

Table S1 Retention time and peak area of the analysts in method validation.

Project	Group	RT (min)						RA (mAu)					
		Loganic acid	6'-O-β-D-glucosylgentiopicoside	Swertiamarine	Gentiopicoside	Sweroside	Loganic acid	6'-O-β-D-glucosylgentiopicoside	Swertiamarine	Gentiopicoside	Sweroside		
Precision	1	14.97	16.58	17.63	20.40	22.11	1067.26	69.65	214.48	2280.31	94.28		
	2	15.00	16.61	17.66	20.42	22.13	1065.54	69.64	214.17	2276.76	94.41		
	3	15.01	16.62	17.66	20.42	22.12	1065.12	69.69	214.02	2277.37	94.90		
	4	14.98	16.59	17.64	20.41	22.12	1064.95	69.17	213.22	2277.57	94.42		
	5	14.98	16.58	17.64	20.41	22.12	1064.79	69.28	212.83	2273.75	94.58		
	6	14.97	16.58	17.64	20.41	22.13	1065.19	69.50	213.33	2280.96	95.00		
	RSD (%)	0.10	0.10	0.07	0.04	0.03	0.09	0.31	0.30	0.11	0.30		
Repeatability	1	14.84	16.43	17.48	20.28	22.01	1049.54	66.64	204.33	2224.10	94.27		
	2	14.90	16.49	17.54	20.33	22.05	1067.21	68.79	211.86	2284.84	95.10		
	3	14.94	16.54	17.58	20.36	22.07	1073.50	69.13	212.77	2293.49	95.28		
	4	14.92	16.55	17.59	20.37	22.08	1100.55	70.65	216.14	2341.47	99.25		
	5	14.93	16.52	17.58	20.36	22.07	1097.94	70.83	216.62	2356.39	99.00		
	6	14.96	16.57	17.61	20.39	22.10	1086.71	69.94	214.65	2335.57	96.94		
	RSD (%)	0.28	0.30	0.26	0.18	0.14	1.82	2.23	2.12	2.12	2.19		
Stability	0 h	14.88	16.48	17.54	20.32	22.04	1055.34	67.67	210.04	2244.22	94.88		
	2 h	14.90	16.50	17.56	20.34	22.05	1056.36	67.83	206.89	2250.74	89.74		
	4 h	15.14	16.72	17.74	20.44	22.12	1057.59	70.23	214.28	2247.66	88.95		
	8 h	14.90	16.49	17.56	20.34	22.05	1053.92	67.77	211.17	2251.41	91.18		
	12 h	14.78	16.37	17.44	20.25	21.97	1084.68	69.74	214.54	2321.49	91.77		
	24 h	14.89	16.48	17.53	20.32	22.04	1058.97	68.39	207.62	2256.31	90.09		
	48 h	14.82	16.41	17.47	20.27	21.99	1094.52	70.20	213.35	2333.03	93.25		
	96 h	14.81	16.39	17.45	20.24	21.96	1071.71	69.02	211.79	2287.03	88.60		
	RSD (%)	0.75	0.66	0.54	0.31	0.23	1.63	1.68	1.59	1.73	1.71		

Table S2 The durability result of the HPLC method.

