
Multivariate HPLC System Assessment and Optimization for Traditional Chinese Medicine: A Case Study of *Gastrodia Elata*

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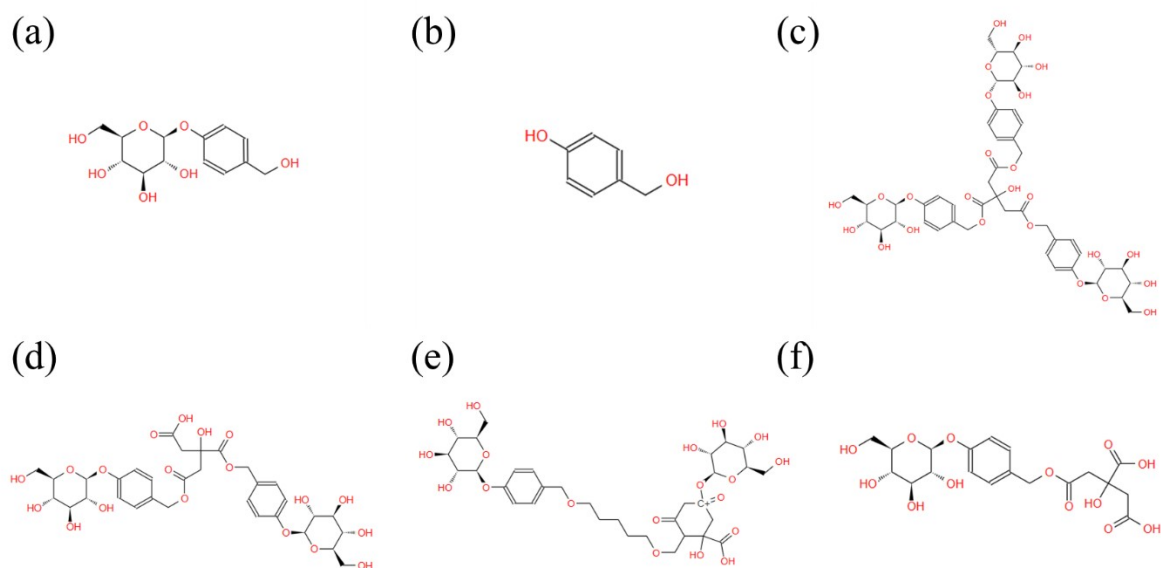


Figure S1 Chemical structures of the standards used: (a) Gastrodin; (b) *p*-Hydroxybenzyl alcohol; (c) Parisin A; (d) Parisin B; (e) Parisin C; (f) Parisin E.

Table S1 HPLC gradient elution program for the analysis of *Gastrodia elata*.

Time (min)	Solvent A (%)	Solvent B (%)
0	3	97
10	3	97
15	12	88
25	18	82
40	18	82
41	95	5
46	95	5
47	3	97

Table S2 The sequence and number of injections of the reference and sample solutions for analyst A/B.

No.	Injected solution	No. of injections	Injected solution	No. of injections
1	RefA1	1	RefB1	1
2	RefA2	1	RefB2	1
3	RefA3	1	RefB3	1
4	RefA4	1	RefB4	1
5	RefA5	1	RefB5	1
6	SampA52	3	SampB52	3
7	SampA42	3	SampB42	3
8	SampA31	3	SampB31	3
9	SampA43	3	SampB43	3
10	SampA33	3	SampB33	3
11	RefA1	1	RefB1	1
12	RefA2	1	RefB2	1
13	RefA3	1	RefB3	1
14	RefA4	1	RefB4	1

Supplementary Material

15	RefA5	1	RefB5	1
16	SampA41	3	SampB41	3
17	SampA22	3	SampB22	3
18	SampA13	3	SampB13	3
19	SampA21	3	SampB21	3
20	SampA53	3	SampB53	3
21	RefA1	1	RefB1	1
22	RefA2	1	RefB2	1
23	RefA3	1	RefB3	1
24	RefA4	1	RefB4	1
25	RefA5	1	RefB5	1
26	SampA51	3	SampB51	3
27	SampA23	3	SampB23	3
28	SampA32	3	SampB32	3
29	SampA11	3	SampB11	3
30	SampA12	3	SampB12	3
31	RefA1	1	RefB1	1
32	RefA2	1	RefB2	1
33	RefA3	1	RefB3	1
34	RefA4	1	RefB4	1
35	RefA5	1	RefB5	1

Samp A_{ij} denotes a sample sequence from analyst A, corresponding to batch i, preparation j, while Samp B_{ij} refers to a sample sequence from analyst B, associated with batch i, preparation j. Ref A/B 1 ~ 5 represents the reference standard solution at five concentration levels.

Table S3 Normalized data of content of chemical components under the condition (1) and (9) (Standard order) of the full factorial experiment.

