.4747
9	9.5587	7.0396	5.1756	6.4144
12	9.5821	7.0208	5.2528	6.4352
24	9.5834	7.1018	5.2370	6.3728
average	9.5632	7.0409	5.2069	6.4353
RSD (%)	0.18	0.45	0.68	0.67

Table 15 The RSDs (%) of standard solution stability (the logarithm of the peak area) for HPLC-ELSD method

Time (h)	Fru	Glu	Mel	Man
0	8.5046	7.2617	5.9784	6.8144
3	8.4940	7.2986	5.9566	6.7969
6	8.5103	7.3288	6.0234	6.8766
9	8.5236	7.3035	5.9781	6.8218
12	8.5255	7.3333	6.0208	6.8217
24	8.5278	7.3766	6.0220	6.8709
average	8.5143	7.3171	5.9966	6.8337
RSD (%)	0.16	0.53	0.48	0.47

3.5 validation for accuracy

Table 16 Accuracy for HPLC-ELSD method

Compounds	samples	C_1 (mg mL^{-1})	C_2 (mg mL^{-1})	C_3 (mg mL^{-1})	Recovery rate (%)	Average recovery rate (%)	RSD %
Fru	1	0.3043	0.2898	0.5952	100.38	100.52	0.97
	2	0.3043	0.2898	0.5918	99.23		
	3	0.3043	0.2898	0.5972	101.09		
	4	0.3043	0.2898	0.5929	99.61		
	5	0.3043	0.2898	0.5993	101.8		
	6	0.3043	0.2898	0.5969	101		
Glu	1	0.0948	0.0953	0.1855	95.16	99.88	2.65
	2	0.0948	0.0953	0.1917	101.71		
	3	0.0948	0.0953	0.1909	100.87		
	4	0.0948	0.0953	0.1906	100.57		
	5	0.0948	0.0953	0.1924	102.4		
	6	0.0948	0.0953	0.1888	98.59		
Mel	1	0.0473	0.0506	0.0969	98.11	97.97	1.19
	2	0.0473	0.0506	0.0963	96.85		
	3	0.0473	0.0506	0.0973	98.91		
	4	0.0473	0.0506	0.0977	99.57		
	5	0.0473	0.0506	0.0961	96.52		

	6	0.0473	0.0506	0.0968	97.85		
Man	1	0.1745	0.1902	0.3696	102.56	101.24	4.05
	2	0.1745	0.1902	0.3664	100.89		
	3	0.1745	0.1902	0.3662	100.77		
	4	0.1745	0.1902	0.3528	93.72		
	5	0.1745	0.1902	0.3726	104.16		
	6	0.1745	0.1902	0.3749	105.36		

C_1: the concentration contained in the sample. C_2: the concentration added in the sample. C_3: the concentration detected in the sample.

4 validation for HPLC-UV method

4.1 validation for HPLC-UV fingerprint methodology

4.1.1 validation for the instrument precision

Table 17 The RSDs (%) of instrument precision (relative peak area) for HPLC-UV fingerprint methodology

Peak number	Samples							RSD %
	1	2	3	4	5	6	average	
1	0.0470	0.0475	0.0467	0.0471	0.0471	0.0475		0.65
2	0.3099	0.3105	0.3084	0.3080	0.3066	0.3055	0.3082	0.62
3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.00
4	61.4748	62.1657	61.7826	61.6987	61.7914	62.2334	61.8578	0.47
5	0.3049	0.3052	0.3037	0.3032	0.3019	0.3018	0.3034	0.47
6	0.0396	0.0399	0.0398	0.0399	0.0397	0.0396	0.0398	0.32
7	0.0529	0.0538	0.0540	0.0546	0.0548	0.0552	0.0542	1.56
8	0.0754	0.0749	0.0749	0.0746	0.0741	0.0735	0.0746	0.92
9	0.0197	0.0195	0.0194	0.0193	0.0195	0.0195	0.0195	0.78
10	0.0309	0.0307	0.0309	0.0310	0.0310	0.0309	0.0309	0.40
11	0.0244	0.0246	0.0246	0.0245	0.0247	0.0247	0.0246	0.43
12	0.0950	0.0949	0.0948	0.0952	0.0954	0.0958	0.0952	0.39
13	0.0355	0.0354	0.0358	0.0355	0.0352	0.0354	0.0355	0.54
14	0.0157	0.0163	0.0167	0.0163	0.0161	0.0159	0.0162	2.11
15	0.0146	0.0143	0.0144	0.0141	0.0144	0.0141	0.0143	1.32
16	0.1092	0.1098	0.1092	0.1099	0.1095	0.1097	0.1096	0.28
17	0.0243	0.0238	0.0241	0.0243	0.0245	0.0240	0.0242	1.00
18	0.5106	0.5124	0.5120	0.5120	0.5118	0.5129	0.5120	0.15

Table 18 The RSDs (%) of instrument precision (relative retention time) for HPLC-UV fingerprint methodology

Peak number	Samples							RSD %
	1	2	3	4	5	6	average	
1	0.7038	0.7015	0.7009	0.7004	0.7027	0.7022	0.7019	0.18
2	0.7585	0.7572	0.7559	0.7560	0.7565	0.7575	0.7569	0.13
3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.00
4	1.2111	1.2093	1.2104	1.2076	1.2099	1.2077	1.2093	0.12
5	1.6396	1.6373	1.6424	1.6383	1.6443	1.6367	1.6398	0.18
6	5.5082	5.4532	5.4991	5.4742	5.5065	5.4696	5.4851	0.41
7	5.5793	5.5232	5.5709	5.5457	5.5774	5.5404	5.5561	0.41
8	5.8702	5.8114	5.8615	5.8358	5.8670	5.8293	5.8459	0.41
9	6.3692	6.3084	6.3622	6.3343	6.3663	6.3230	6.3439	0.40
10	6.6126	6.5484	6.6047	6.5750	6.6091	6.5607	6.5851	0.42
11	6.7192	6.6502	6.7097	6.6774	6.7125	6.6622	6.6885	0.44
12	7.3741	7.2996	7.3630	7.3305	7.3691	7.3130	7.3416	0.43
13	7.5884	7.5095	7.5749	7.5422	7.5819	7.5229	7.5533	0.44
14	8.1243	8.0411	8.1107	8.0752	8.1193	8.0605	8.0885	0.43
15	8.5913	8.5046	8.5793	8.5409	8.5864	8.5268	8.5549	0.42
16	9.1333	9.0422	9.1185	9.0801	9.1245	9.0664	9.0942	0.40
17	9.3253	9.2334	9.3096	9.2714	9.3155	9.2565	9.2853	0.40
18	10.0885	9.9908	10.0721	10.0307	10.0749	10.0092	10.0444	0.40

4.1.2 validation for repeatability

Table 19 The RSDs (%) of repeatability (relative peak area) for HPLC-UV fingerprint methodology

Peak number	Samples							RSD %
	1	2	3	4	5	6	average	
1	0.0474	0.0475	0.0478	0.0479	0.0480	0.0484	0.0478	0.77
2	0.3083	0.3101	0.3028	0.3170	0.3231	0.3375	0.3165	3.95
3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.00
4	62.3090	61.5763	62.8562	63.2322	63.0863	62.7217	62.6303	0.97
5	0.3025	0.3036	0.3018	0.3086	0.3132	0.3201	0.3083	2.35
6	0.0397	0.0395	0.0397	0.0400	0.0403	0.0399	0.0398	0.73
7	0.0556	0.0561	0.0537	0.0586	0.0590	0.0594	0.0571	3.99
8	0.0728	0.0726	0.0755	0.0707	0.0704	0.0701	0.0720	2.86
9	0.0192	0.0195	0.0195	0.0198	0.0195	0.0194	0.0195	1.08