Analyte	Instrument	Column	Loganic acid		6'-O- β -D-glucosylgentiopicroside		Swertiamarine		Gentiopicroside		Sweroside	
			RRT (min)	RPA (mAu)	RRT (min)	RPA (mAu)	RRT (min)	RPA (mAu)	RRT (min)	RPA (mAu)	RRT (min)	RPA (mAu)
Sample	Agilent 1260	Waters Atlantis® T3, 4.6×250mm, 5 μ m	0.73	0.47	0.81	0.03	0.86	0.09	1.00	1.00	1.08	0.04
	Infinity II	Agilent 5 TC-C18, 4.6×250 mm	0.73	0.46	0.81	0.03	0.84	0.09	1.00	1.00	1.09	0.04
		YMC-PACK ODS-A, 150×4.6mm, 5 μ m	0.66	0.46	0.75	0.03	0.81	0.09	1.00	1.00	1.15	0.04
	SHIMADZU	Waters Atlantis® T3, 4.6×250mm, 5 μ m	0.75	0.43	0.82	0.03	0.87	0.09	1.00	1.00	1.08	0.04
	Nexera LC-40D	Agilent 5 TC-C18, 4.6×250 mm	0.74	0.43	0.82	0.03	0.85	0.08	1.00	1.00	1.09	0.04
		YMC-PACK ODS-A, 150×4.6mm, 5 μ m	0.66	0.45	0.76	0.03	0.81	0.08	1.00	1.00	1.15	0.04
Mixed standards	Agilent 1260	Waters Atlantis® T3, 4.6×250mm, 5 μ m	0.73	0.33	0.81	0.09	0.86	0.10	1.00	1.00	1.08	0.08
	Infinity II	Agilent 5 TC-C18, 4.6×250 mm	0.73	0.33	0.81	0.10	0.84	0.10	1.00	1.00	1.09	0.08
		YMC-PACK ODS-A, 150×4.6mm, 5 μ m	0.67	0.33	0.76	0.10	0.81	0.10	1.00	1.00	1.15	0.08
	SHIMADZU	Waters Atlantis® T3, 4.6×250mm, 5 μ m	0.75	0.30	0.82	0.09	0.87	0.10	1.00	1.00	1.08	0.08
	Nexera LC-40D	Agilent 5 TC-C18, 4.6×250 mm	0.74	0.30	0.82	0.10	0.85	0.10	1.00	1.00	1.09	0.08
		YMC-PACK ODS-A, 150×4.6mm, 5 μ m	0.67	0.31	0.76	0.10	0.81	0.09	1.00	1.00	1.15	0.08

Table S3 Co-relationships of five active compounds in *Gentianae Macrophyllae Radix*.

	Sweroside	6'- <i>O</i> - β -D-glucosylgentiopicroside	Loganic acid	Swertiamarine	Gentiopicroside
Sweroside	1	-0.4211*	-0.061793	-0.048271	-0.15493
6'- <i>O</i> - β -D-glucosylgentiopicroside		1	0.3788*	0.33998	0.40388*
Loganic acid			1	0.66155**	0.65182**
Swertiamarine				1	0.97185**
Gentiopicroside					1

* $p < 0.05$; ** $p < 0.01$.

Table S4 Response factors for the compounds.

No.	Loganic acid			6'-O-β-D-Glucosylgentiopicroside			Swertiamarine			Gentiopicroside		Sweroside		
	C ₁ (mg/ml)	A ₁	F ₁	C ₂ (mg/ml)	A ₂	F ₂	C ₃ (mg/ml)	A ₃	F ₃	C ₄ (mg/ml)	A ₄	C ₅ (mg/ml)	A ₅	F ₅
1	0.006563	53.846	0.76	0.0046875	14.742	1.98	0.00189375	20.365	0.58	0.0253125	157.513	0.00165625	12.4	0.83
2	0.013125	105.739	0.77	0.009375	29.283	2.00	0.0037875	37.561	0.63	0.050625	316.052	0.0033125	25.005	0.83
3	0.02625	205.197	0.79	0.01875	57.995	1.98	0.007575	69.333	0.67	0.10125	621.521	0.006625	49.765	0.82
4	0.0525	390.141	0.78	0.0375	110.802	1.96	0.01515	126.308	0.70	0.2025	1175.249	0.01325	95.474	0.81
5	0.105	764.331	0.78	0.075	218.099	1.96	0.0303	241.062	0.72	0.405	2311.412	0.0265	188.231	0.80
6	0.21	1582.085	0.78	0.15	453.939	1.95	0.0606	496.561	0.72	0.81	4780.446	0.053	393.587	0.79
Average			0.78			1.97			0.67					0.81
RSD (%)			1.31			0.89			0.08					1.76