Analyst	Sample No.	Sample solution	Gastrodin	<i>p</i> -hydroxybenzyl alcohol	Parishin E	Parishin B	Parishin C	Parishin A
A	1	1	0.3596	-0.9103	0.2923	-0.6610	1.603	-0.5631
A	1	2	0.3079	-0.9285	0.2594	-0.6662	1.486	-0.5784
A	1	3	-0.0648	-1.668	1.009	-0.5306	2.004	-0.0677
A	2	1	1.136	-1.043	-1.149	1.337	0.2212	2.392
A	2	2	1.358	-0.9901	-1.101	1.652	0.5587	2.641
A	2	3	1.275	0.2067	-1.424	0.6500	-0.5140	1.074
A	3	1	-1.459	-0.2726	-0.0821	-0.9789	-0.6358	-0.8095
A	3	2	-1.557	1.608	-0.7194	-1.441	-1.353	-1.263
A	3	3	-1.877	-0.5691	-0.4692	-1.604	-1.511	-1.083
A	4	1	0.4532	-0.9116	0.4838	0.2707	0.7383	0.5775
A	4	2	0.5023	-0.8963	0.5037	0.0991	0.7814	0.5871
A	4	3	-0.0831	-1.083	0.0958	-0.5614	-0.3406	0.1120
A	5	1	-0.5417	1.139	0.9716	0.8597	-0.7539	-0.3788
A	5	2	-0.5461	-0.4477	2.467	1.727	0.1128	0.4345
A	5	3	-0.5771	-0.4469	2.383	1.644	0.0618	0.4008
B	1	1	0.3394	-0.9721	0.2248	-0.6825	1.564	-0.5719
B	1	2	0.3625	-0.9601	0.2421	-0.6413	1.607	-0.5522
B	1	3	0.3746	-0.9456	0.2447	-0.7615	1.630	-0.5433
B	2	1	1.431	0.1496	-1.403	0.8486	-0.3038	1.203
B	2	2	1.563	0.2036	-1.388	0.9863	-0.1577	1.295
B	2	3	1.586	0.1995	-1.382	1.0302	-0.1797	1.306
B	3	1	-1.452	1.719	-0.6781	-1.321	-1.223	-1.221

Supplementary Material

B	3	2	-1.521	1.708	-0.6817	-1.319	-1.214	-1.223
B	3	3	-1.405	1.744	-0.6590	-1.280	-1.175	-1.206
B	4	1	0.5922	0.3622	-0.2885	-0.2718	-0.1731	-0.2192
B	4	2	0.5553	0.3392	-0.2728	-0.3012	-0.1983	-0.2297
B	4	3	0.4870	0.2840	-0.3310	-0.3911	-0.2826	-0.2689
B	5	1	-0.5316	1.178	0.9919	0.8591	-0.7109	-0.3833
B	5	2	-0.5993	1.043	0.8826	0.6085	-0.9180	-0.4723
B	5	3	-0.4686	1.161	0.9773	0.8416	-0.7227	-0.3884

Table S4 Results of Pearson correlation analysis for different components.

	Gastrodin	<i>p</i> -hydroxybenzyl alcohol	Parishin E	Parishin B	Parishin C
<i>p</i> -hydroxybenzyl alcohol	0.456	–	–	–	–
Parishin E	0.344	0.171	–	–	–
Parishin B	0.545	0.137	0.216	–	–
Parishin C	0.505	0.798	0.244	0.070	–
Parishin A	0.786	0.417	0.268	0.783	0.223

Table S5 Prioritization matrix: score for all critical variables

NO.	Critical variables	Weight (rating = 10 for each response)			Score
		% R&R	ndc	Defect rate	
1	Analytical chromatography method	3	3	3	90
2	Integration method	5	5	5	150
3	Analyst experience	5	5	5	150
4	Technique of weighing	7	7	7	210
5	Technique of integration	5	5	5	150
6	Sample injection device	3	3	3	90
7	Detector	5	5	5	150
8	Chromatographic column	5	5	5	150
9	Pump module	5	5	5	150
10	Column temperature box	5	5	5	150
11	Number of concentration levels of reference substance	9	9	9	270
12	Weight of the control substance	7	7	7	210
13	Selection method of reference substance	9	9	9	270
14	The number of injections was repeated	9	9	9	270
15	Weight of the sample	7	7	7	210
16	The homogeneity of the samples	5	5	5	150
17	The number of sample preparation repetitions	9	9	9	270
18	Reagent	5	5	5	150
19	Pipette of water	5	5	5	150
20	Volumetric flask	5	5	5	150
21	Illumination	1	1	1	30
22	Temperature	3	3	3	90
23	Humidity	3	3	3	90

Supplementary Material

Table S6 Risk priority number for the critical variables.

No.	Critical variables	Severity	Occurrence	Detection	Risk priority number
1	Number of concentration levels of reference substance	9	9	9	729
2	Selection method of reference substance	9	9	9	729
3	The number of injections was repeated	9	9	9	729
4	The number of sample preparations	9	9	9	729
5	Technique of weighing	9	7	9	567
6	Weight of the reference substance	9	5	7	315
7	Weight of the sample	9	5	7	315

Supplementary Material

Table S7 The content of chemical components measured under various conditions (Standard order) in the full factorial experiment.