10	0.0309	0.0308	0.0308	0.0311	0.0311	0.0312	0.0310	0.50
11	0.0245	0.0247	0.0250	0.0247	0.0249	0.0247	0.0247	0.66
12	0.0963	0.0961	0.0950	0.0975	0.0957	0.0961	0.0961	0.87
13	0.0355	0.0360	0.0360	0.0359	0.0354	0.0354	0.0357	0.83
14	0.0151	0.0163	0.0165	0.0158	0.0165	0.0159	0.0160	3.29
15	0.0136	0.0142	0.0147	0.0139	0.0144	0.0144	0.0142	2.66
16	0.1093	0.1103	0.1098	0.1105	0.1107	0.1107	0.1102	0.50
17	0.0244	0.0243	0.0243	0.0235	0.0247	0.0245	0.0243	1.69
18	0.5124	0.5132	0.5112	0.5138	0.5156	0.5158	0.5137	0.35

Table 20 The RSDs (%) of repeatability (relative retention time) for HPLC-UV fingerprint methodology

Peak	Samples							RSD
number	1	2	3	4	5	6	average	%
1	0.7020	0.7008	0.7111	0.7045	0.7039	0.7039	0.7044	0.51
2	0.7580	0.7564	0.7663	0.7611	0.7602	0.7603	0.7604	0.45
3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.00
4	1.2077	1.2063	1.2071	1.2073	1.2072	1.2052	1.2068	0.07
5	1.6377	1.6393	1.6248	1.6325	1.6338	1.6338	1.6337	0.31
6	5.4761	5.4810	5.4108	5.4328	5.4462	5.4386	5.4476	0.49
7	5.5466	5.5506	5.4804	5.5028	5.5177	5.5099	5.5180	0.49
8	5.8344	5.8370	5.7666	5.7900	5.8067	5.7976	5.8054	0.46
9	6.3316	6.3307	6.2571	6.2841	6.3021	6.2909	6.2994	0.46
10	6.5716	6.5707	6.4943	6.5218	6.5409	6.5300	6.5382	0.46
11	6.6756	6.6713	6.5966	6.6247	6.6451	6.6330	6.6410	0.45
12	7.3273	7.3242	7.2415	7.2736	7.2918	7.2834	7.2903	0.44
13	7.5375	7.5318	7.4510	7.4851	7.5013	7.4926	7.4999	0.43
14	8.0712	8.0652	7.9804	8.0159	8.0347	8.0243	8.0319	0.42
15	8.5367	8.5305	8.4411	8.4763	8.4958	8.4841	8.4941	0.42
16	9.0725	9.0645	8.9763	9.0102	9.0322	9.0183	9.0290	0.40
17	9.2635	9.2519	9.1649	9.1980	9.2218	9.2069	9.2178	0.39
18	10.0192	10.0049	9.9121	9.9487	9.9746	9.9585	9.9697	0.39

4.1.3 validation for stability

Table 21 The RSDs (%) of stability (relative peak area) for HPLC-UV fingerprint methodology

Peak	Sample							RSD
number	0	3.0	6.0	12.0	18.0	24.0	average	%
1	0.0488	0.0485	0.0485	0.0491	0.0487	0.0491	0.0488	0.63
2	0.3289	0.3285	0.3295	0.3251	0.3298	0.3371	0.3298	1.19
3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.00
4	62.9221	62.3882	62.3987	63.3738	62.9963	63.4018	62.9135	0.71

5	0.3135	0.3114	0.3111	0.3111	0.3125	0.3169	0.3127	0.72
6	0.0400	0.0396	0.0396	0.0397	0.0396	0.0397	0.0397	0.45
7	0.0534	0.0542	0.0552	0.0569	0.0579	0.0597	0.0562	4.24
8	0.0758	0.0743	0.0737	0.0719	0.0704	0.0700	0.0727	3.18
9	0.0194	0.0196	0.0196	0.0193	0.0192	0.0197	0.0195	1.05
10	0.0313	0.0305	0.0309	0.0309	0.0311	0.0315	0.0310	1.12
11	0.0250	0.0247	0.0245	0.0248	0.0241	0.0242	0.0245	1.43
12	0.0954	0.0963	0.0948	0.0954	0.0943	0.0946	0.0951	0.76
13	0.0353	0.0349	0.0351	0.0350	0.0348	0.0351	0.0350	0.48
14	0.0159	0.0154	0.0153	0.0155	0.0152	0.0147	0.0153	2.50
15	0.0150	0.0143	0.0144	0.0144	0.0145	0.0149	0.0146	2.18
16	0.1098	0.1093	0.1096	0.1098	0.1094	0.1095	0.1096	0.17
17	0.0242	0.0241	0.0243	0.0242	0.0242	0.0237	0.0241	0.86
18	0.5120	0.5088	0.5079	0.5104	0.5104	0.5131	0.5104	0.38

Table 22 The RSDs (%) of stability (relative retention time) for HPLC-UV fingerprint methodology

Peak number	Testing time(h)							RSD %
	0	3.0	6.0	12.0	18.0	24.0	average	
1	0.7029	0.7029	0.7029	0.7092	0.7032	0.7020	0.7039	0.38
2	0.7591	0.7589	0.7592	0.7645	0.7598	0.7591	0.7601	0.29
3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.00
4	1.2052	1.2066	1.2067	1.2077	1.2063	1.2067	1.2065	0.07
5	1.6357	1.6378	1.6363	1.6251	1.6317	1.6335	1.6334	0.28
6	5.4640	5.4801	5.4708	5.4011	5.4207	5.4406	5.4462	0.57
7	5.5345	5.5507	5.5412	5.4708	5.4910	5.5108	5.5165	0.57
8	5.8242	5.8405	5.8302	5.7570	5.7788	5.7996	5.8051	0.56
9	6.3206	6.3363	6.3282	6.2485	6.2712	6.2932	6.2997	0.55
10	6.5586	6.5755	6.5682	6.4857	6.5086	6.5313	6.5380	0.55
11	6.6626	6.6788	6.6730	6.5884	6.6118	6.6335	6.6413	0.55
12	7.3130	7.3312	7.3270	7.2311	7.2583	7.2831	7.2906	0.55
13	7.5228	7.5418	7.5409	7.4377	7.4672	7.4917	7.5004	0.56
14	8.0582	8.0744	8.0774	7.9646	7.9981	8.0240	8.0328	0.56
15	8.5202	8.5374	8.5423	8.4250	8.4583	8.4851	8.4947	0.55
16	9.0569	9.0773	9.0801	8.9580	8.9923	9.0200	9.0308	0.55
17	9.2458	9.2671	9.2709	9.1470	9.1819	9.2081	9.2201	0.54
18	9.9986	10.0275	10.0293	9.8928	9.9337	9.9585	9.9734	0.55