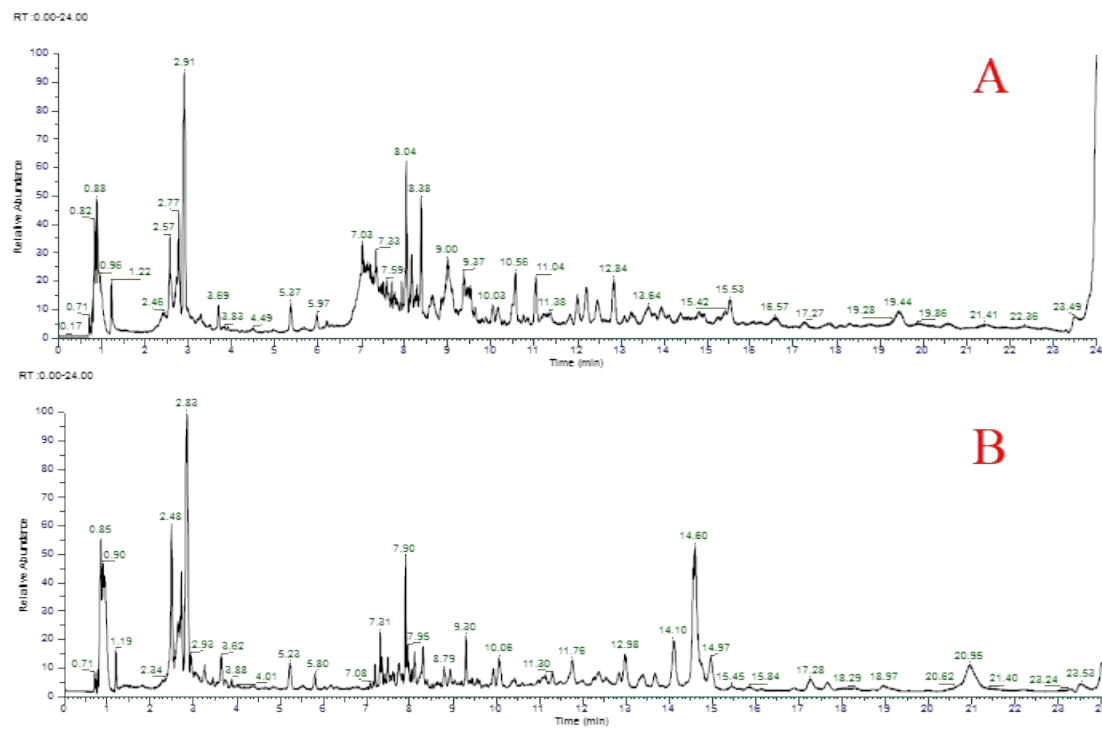


Figure S1 The comparison of positive (A) and negative mode (B) of LC-MS.