Analyst	Sample No.	Sample solution	Component	(1)(9) (mg·g ⁻¹)	(2)(10) (mg·g ⁻¹)	(3)(11) (mg·g ⁻¹)	(4)(12) (mg·g ⁻¹)	(5)(13) (mg·g ⁻¹)	(6)(14) (mg·g ⁻¹)	(7)(15) (mg·g ⁻¹)	(8)(16) (mg·g ⁻¹)
A	1	1	Ga	5.519	5.519	5.543	5.542	5.505	5.504	5.514	5.513
			Ph	0.6741	0.6733	0.3699	0.3695	0.3568	0.3564	0.3688	0.3685
			PE	4.530	4.529	6.748	6.747	6.691	6.689	6.696	6.695
			PB	3.294	3.284	3.838	3.827	3.779	3.768	3.817	3.806
			PC	1.209	1.208	1.487	1.486	1.476	1.475	1.477	1.477
			PA	5.924	5.920	8.402	8.396	8.420	8.414	8.356	8.350
A	1	2	Ga	5.452	5.439	5.475	5.463	5.437	5.425	5.447	5.434
			Ph	0.6655	0.6645	0.3655	0.3650	0.3522	0.3517	0.3645	0.3640
			PE	4.466	4.458	6.654	6.642	6.597	6.585	6.603	6.591
			PB	3.290	3.284	3.835	3.828	3.775	3.768	3.814	3.807
			PC	1.186	1.185	1.459	1.458	1.448	1.447	1.450	1.448
			PA	5.872	5.859	8.328	8.308	8.346	8.327	8.283	8.263
A	1	3	Ga	4.963	4.950	4.974	4.961	4.976	4.963	4.999	4.986
			Ph	0.3165	0.3167	0.3287	0.3289	0.5979	0.5983	0.3293	0.3295
			PE	5.914	5.891	5.924	5.901	4.004	3.988	5.968	5.945
			PB	3.391	3.381	3.431	3.421	2.955	2.947	3.449	3.439
			PC	1.287	1.283	1.289	1.286	1.054	1.051	1.298	1.294
			PA	7.587	7.556	7.526	7.496	5.338	5.317	7.566	7.535
A	2	1	Ga	6.537	6.538	6.544	6.545	6.554	6.555	6.580	6.581
			Ph	0.6115	0.6222	0.6229	0.6335	1.1553	1.1754	0.6266	0.6373
			PE	1.746	1.761	1.778	1.793	1.182	1.192	1.780	1.796
			PB	4.789	4.778	4.825	4.813	4.174	4.164	4.853	4.842
			PC	0.9389	0.9434	0.9424	0.9468	0.7689	0.7726	0.9480	0.9525
			PA	15.84	15.87	15.75	15.78	11.15	11.17	15.85	15.88
A	2	2	Ga	6.827	6.848	6.833	6.854	6.845	6.866	6.871	6.893
			Ph	0.6364	0.6397	0.6478	0.6511	1.2023	1.2086	0.6517	0.6551
			PE	1.839	1.847	1.871	1.879	1.245	1.250	1.874	1.882

Supplementary Material

A	2	3	PB	5.025	5.044	5.060	5.079	4.380	4.396	5.090	5.110
			PC	1.005	1.007	1.008	1.010	0.823	0.824	1.014	1.016
			PA	16.68	16.69	16.58	16.59	11.73	11.74	16.69	16.70
			Ga	6.718	6.736	6.744	6.763	6.700	6.719	6.707	6.725
			Ph	1.201	1.204	0.651	0.653	0.636	0.637	0.647	0.649
			PE	1.215	1.219	1.829	1.835	1.795	1.800	1.827	1.832
			PB	4.275	4.290	4.970	4.987	4.905	4.923	4.940	4.957
			PC	0.7953	0.7975	0.9804	0.9831	0.9712	0.9739	0.9745	0.9772
			PA	11.42	11.46	16.24	16.29	16.23	16.28	16.13	16.19
			Ga	3.137	3.106	3.158	3.127	3.165	3.134	3.181	3.150
A	3	1	Ph	0.9752	0.9652	0.9908	0.9809	1.853	1.834	0.9963	0.9863
			PE	3.807	3.781	3.838	3.812	2.601	2.583	3.875	3.849
			PB	3.056	3.069	3.107	3.120	2.685	2.697	3.135	3.148
			PC	0.7716	0.7866	0.7780	0.7929	0.6365	0.6488	0.7847	0.7998
			PA	5.097	5.085	5.053	5.041	3.613	3.604	5.099	5.088
			Ga	3.009	3.007	3.028	3.026	3.001	2.999	3.016	3.014
			Ph	1.863	1.862	1.004	1.003	0.986	0.985	0.996	0.996
			PE	2.576	2.526	3.849	3.775	3.804	3.730	3.826	3.752
			PB	2.709	2.686	3.166	3.139	3.109	3.082	3.150	3.124
			PC	0.6315	0.6350	0.7795	0.7838	0.7711	0.7754	0.7753	0.7795
A	3	2	PA	3.576	3.591	5.052	5.073	5.082	5.103	5.031	5.052
			Ga	2.590	2.588	2.611	2.609	2.614	2.612	2.630	2.628
			Ph	0.8352	0.8363	0.8511	0.8521	1.5872	1.5893	0.8552	0.8563
			PE	3.059	3.091	3.091	3.122	2.090	2.112	3.121	3.153
			PB	2.587	2.566	2.638	2.618	2.274	2.255	2.663	2.642
			PC	0.6006	0.6175	0.6069	0.6238	0.4954	0.5093	0.6124	0.6294
			PA	4.179	4.174	4.133	4.128	2.962	2.958	4.174	4.169
			Ga	5.642	5.637	5.651	5.646	5.657	5.652	5.681	5.676
			Ph	0.6735	0.6730	0.6847	0.6842	1.272	1.271	0.6890	0.6885
			PE	4.900	4.894	4.915	4.910	3.317	3.314	4.949	4.944