4.2 validation for HPLC-UV quantitative analysis method

4.2.1 validation for instrument precision

Table 23 The RSDs (%) of the instrument precision (peak area) for HPLC-UV method

Samples	Peak area				
	DSS	PA	RA	SAB	SAA
1	597.90	183.40	56.80	65.30	305.30
2	596.40	183.00	56.60	65.50	305.60
3	597.90	181.60	56.70	65.30	306.10
4	596.70	180.90	56.80	65.60	305.50
5	596.20	180.00	56.90	65.30	305.20
6	596.00	180.90	57.10	65.40	305.70
average	596.85	181.63	56.82	65.40	305.57
RSD (%)	0.14	0.73	0.30	0.19	0.10

Table 24 The RSDs (%) of the instrument precision (retention time) for HPLC-UV method

Samples	Retention time				
	DSS	PA	RA	SAB	SAA
1	6.249	10.246	46.081	57.074	63.043
2	6.298	10.312	45.973	56.948	62.922
3	6.259	10.280	46.085	57.073	63.041
4	6.278	10.285	46.021	57.005	62.973
5	6.247	10.272	46.035	57.001	62.938
6	6.297	10.306	46.050	57.091	63.028
average	6.271	10.284	46.041	57.032	62.991
RSD (%)	0.37	0.23	0.09	0.10	0.09

4.2.2 validation for intra-day precision

Table 25 The RSDs (%) of the intra-day precision (concentration) for HPLC-UV method

Samples	Concentration (mg g^{-1})				
	DSS	PA	RA	SAB	SAA
1	0.4116	0.0191	0.0150	0.0297	0.0553
2	0.4121	0.0192	0.0150	0.0301	0.0554
3	0.4135	0.0192	0.0149	0.0300	0.0554
4	0.4104	0.0194	0.0152	0.0300	0.0553
5	0.4111	0.0198	0.0149	0.0301	0.0556
6	0.4125	0.0203	0.0150	0.0302	0.0558
average	0.4119	0.0195	0.0150	0.0300	0.0555
RSD (%)	0.27	2.35	0.69	0.51	0.34

4.2.3 validation for inter-day precision

Table 26 The RSDs (%) of the inter-day precision (concentration mg mL^{-1}) for HPLC-UV method

Time (day)	Concentration (mg mL^{-1})				
	DSS	PA	RA	SAB	SAA
1	0.4198	0.0199	0.0149	0.0308	0.0573
2	0.4108	0.0196	0.0148	0.0302	0.0559
3	0.4079	0.0171	0.0147	0.0300	0.0568
4	0.4020	0.0190	0.0146	0.0296	0.0581
5	0.4033	0.0195	0.0148	0.0307	0.0607
6	0.4058	0.0191	0.0150	0.0308	0.0635
average	0.4083	0.0190	0.0148	0.0303	0.0587
RSD (%)	1.59	5.33	0.80	1.62	4.88

4.2.4 validation for stability

Table 27 The RSDs (%) of the stability (concentration mg g^{-1}) for HPLC-UV method

Time (h)	Concentration (mg g^{-1})				
	DSS	PA	RA	SAB	SAA
0	0.2263	0.0109	0.0082	0.0164	0.0304
3	0.2279	0.0109	0.0083	0.0165	0.0304
6	0.2278	0.0109	0.0082	0.0165	0.0303
12	0.2263	0.0108	0.0082	0.0164	0.0303
18	0.2270	0.0109	0.0081	0.0164	0.0304
24	0.2256	0.0110	0.0081	0.0163	0.0303
average	0.2268	0.0109	0.0082	0.0164	0.0304
RSD (%)	0.41	0.54	1.00	0.38	0.14

4.2.5 validation for accuracy

Table 28 Accuracy for HPLC-ELSD method

Compounds	Samples	m_1 (mg)	m_2 (mg)	m_3 (mg)	Recovery rate (%)	Average recovery rate (%)	RSD %
DSS	1	1.0781	0.6299	1.6819	95.87	98.01	1.59
	2	1.0775	0.6299	1.6902	97.26		
	3	1.0776	0.6299	1.6812	95.82		
	4	1.0773	1.0499	2.1169	99.02		
	5	1.0764	1.0499	2.1027	97.75		
	6	1.0768	1.0499	2.1083	98.25		
	7	1.0767	1.4698	2.5346	99.19		
	8	1.0787	1.4698	2.5492	100.04		
	9	1.0772	1.4698	2.5312	98.93		
PA	1	0.0530	0.0451	0.0982	100.23	95.10	4.18
	2	0.0530	0.0451	0.0982	100.15		
	3	0.0530	0.0451	0.0979	99.48		
	4	0.0530	0.0751	0.1247	95.38		
	5	0.0530	0.0751	0.1238	94.23		
	6	0.0530	0.0751	0.1233	93.65		
	7	0.0530	0.1052	0.1482	90.54		
	8	0.0531	0.1052	0.1493	91.53		

	9	0.0530	0.1052	0.1484	90.74		
RA	1	0.0402	0.0355	0.0754	99.24	99.50	0.91
	2	0.0402	0.0355	0.0754	99.30		
	3	0.0402	0.0355	0.0751	98.44		
	4	0.0402	0.0591	0.0990	99.45		
	5	0.0401	0.0591	0.0985	98.74		
	6	0.0401	0.0591	0.0985	98.72		
	7	0.0401	0.0828	0.1233	100.44		
	8	0.0402	0.0828	0.1240	101.27		
	9	0.0401	0.0828	0.1228	99.87		
SAB	1	0.0807	0.1366	0.2129	96.76	98.74	1.21
	2	0.0807	0.1366	0.2147	98.13		
	3	0.0807	0.1366	0.2142	97.74		
	4	0.0807	0.2276	0.3086	100.11		
	5	0.0806	0.2276	0.3057	98.88		
	6	0.0806	0.2276	0.3060	98.99		
	7	0.0806	0.3187	0.3988	99.83		
	8	0.0808	0.3187	0.4004	100.27		
	9	0.0807	0.3187	0.3928	97.94		
SAA	1	0.1547	0.1204	0.2791	103.36	104.49	0.92
	2	0.1546	0.1204	0.2802	104.34		
	3	0.1546	0.1204	0.2790	103.33		
	4	0.1546	0.2006	0.3660	105.40		
	5	0.1544	0.2006	0.3642	104.56		
	6	0.1545	0.2006	0.3646	104.73		
	7	0.1545	0.2809	0.4501	105.26		
	8	0.1548	0.2809	0.4524	105.95		
	9	0.1545	0.2809	0.4451	103.43		

m_1: the content contained in the sample.m_2: the content added in the sample. m_3: the content detected in the sample.、

5 Linearity for different methods

Table 29 The linearity for different methods

Method	Compounds	Regression equation	R^2	Linearity range (μg mL^{-1})
HPLC	macromolecule	Y=-0.2265X+7.4854	0.9996	Mw:1638~13700
The phenol sulfuric acid assay	Glu	Y=9.3201X-0.0061	0.9995	20.7～93.2
HPLC-ELSD	Fru	Y=1.4544X+0.2334	0.9993	81.2～677
	Glu	Y=1.4493 X-0.4727	0.9998	66.5～554
	Mel	Y=1.5211 X-1.7741	0.9994	50.6～422
	Man	Y=1.5224X-2.3766	0.9998	121～1010

	DSS	Y=2606.0X+2.3184	0.9999	7.42～473
	PA	Y=16845.2X+2.1490	0.9999	1.16～73.9
HPLC	RA	Y=6758.1X+1.2019	0.9999	1.18～75.1
	SA B	Y=3881.4X+1.2153	0.9999	2.31～148
	SA A	Y=10057.0X-2.4057	0.9997	2.02～129

6 Determination of indicators

6.1 Macromolecular impurity inspection

13 batches of injections were tested, which were shown as followed. And no macromolecular impurity was detected. Each sample was measured in parallel twice.