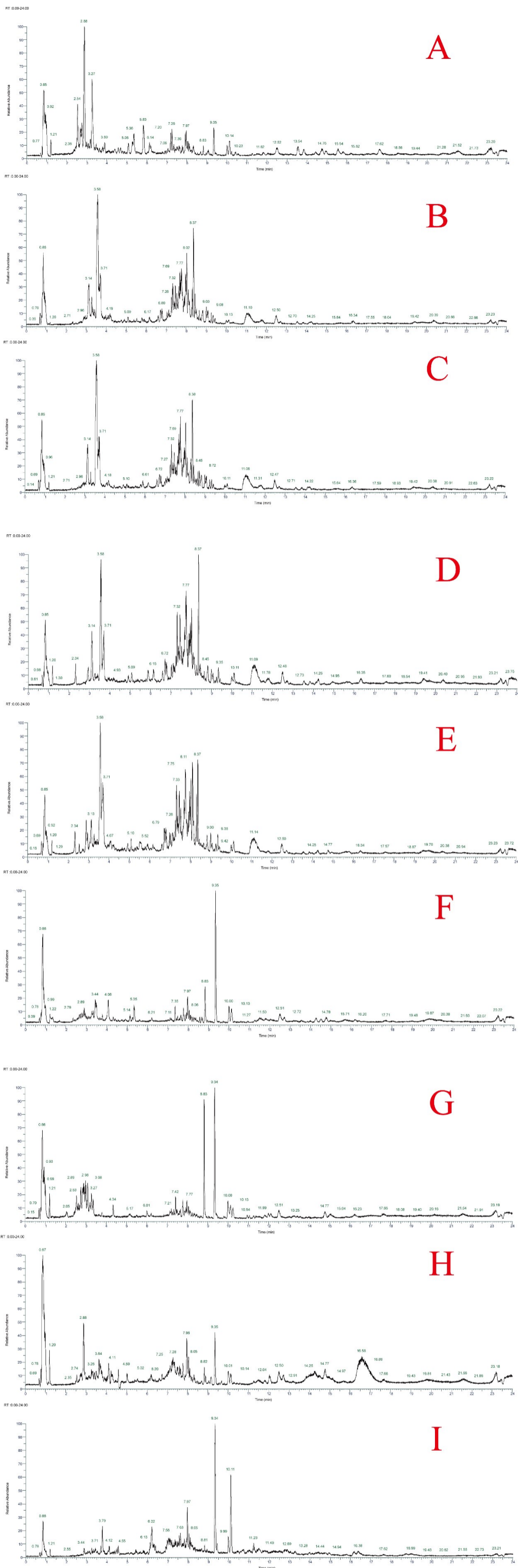


Fig. S2 TIC chromatogram of the adulterants. (A~I represent the adulterant samples A-1~9).

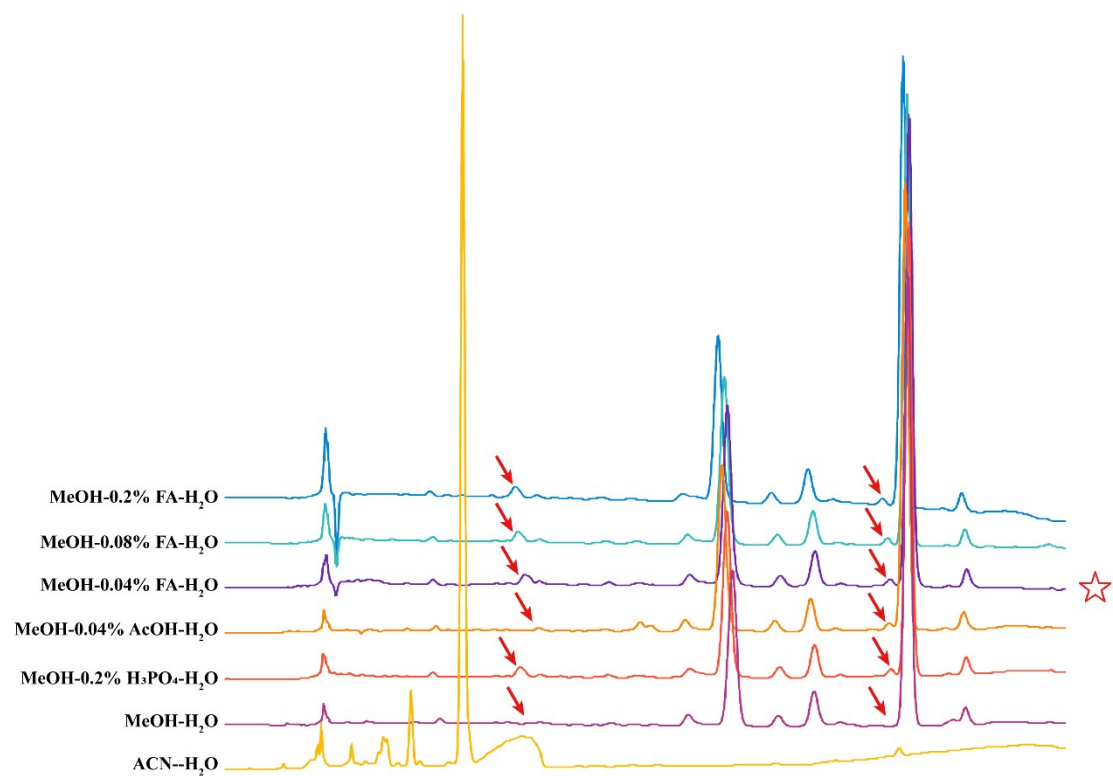


Figure S3 The influence of different acids and PH on the chromatogram.