Supplementary Material

A	4	2	PB	3.991	3.984	4.029	4.022	3.478	3.472	4.052	4.044
			PC	1.040	1.020	1.043	1.024	0.8516	0.8356	1.049	1.030
			PA	9.752	9.749	9.683	9.679	6.862	6.859	9.738	9.735
			Ga	5.706	5.727	5.731	5.752	5.758	5.778	5.772	5.792
			Ph	0.6807	0.6087	0.6967	0.6248	1.294	1.157	0.6993	0.6267
			PE	4.938	4.949	4.970	4.981	3.374	3.382	5.017	5.029
			PB	3.863	3.888	3.913	3.939	3.394	3.417	3.948	3.974
			PC	1.048	1.033	1.055	1.039	0.8648	0.8520	1.064	1.048
			PA	9.785	9.778	9.747	9.740	6.935	6.931	9.825	9.818
			Ga	4.939	4.927	4.963	4.951	4.984	4.972	4.998	4.986
A	4	3	Ph	0.5923	0.5911	0.6084	0.6073	1.126	1.123	0.6102	0.6090
			PE	4.150	4.141	4.182	4.173	2.836	2.829	4.222	4.213
			PB	3.368	3.360	3.419	3.410	2.960	2.952	3.449	3.441
			PC	0.8292	0.8270	0.8356	0.8333	0.6840	0.6822	0.8427	0.8405
			PA	8.190	8.171	8.150	8.131	5.805	5.791	8.217	8.198
			Ga	4.339	4.343	4.360	4.364	4.327	4.331	4.339	4.343
			Ph	1.642	1.641	0.8860	0.8857	0.8690	0.8687	0.8796	0.8793
			PE	5.842	5.841	8.696	8.695	8.629	8.628	8.624	8.623
			PB	4.432	4.428	5.150	5.146	5.086	5.081	5.120	5.115
			PC	0.7485	0.7489	0.9230	0.9235	0.9140	0.9145	0.9176	0.9180
A	5	1	PA	6.543	6.544	9.284	9.285	9.299	9.301	9.232	9.233
			Ga	4.333	4.354	4.356	4.377	4.372	4.393	4.387	4.408
			Ph	0.8925	0.9041	0.9083	0.9199	1.696	1.718	0.9130	0.9247
			PE	8.730	8.699	8.761	8.730	5.965	5.943	8.844	8.813
			PB	5.081	5.070	5.132	5.121	4.465	4.456	5.176	5.165
			PC	0.9178	0.9192	0.9242	0.9256	0.7571	0.7583	0.9320	0.9335
			PA	9.272	9.281	9.234	9.243	6.572	6.578	9.308	9.317
			Ga	4.292	4.290	4.305	4.302	4.304	4.302	4.325	4.323
			Ph	0.8929	0.8922	0.9034	0.9027	1.687	1.686	0.9101	0.9094
			PE	8.568	8.567	8.564	8.562	5.801	5.800	8.635	8.634

Supplementary Material

			PB	5.019	5.018	5.054	5.052	4.374	4.373	5.084	5.082
			PC	0.9078	0.9072	0.9114	0.9108	0.7434	0.7429	0.9168	0.9162
			PA	9.159	9.162	9.092	9.095	6.444	6.446	9.143	9.146
			Ga	5.493	5.488	5.494	5.490	5.522	5.517	5.531	5.526
			Ph	0.6449	0.6460	0.3457	0.3462	0.6483	0.6494	0.3410	0.3416
B	1	1	PE	4.399	4.397	6.690	6.685	4.423	4.420	6.697	6.693
			PB	3.277	3.277	3.807	3.806	3.295	3.294	3.807	3.807
			PC	1.201	1.200	1.491	1.490	1.208	1.206	1.494	1.493
			PA	5.894	5.892	8.143	8.139	5.925	5.923	8.154	8.150
			Ga	5.523	5.520	5.524	5.522	5.552	5.549	5.561	5.558
			Ph	0.6506	0.6523	0.3487	0.3496	0.6540	0.6557	0.3440	0.3450
B	1	2	PE	4.433	4.431	6.740	6.738	4.456	4.454	6.748	6.745
			PB	3.308	3.274	3.842	3.803	3.326	3.292	3.843	3.803
			PC	1.210	1.209	1.501	1.500	1.216	1.215	1.505	1.504
			PA	5.961	5.955	8.234	8.227	5.992	5.987	8.245	8.238
			Ga	5.539	5.497	5.548	5.506	5.510	5.468	5.511	5.469
			Ph	0.6574	0.6507	0.3459	0.3423	0.6540	0.6473	0.3505	0.3470
B	1	3	PE	4.438	4.410	6.720	6.679	4.414	4.387	6.712	6.671
			PB	3.218	3.231	3.719	3.733	3.201	3.214	3.719	3.733
			PC	1.214	1.206	1.502	1.492	1.208	1.200	1.499	1.489
			PA	5.991	5.944	8.243	8.180	5.959	5.913	8.231	8.169
			Ga	6.923	6.938	6.939	6.954	6.887	6.902	6.890	6.905
			Ph	1.175	1.177	0.6217	0.6230	1.168	1.171	0.6259	0.6272
B	2	1	PE	1.255	1.257	1.908	1.911	1.248	1.250	1.916	1.919
			PB	4.424	4.430	5.111	5.119	4.401	4.407	5.104	5.111
			PC	0.8364	0.8372	1.034	1.035	0.8320	0.8328	1.033	1.034
			PA	11.85	11.87	16.26	16.30	11.79	11.81	16.19	16.22
			Ga	7.095	7.055	7.112	7.072	7.058	7.018	7.061	7.021
B	2	2	Ph	1.200	1.196	0.6353	0.6332	1.194	1.190	0.6394	0.6373
			PE	1.284	1.278	1.952	1.944	1.277	1.272	1.960	1.952