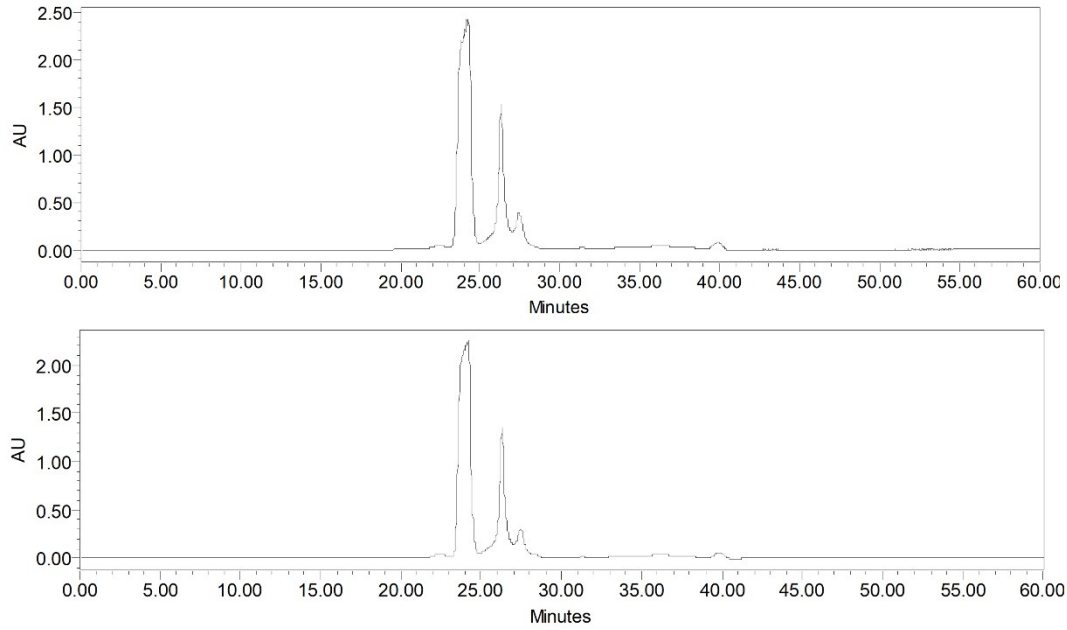

Fig. 1 HPLC-UV chromatograms of batch 1

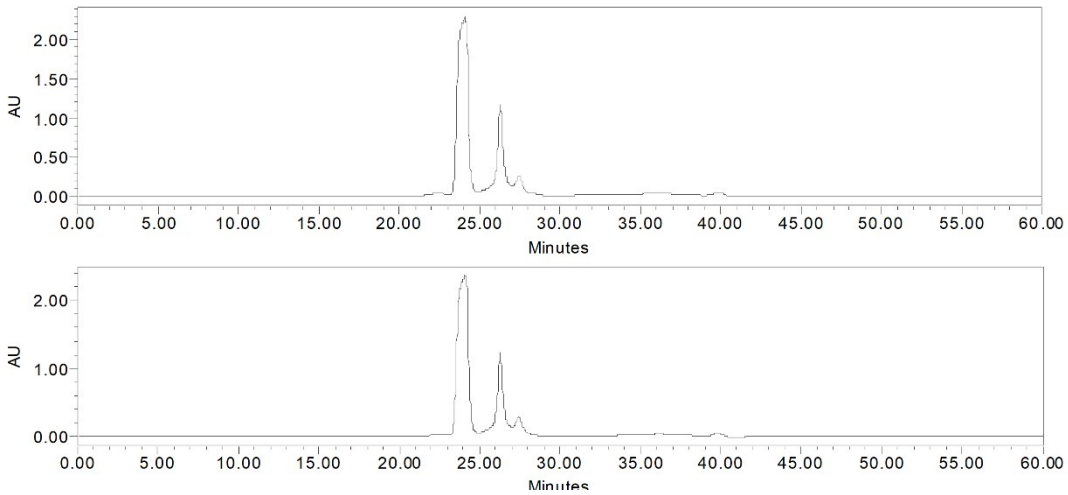

Fig. 2 HPLC-UV chromatograms of batch 2

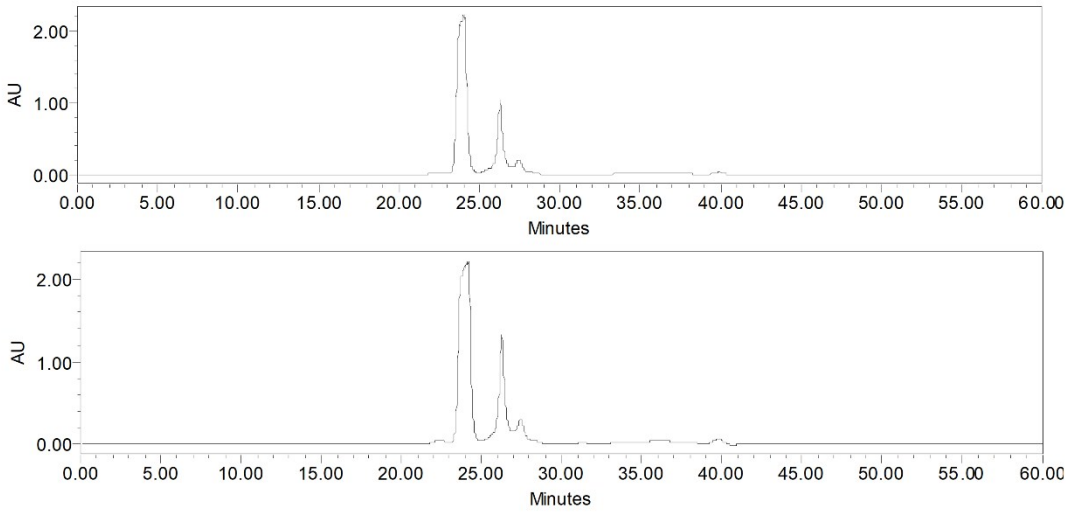

Fig. 3 HPLC-UV chromatograms of batch 3

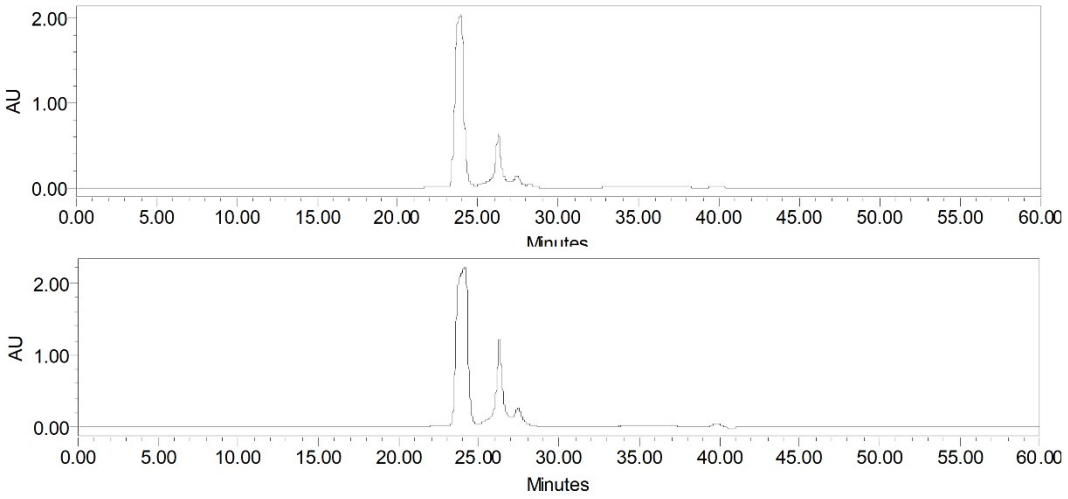

Fig. 4 HPLC-UV chromatograms of batch 4

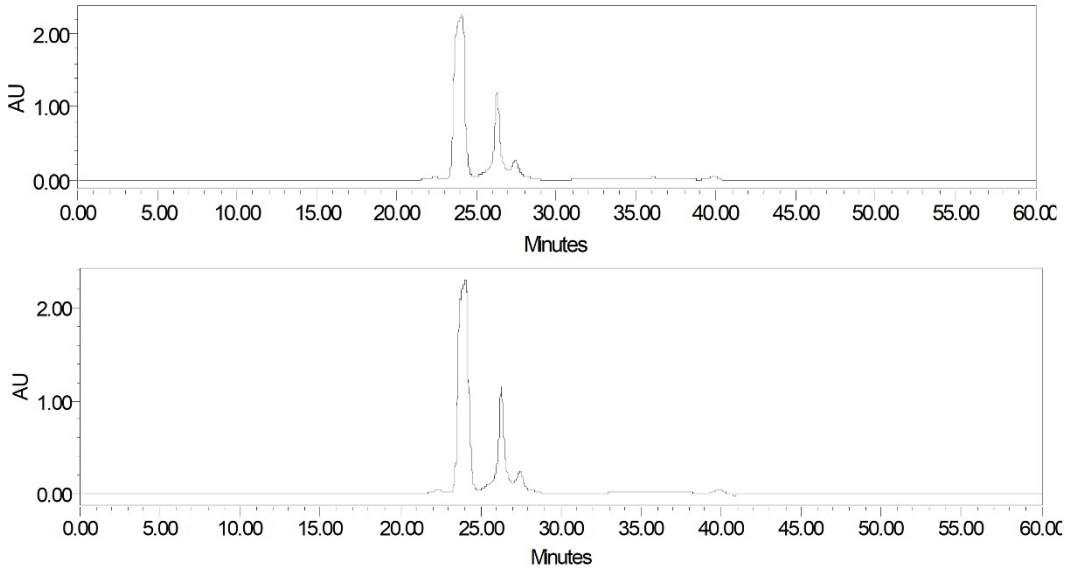

Fig. 5 HPLC-UV chromatograms of batch 5

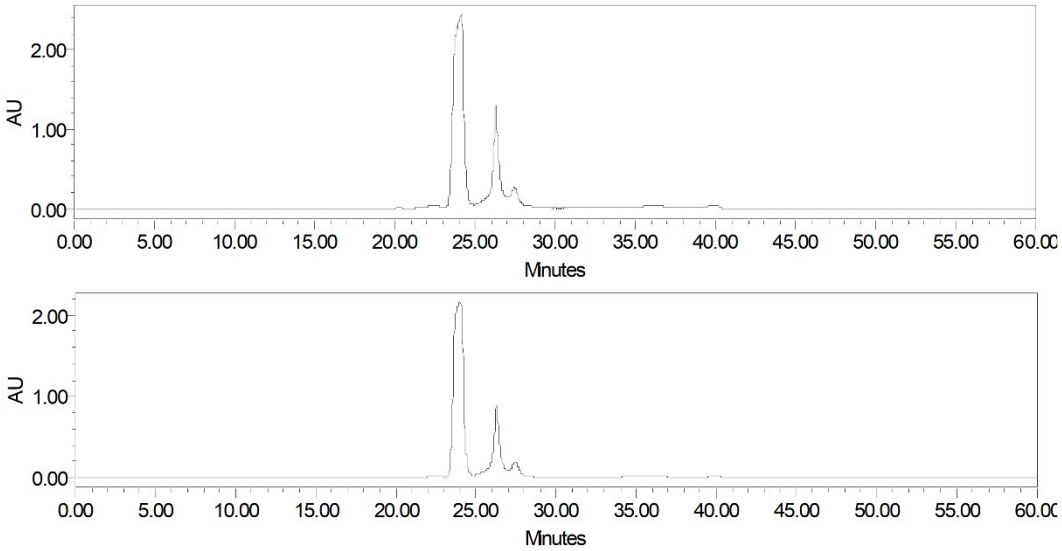

Fig. 6 HPLC-UV chromatograms of batch 6

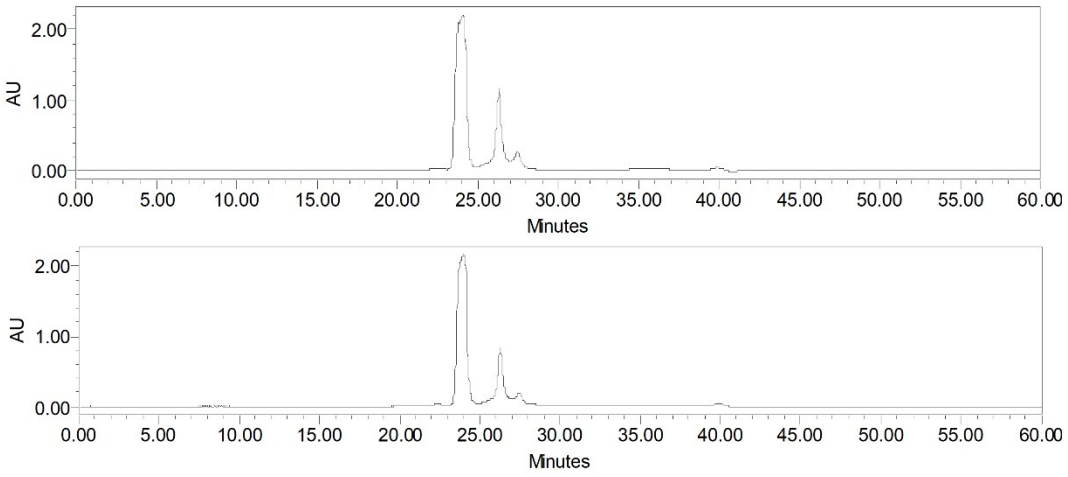

Fig. 7 HPLC-UV chromatograms of batch 7

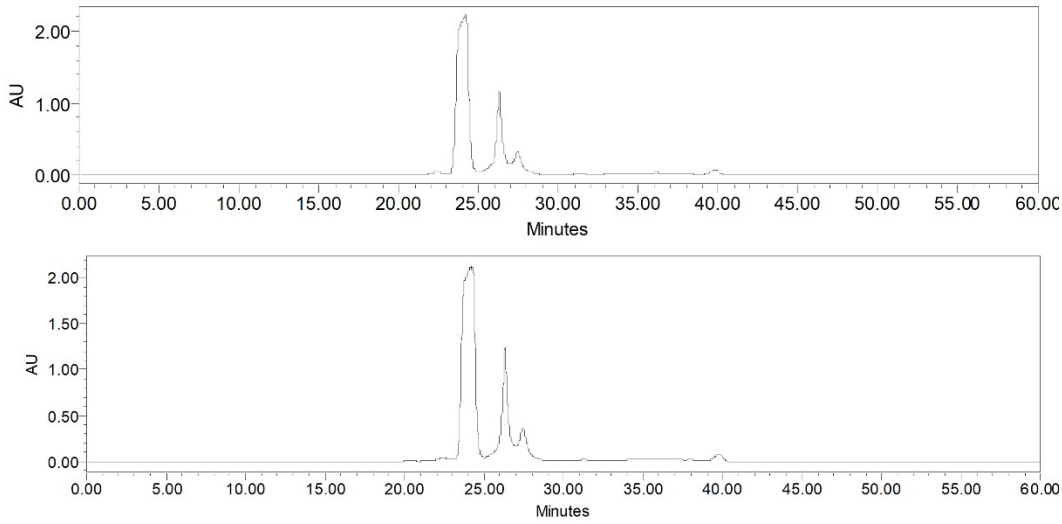

Fig. 8 HPLC-UV chromatograms of batch 8

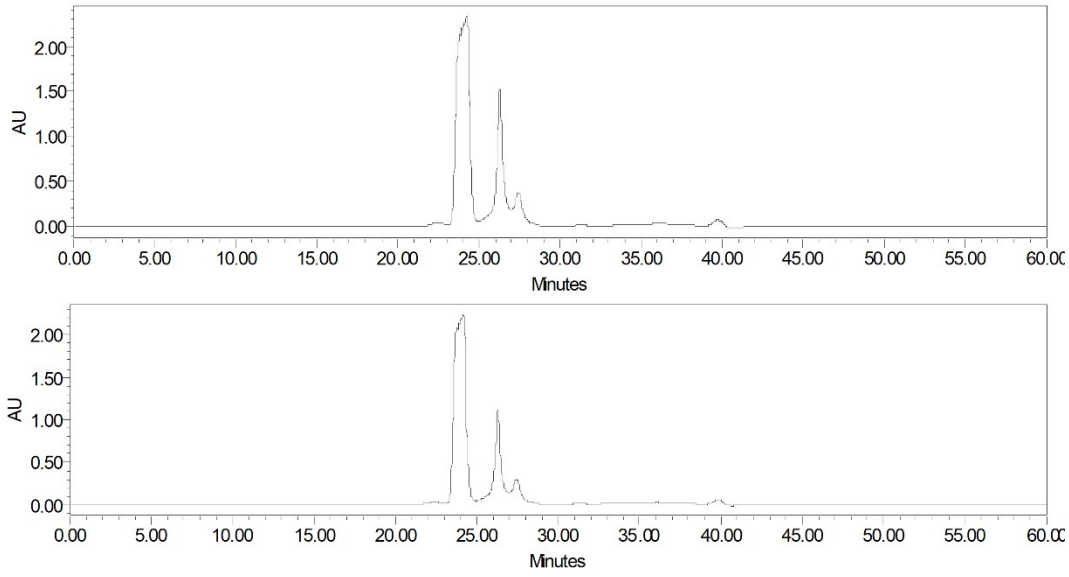

Fig. 9 HPLC-UV chromatograms of batch 9

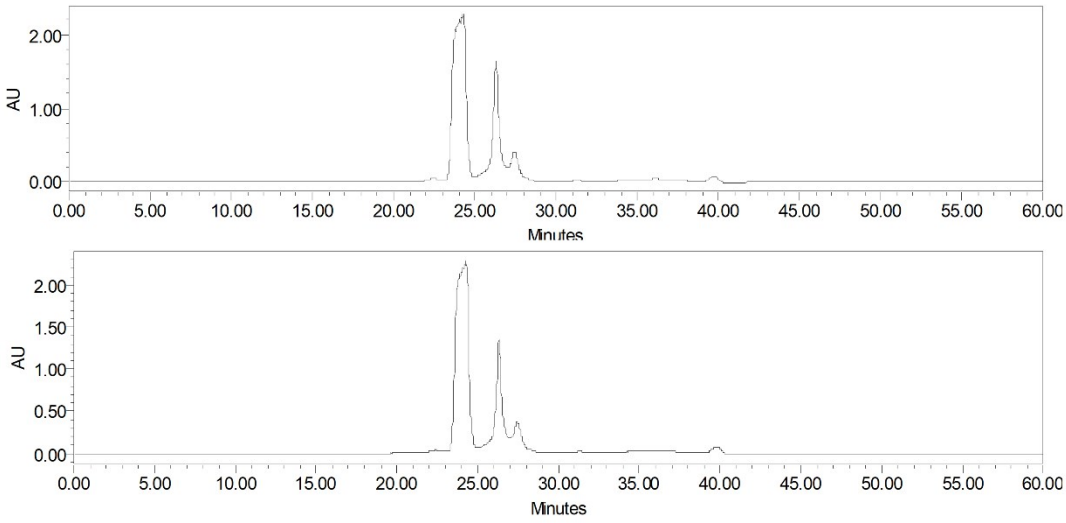

Fig. 10 HPLC-UV chromatograms of batch 10

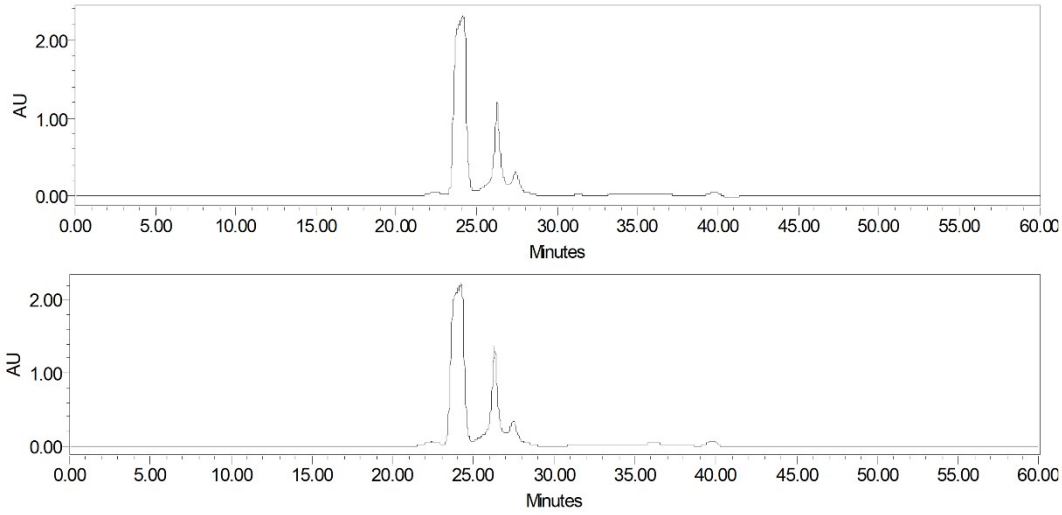

Fig. 11 HPLC-UV chromatograms of batch 11

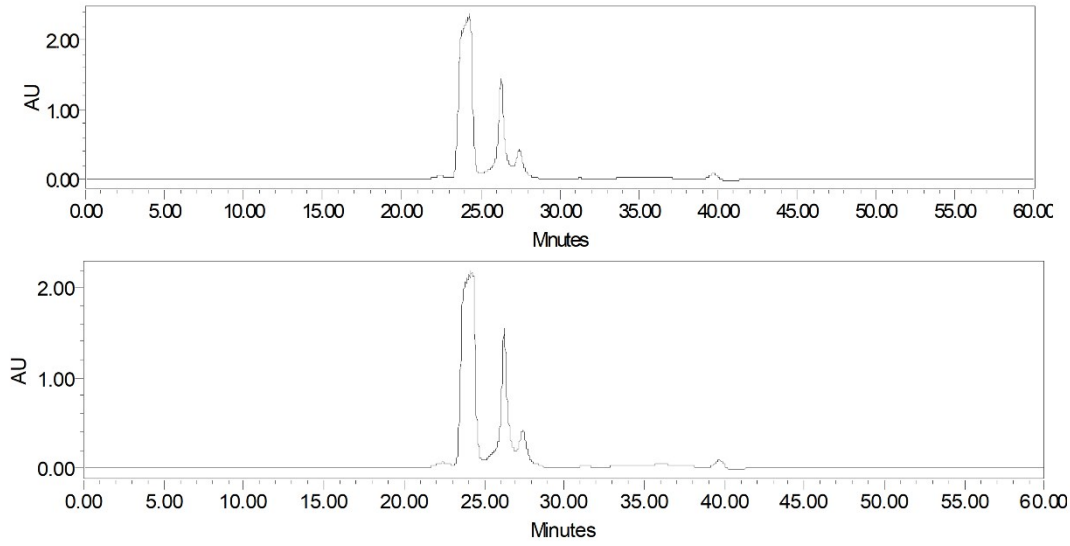

Fig. 12 HPLC-UV chromatograms of batch 12