Supplementary Material

B	2	3	PB	4.527	4.512	5.231	5.214	4.503	4.489	5.222	5.206
			PC	0.8649	0.8609	1.070	1.065	0.8604	0.8564	1.068	1.063
			PA	12.16	12.11	16.69	16.61	12.10	12.04	16.61	16.54
			Ga	7.125	7.129	7.128	7.132	7.163	7.167	7.180	7.184
			Ph	1.198	1.200	0.6418	0.6428	1.204	1.206	0.6376	0.6386
			PE	1.295	1.295	1.988	1.987	1.302	1.302	1.980	1.979
			PB	4.560	4.567	5.288	5.297	4.584	4.591	5.296	5.305
			PC	0.8606	0.8618	1.069	1.070	0.8652	0.8663	1.070	1.071
			PA	12.20	12.21	16.75	16.77	12.26	12.28	16.82	16.85
			Ga	3.147	3.155	3.147	3.155	3.222	3.230	3.200	3.208
B	3	1	Ph	1.916	1.917	1.020	1.020	1.961	1.962	1.029	1.030
			PE	2.655	2.658	4.031	4.035	2.718	2.721	4.091	4.095
			PB	2.799	2.802	3.243	3.247	2.865	2.869	3.270	3.273
			PC	0.6569	0.6576	0.8140	0.8149	0.6724	0.6731	0.8269	0.8279
			PA	3.715	3.721	5.151	5.158	3.803	3.809	5.175	5.183
			Ga	3.056	3.064	3.055	3.063	3.072	3.080	3.069	3.077
			Ph	1.910	1.911	1.023	1.023	1.920	1.921	1.020	1.020
			PE	2.648	2.649	4.037	4.039	2.662	2.663	4.036	4.037
			PB	2.801	2.803	3.256	3.259	2.816	2.818	3.254	3.256
			PC	0.6586	0.6689	0.8180	0.8308	0.6621	0.6724	0.8184	0.8312
B	3	2	PA	3.710	3.711	5.161	5.162	3.730	3.731	5.149	5.150
			Ga	3.208	3.131	3.208	3.131	3.284	3.205	3.263	3.183
			Ph	1.928	1.917	1.026	1.021	1.973	1.963	1.036	1.030
			PE	2.692	2.664	4.087	4.044	2.756	2.726	4.149	4.104
			PB	2.830	2.807	3.279	3.252	2.897	2.873	3.307	3.279
			PC	0.6662	0.6595	0.8255	0.8173	0.6819	0.6751	0.8388	0.8303
			PA	3.767	3.723	5.222	5.161	3.856	3.811	5.249	5.186
			Ga	5.824	5.824	5.834	5.834	5.793	5.793	5.795	5.795
			Ph	1.275	1.276	0.6753	0.6757	1.268	1.269	0.6793	0.6797
			PE	3.408	3.407	5.163	5.162	3.390	3.389	5.161	5.159

Supplementary Material

B	4	2	PB	3.585	3.587	4.142	4.145	3.566	3.568	4.140	4.143
			PC	0.8619	0.8617	1.066	1.066	0.8574	0.8572	1.065	1.064
			PA	7.078	7.079	9.732	9.732	7.041	7.042	9.709	9.709
			Ga	5.776	5.791	5.776	5.792	5.912	5.927	5.924	5.939
			Ph	1.264	1.265	0.6741	0.6746	1.294	1.295	0.6698	0.6702
			PE	3.438	3.429	5.210	5.196	3.519	3.510	5.313	5.299
			PB	3.563	3.572	4.120	4.131	3.647	3.656	4.182	4.193
			PC	0.8570	0.8579	1.061	1.062	0.8772	0.8781	1.083	1.084
			PA	7.043	7.061	9.682	9.706	7.209	7.228	9.876	9.901
			Ga	5.686	5.717	5.687	5.718	5.820	5.852	5.831	5.863
B	4	3	Ph	1.238	1.243	0.6603	0.6629	1.267	1.272	0.6554	0.6582
			PE	3.326	3.346	5.041	5.071	3.404	3.425	5.138	5.169
			PB	3.496	3.516	4.043	4.066	3.578	3.599	4.102	4.126
			PC	0.8405	0.8449	1.041	1.046	0.8604	0.8649	1.062	1.068
			PA	6.911	6.948	9.502	9.552	7.074	7.112	9.690	9.741
			Ga	4.352	4.315	4.352	4.315	4.375	4.338	4.378	4.341
			Ph	1.660	1.647	0.8889	0.8819	1.669	1.655	0.8853	0.8783
			PE	5.881	5.841	8.934	8.873	5.912	5.872	8.949	8.888
			PB	4.432	4.400	5.140	5.103	4.455	4.423	5.147	5.111
			PC	0.7569	0.7524	0.9399	0.9343	0.7609	0.7564	0.9409	0.9353
B	5	1	PA	6.527	6.482	9.007	8.946	6.562	6.517	9.025	8.963
			Ga	4.263	4.298	4.264	4.299	4.364	4.400	4.357	4.393
			Ph	1.596	1.611	0.8502	0.8581	1.634	1.649	0.8530	0.8612
			PE	5.670	5.684	8.572	8.592	5.804	5.818	8.796	8.817
			PB	4.244	4.269	4.903	4.932	4.344	4.370	4.995	5.025
			PC	0.7165	0.7214	0.8876	0.8936	0.7334	0.7384	0.9033	0.9095
			PA	6.229	6.268	8.573	8.626	6.376	6.416	8.726	8.781
			Ga	4.435	4.342	4.438	4.345	4.411	4.319	4.412	4.319
			Ph	1.652	1.639	0.8765	0.8695	1.644	1.630	0.8802	0.8732
			PE	5.853	5.819	8.860	8.809	5.822	5.789	8.845	8.794