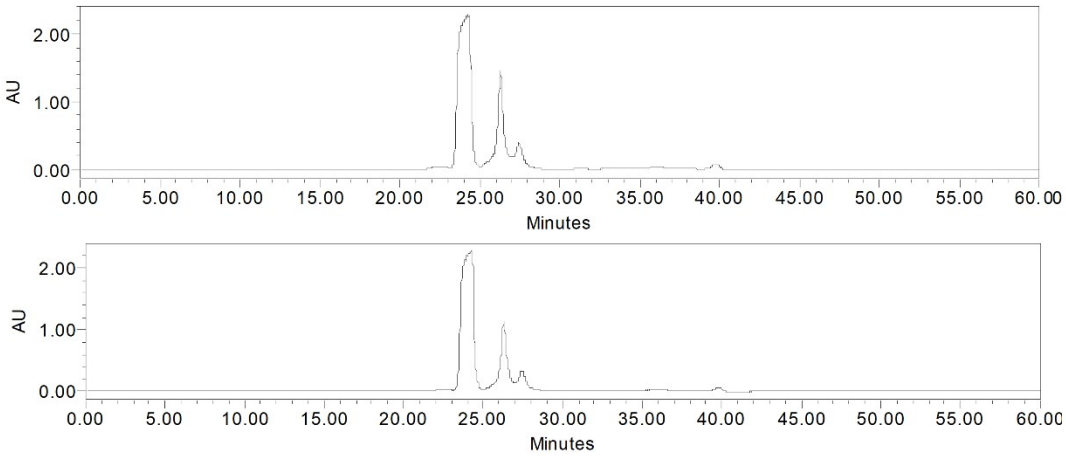

Fig. 13 HPLC-UV chromatograms of batch 13

6.2 Content data of total sugar in SL I

Total sugar content of 13 batches of injection were shown as followed. Each sample was measured in parallel 3 times.

Table 30 Total sugar content of 13 batches of injection

Batch number	Content (%)			average	RSD %
	1	2	3		
1	0.3171	0.3073	0.3163	0.3136	1.73
2	0.3000	0.2967	0.3020	0.2996	0.90
3	0.3008	0.3111	0.3102	0.3073	1.86
4	0.3076	0.3078	0.3079	0.3078	0.05
5	0.3021	0.3034	0.2995	0.3017	0.66
6	0.3112	0.3114	0.3218	0.3148	1.93
7	0.3136	0.3168	0.3131	0.3145	0.63
8	0.3009	0.3016	0.3083	0.3036	1.35
9	0.2947	0.2960	0.3002	0.2970	0.97
10	0.3075	0.3157	0.3047	0.3093	1.85
11	0.3060	0.2972	0.2976	0.3003	1.65
12	0.3136	0.3144	0.3159	0.3146	0.38
13	0.2928	0.3012	0.3009	0.2983	1.61

6.3 Content data of saccharides in SL I

Saccharides content of 13 batches of injection were shown as followed. Each sample was measured in parallel 3 times.

Table 31 Fru content of 13 batches of injection

Batch number	content (%)			