Supplementary Material

PB	4.419	4.390	5.105	5.072	4.395	4.367	5.098	5.065
PC	0.7546	0.7487	0.9330	0.9258	0.7506	0.7448	0.9321	0.9249
PA	6.510	6.466	8.954	8.894	6.476	6.432	8.937	8.877

^a Ga, Gastrodin; Ph; *p*-hydroxybenzyl alcohol; PE, Parishin E; PB, Parishin B; PC, Parishin C; PA, Parishin A.

Table S8 Results for calculations of % R&R and ndc indexes by ANOVA and Xbar-R method at the conditions of the full factorial designed experiment

Index	Method	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
		(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
%R&R	ANOVA	26.43	26.59	17.87	8.75	29.95	29.57	8.73	17.85
	Xbar-R	20.64	20.36	14.01	6.87	21.92	21.44	7.25	13.80
ndc	ANOVA	5	5	7	16	4	4	16	7
	Xbar-R	7	7	10	20	6	6	19	10

Table S9 Results of weighted principal component analysis for six CTQs under conditions (1) and (9) (Standard order) from the full factorial experiment

Sample No.	Analyst A			Analyst B		
	1 st	2 nd	3 rd	1 st	2 nd	3 rd
1	-1.761	-1.906	-1.187	-1.854	-1.677	-1.910
2	10.18	11.84	6.003	7.026	7.774	7.931
3	-6.473	-8.757	-9.245	-8.207	-8.322	-7.997
4	2.675	2.389	-1.207	-0.0561	-0.2138	-0.6178
5	-0.0968	3.533	3.227	-0.0886	-0.9683	-0.0293

1st: first sample preparations; 2nd: second sample preparations; 3rd: third sample preparations.

Table S10 ANOVA for the Box-Behnken design experiment.

Response	Source ^a	Sum of squares	Freedom	Mean Square	F-value
Y ₁ ^b	Model	316.7	5	52.78	36.04
	X ₁	209.1	1	209.1	142.8
	X ₃	19.45	1	19.45	13.28
	X ₁ X ₃	16.36	1	16.36	11.17
	X ₁ ²	49.86	1	49.86	34.05
	X ₃ ²	7.210	1	7.210	4.920
	Residual	29.29	21	1.460	-
	Lack of fit	29.25	19	1.630	90.23
Y ₂ ^b	Model	200.9	5	40.17	189.3
	X ₂	139.7	1	139.7	658.5
	X ₃	19.76	1	19.76	93.10
	X ₄	34.48	1	34.48	162.5
	X ₃ X ₄	1.780	1	1.780	8.370
	X ₂ ²	2.540	1	2.540	11.98
	Residual	4.460	21	0.2100	-
	Lack of fit	4.400	19	0.2300	8.710

^a X₁, Proportion of phosphoric acid in the mobile phase; X₂, Flow rate; X₃, Column temperature; X₄, Time of gradient switch.

^b Y₁, Resolution between critical peaks; Y₂, Retention time of the last chromatographic peak

Table S11 Validation experiment parameters and results.

No.	Verify parameters					Y ₁		Y ₂ (min)	
	X ₁ (%))	X ₂ (mL·min ⁻¹)	X ₃ (°C)	X ₄ (min)	Probability to attain goals	Experimental value	Predicted value	Experimental value	Predicted value
1	0.1	0.9	30	0	1	1.55	3.22	34.19	34.49
2	0.2	1	30	0	0.9014	2.713	1.55	33.29	33.2
3	0.1	1	35	0	1	2.657	3.42	32.25	31.9

Table S12 Regression equations, correlation coefficients, and linear analysis range for each component.

Component	Linear equation	R	Linear range (μg/mL)	Detection limit (μg/mL)	Limit of quantitation (μg/mL)
Gastrodin	$Y=3.859 \times 10^{-5}X+1.224$	0.9999	28.64-257.8	0.3182	1.253
<i>p</i> -hydroxybenzyl alcohol	$Y=2.160 \times 10^{-5}X+0.2090$	0.9999	3.328-29.95	0.1726	0.7488
Parishin E	$Y=5.828 \times 10^{-5}X+2.685$	0.9998	21.96-197.6	0.8133	2.196
Parishin B	$Y=4.244 \times 10^{-5}X+2.371$	0.9997	20.26-182.3	0.7502	2.476
Parishin C	$Y=4.903 \times 10^{-5}X+0.5669$	0.9997	5.040-45.36	0.616	2.016
Parishin A	$Y=5.332 \times 10^{-5}X+2.692$	0.9999	56.36-507.2	0.6262	2.087

Table S13 RSD values and recovery results for intra-day precision, inter day precision, repeatability, stability.