	1	2	3	average
1	0.1554	0.1535	0.1538	0.1543
2	0.1536	0.1516	0.1525	0.1526
3	0.1519	0.1503	0.1502	0.1508
4	0.1568	0.1554	0.1561	0.1561
5	0.1556	0.1554	0.1545	0.1551
6	0.1540	0.1529	0.1525	0.1531
7	0.1586	0.1589	0.1575	0.1583
8	0.1533	0.1554	0.1542	0.1543
9	0.1533	0.1540	0.1521	0.1531
10	0.1539	0.1538	0.1531	0.1536
11	0.1392	0.1379	0.1376	0.1382
12	0.1392	0.1398	0.1392	0.1394
13	0.1376	0.1366	0.1364	0.1369

Table 32 Glu content of 13 batches of injection

Batch number	content (%)			
	1	2	3	average
1	0.0552	0.0550	0.0565	0.0556
2	0.0555	0.0552	0.0561	0.0556
3	0.0554	0.0552	0.0545	0.0550
4	0.0573	0.0563	0.0574	0.0570
5	0.0570	0.0567	0.0569	0.0569
6	0.0549	0.0559	0.0559	0.0556
7	0.0570	0.0563	0.0577	0.0570
8	0.0554	0.0559	0.0563	0.0558

9	0.0555	0.0552	0.0546	0.0551
10	0.0530	0.0562	0.0542	0.0545
11	0.0562	0.0564	0.0577	0.0568
12	0.0572	0.0565	0.0577	0.0571
13	0.0575	0.0583	0.0577	0.0578

Table 33 Mel content of 13 batches of injection

Batch number	content (%)			
	1	2	3	average
1	0.0178	0.0176	0.0180	0.0178
2	0.0182	0.0180	0.0184	0.0182
3	0.0165	0.0157	0.0159	0.0160
4	0.0190	0.0189	0.0188	0.0189
5	0.0182	0.0179	0.0187	0.0183
6	0.0170	0.0160	0.0170	0.0167
7	0.0192	0.0189	0.0191	0.0190
8	0.0188	0.0184	0.0185	0.0185
9	0.0168	0.0166	0.0158	0.0164
10	0.0201	0.0204	0.0196	0.0200
11	0.0209	0.0205	0.0202	0.0205
12	0.0207	0.0201	0.0199	0.0202
13	0.0196	0.0194	0.0193	0.0194

Table 34 Man content of 13 batches of injection

Batch number	content (%)			
	1	2	3	average
1	0.0661	0.0705	0.0703	0.0690

2	0.0712	0.0709	0.0730	0.0717
3	0.0604	0.0616	0.0609	0.0610
4	0.0727	0.0701	0.0720	0.0716
5	0.0741	0.0747	0.0733	0.0740
6	0.0619	0.0621	0.0616	0.0619
7	0.0753	0.0741	0.0735	0.0743
8	0.0718	0.0726	0.0731	0.0725
9	0.0644	0.0637	0.0616	0.0632
10	0.0822	0.0801	0.0793	0.0805
11	0.0848	0.0840	0.0814	0.0834
12	0.0818	0.0800	0.0799	0.0806
13	0.0770	0.0769	0.0756	0.0765

6.4 Content data of phenolic acids in SL I

phenolic acids content of 13 batches of injection were shown as followed. Each sample was measured in parallel 3 times.