Component	Intraday precision RSD (%)	Daytime precision RSD (%)	Repeatability RSD (%)	Stability RSD (%)	Recovery rate (%)
Gastrodin	1.94	1.82	2.42	3.45	98.95
<i>p</i> -hydroxybenzyl alcohol	1.25	1.91	2.32	3.36	91.35
Parishin E	2.40	2.93	3.10	3.71	98.48
Parishin B	2.19	1.49	3.60	2.83	96.98
Parishin C	2.21	1.60	3.52	3.25	94.26
Parishin A	2.04	1.54	2.89	2.95	98.24

Table S14 Results of content determination of GE samples from different geographical sources.

Sample No.	Location	W/C ^a	Gastrodin (mg·g ⁻¹)	<i>p</i> -hydroxybenzyl alcohol(mg·g ⁻¹)	Parishin E (mg·g ⁻¹)	Parishin B (mg·g ⁻¹)	Parishin C (mg·g ⁻¹)	Parishin A (mg·g ⁻¹)
1	Guizhou	W	1.658	0.5303	5.174	3.755	0.8971	9.725
2	Henan	W	3.527	0.9989	4.929	4.993	1.615	22.75
3	Gansu	W	6.990	0.9771	8.754	6.173	1.628	19.03
4	Xizang	W	3.459	0.7715	7.047	4.883	1.684	19.02
5	Anhui	W	2.142	0.9079	3.792	3.847	1.366	11.43
6	Jilin	W	4.552	0.7810	9.686	4.649	1.173	9.805
7	Shanxi	W	4.963	0.4254	7.816	3.527	1.731	8.833
8	Sichuan	W	2.398	0.9580	4.222	3.236	0.8568	5.581
9	Hubei	W	3.064	1.025	4.356	2.782	0.9157	5.196
10	Yunan	W	5.397	0.6469	5.732	3.667	1.265	10.44
11	Shangluo, Shanxi	W	2.214	0.8365	4.786	4.530	1.473	13.82
12	Dabieshan, Hubei	W	5.813	0.2657	4.667	1.736	0.5544	1.813
13	Lijiang, Yunnan	W	1.733	1.059	10.44	2.769	0.5407	3.099
14	Hanzhong, Shanxi	W	1.940	0.9010	4.604	3.631	1.170	7.340
15	Shiyan, Hubei	W	1.827	0.7581	5.351	4.108	1.410	10.73
16	Guangyuan, Sichuan	W	1.783	0.7025	5.323	3.209	1.051	6.882
17	Nanchong, Sichuan	W	1.435	0.5509	2.079	1.124	0.4727	3.627
18	Yichang, Hubei	W	1.095	0.4191	2.119	1.700	0.5577	4.954
19	Enshi, Hubei	W	2.245	0.2011	4.284	1.364	0.3936	3.106
20	Ankang, Shanxi	W	1.838	0.1480	1.635	1.359	0.3770	2.175

21	Yichang, Hubei	C	1.082	0.2492	3.851	1.786	0.4632	4.817
22	Enshi, Hubei	C	3.138	1.761	2.889	3.409	1.084	12.01
23	Ankang, Shanxi	C	1.324	0.4143	3.191	2.218	0.6895	7.961
24	Kangxian, Gansu	C	1.581	0.2811	3.088	2.250	0.7751	10.38
25	Wudu, Gansu	C	1.069	0.3029	2.742	1.825	0.4749	3.39
26	Liangshan, Sichuan	C	0.8143	0.4571	2.304	1.878	0.4977	5.181

^aW/C: W: wild; C: cultivated

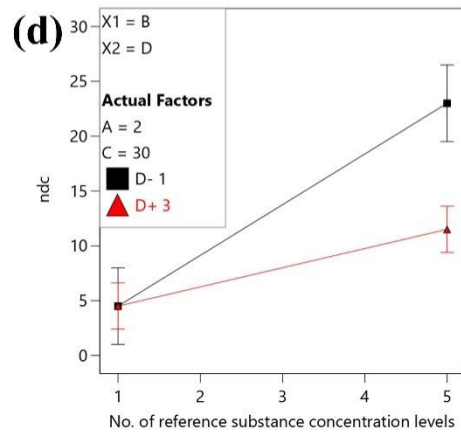
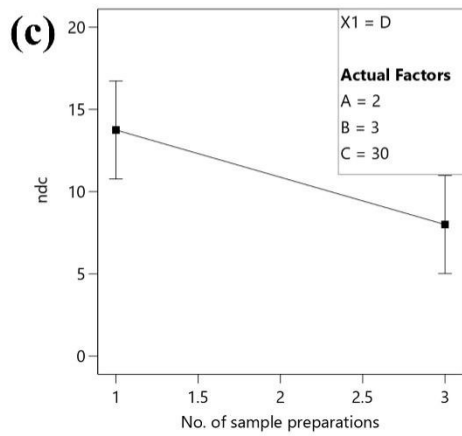
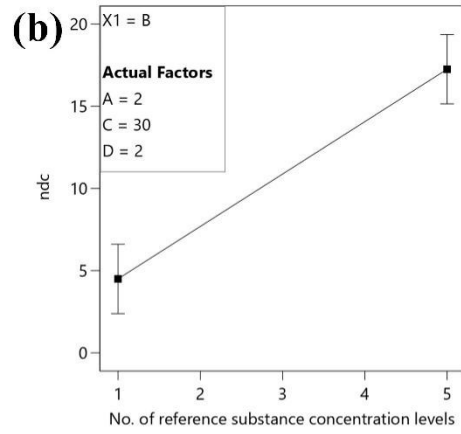
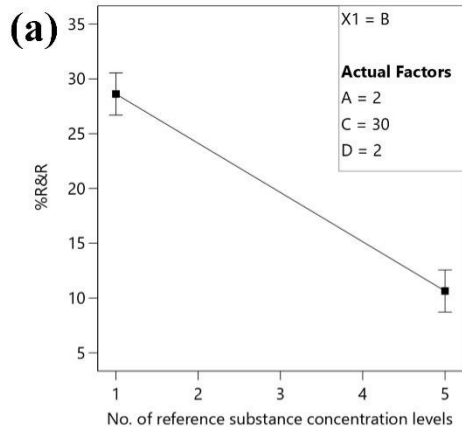