Table 35 DSS content of 13 batches of injection

Batch	content (%)			
number	1	2	3	average
1	0.0434	0.0436	0.0435	0.0435
2	0.0428	0.0427	0.0428	0.0428
3	0.0432	0.0430	0.0430	0.0430
4	0.0433	0.0432	0.0430	0.0432
5	0.0433	0.0433	0.0433	0.0433
6	0.0434	0.0432	0.0432	0.0432
7	0.0449	0.0446	0.0447	0.0448
8	0.0440	0.0440	0.0439	0.0440
9	0.0435	0.0437	0.0437	0.0436
10	0.0436	0.0436	0.0436	0.0436
11	0.0438	0.0439	0.0437	0.0438
12	0.0443	0.0441	0.0440	0.0441
13	0.0437	0.0435	0.0436	0.0436

Table 36 PA content of 13 batches of injection

Batch number	content (%)			
	1	2	3	average
1	0.0024	0.0023	0.0024	0.0024
2	0.0024	0.0023	0.0023	0.0023
3	0.0024	0.0024	0.0024	0.0024
4	0.0023	0.0024	0.0024	0.0024
5	0.0024	0.0024	0.0024	0.0024
6	0.0025	0.0024	0.0024	0.0024
7	0.0025	0.0025	0.0025	0.0025
8	0.0024	0.0025	0.0025	0.0025
9	0.0024	0.0024	0.0024	0.0024
10	0.0023	0.0023	0.0023	0.0023
11	0.0019	0.0019	0.0019	0.0019
12	0.0019	0.0019	0.0019	0.0019
13	0.0018	0.0019	0.0018	0.0018

Table 37 RA content of 13 batches of injection

Batch number	content (%)			
	1	2	3	average
1	0.0022	0.0022	0.0022	0.0022
2	0.0022	0.0022	0.0022	0.0022
3	0.0022	0.0022	0.0022	0.0022
4	0.0022	0.0022	0.0022	0.0022
5	0.0022	0.0022	0.0022	0.0022
6	0.0022	0.0022	0.0022	0.0022
7	0.0023	0.0023	0.0023	0.0023
8	0.0023	0.0023	0.0023	0.0023
9	0.0022	0.0022	0.0022	0.0022
10	0.0022	0.0022	0.0022	0.0022
11	0.0016	0.0016	0.0016	0.0016
12	0.0016	0.0016	0.0016	0.0016
13	0.0016	0.0016	0.0016	0.0016

Table 38 SAB content of 13 batches of injection

Batch number	content (%)			
	1	2	3	average
1	0.0050	0.0052	0.0052	0.0051
2	0.0049	0.0048	0.0050	0.0049
3	0.0050	0.0048	0.0050	0.0049
4	0.0049	0.0049	0.0049	0.0049

5	0.0052	0.0052	0.0052	0.0052
6	0.0050	0.0050	0.0051	0.0050
7	0.0054	0.0053	0.0054	0.0053
8	0.0051	0.0052	0.0051	0.0051
9	0.0049	0.0052	0.0052	0.0051
10	0.0052	0.0051	0.0053	0.0052
11	0.0056	0.0056	0.0056	0.0056
12	0.0057	0.0057	0.0057	0.0057
13	0.0055	0.0055	0.0055	0.0055

Table 39 SAA content of 13 batches of injection

Batch number	content (%)			
	1	2	3	average
1	0.0066	0.0066	0.0066	0.0066
2	0.0065	0.0064	0.0065	0.0064
3	0.0063	0.0062	0.0063	0.0062
4	0.0062	0.0062	0.0061	0.0062
5	0.0066	0.0066	0.0066	0.0066
6	0.0064	0.0062	0.0063	0.0063
7	0.0068	0.0068	0.0068	0.0068
8	0.0065	0.0066	0.0067	0.0066
9	0.0066	0.0066	0.0066	0.0066
10	0.0067	0.0067	0.0067	0.0067
11	0.0040	0.0041	0.0040	0.0040
12	0.0041	0.0041	0.0041	0.0041
13	0.0038	0.0038	0.0038	0.0038

6.5 Similarity evaluation of 13 batches of SL I

Table 40 The similarity evaluation result of the 13 batch SL I (including the Ligustrazine Hydrochloride)

Batch numbers	Similarity	Batch numbers	Similarity
1	1	8	1
2	1	9	1
3	1	10	1
4	1	11	1
5	1	12	1
6	1	13	1
7	1		

Similarity: comparing the injection chromatogram with the control fingerprint.

Table 41 The similarity evaluation result of the 13 batch SL I (without the Ligustrazine Hydrochloride)

Batch numbers	Similarity	Batch numbers	Similarity
1	0.999	8	0.999
2	0.999	9	0.999
3	0.999	10	0.999
4	0.999	11	0.988
5	0.998	12	0.988
6	0.999	13	0.985
7	0.999		

Similarity: comparing the injection chromatogram with the control fingerprint.

6.6 Content data of inorganic salt ions in SL I

Table 42 Anion content of 13 batches SL I

Batch number	Cl^- (mg/L)	NO_3^- (mg/L)	SO_4^{2-} (mg/L)
1	3.2779	0.0721	0.1171
2	3.1748	0.0694	0.1225
3	3.2534	0.072	0.1492
4	3.2827	0.0707	0.1187
5	3.2052	0.0706	0.1265
6	3.2927	0.0717	0.1341
7	3.1201	0.0747	0.1167
8	3.2347	0.0768	0.1052
9	3.2533	0.0728	0.1242
10	3.2353	0.0716	0.123
11	3.2878	0.0679	0.1025
12	3.2311	0.0687	0.0945
13	3.3242	0.0683	0.1013

Table 43 Cation content of 13 batches SL I

Batch number	Na^+ (mg/L)	K^+ (mg/L)	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)	Fe^+ (mg/L)
1	318.7	162.6	7.380	41.79	0.109
2	275.3	119.0	7.350	41.75	0.103
3	277.3	129.9	6.920	41.76	0.152
4	387.9	157.2	8.460	58.80	0.106
5	273.7	116.5	7.050	41.78	0.118

6	267.1	98.60	7.040	42.50	0.075
7	261.5	133.7	7.380	42.59	0.105
8	266.6	127.8	6.980	42.21	0.099
9	266.7	127.0	7.020	41.84	0.106
10	292.9	143.7	6.870	42.21	0.079
11	277.9	124.8	7.490	43.00	0.097
12	267.1	118.9	7.270	40.96	0.066
13	299.4	140.9	7.850	45.24	0.067