Figure S2 (a) Main effect plot for % R&R; (b)(c) Main effect plot for ndc; (d) two-factor interaction effect plots for ndc (A: No. of sample injections, B: No. of reference substance concentration levels, C: Selection method of reference substance, D: No. of sample preparations)

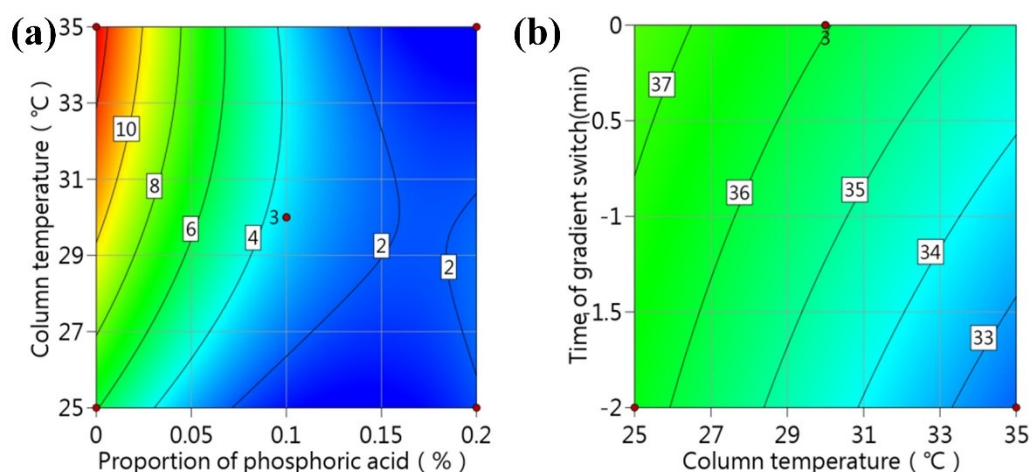


Figure S3 Contour plots showing the significant interactive effects between independent variables on responses: (a) Contour plot of the critical resolution Contour line diagram (flow rate $0.8 \text{ mL} \cdot \text{min}^{-1}$, gradient time change 0 min) Flow rate $0.6 \text{ ml} \cdot \text{min}^{-1}$; (b) Contour line diagram of the retention time of the last chromatographic peak (phosphoric acid ratio in mobile phase 0.1%, flow rate $0.8 \text{ mL} \cdot \text{min}^{-1}$)

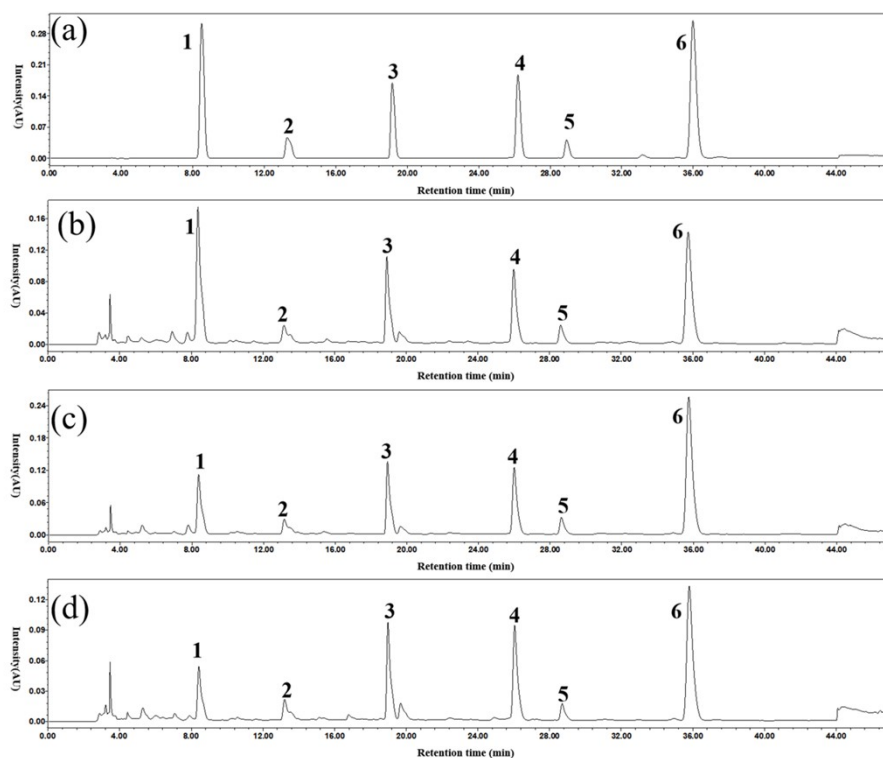


Figure S4 Typical HPLC chromatograms for standards and samples. (a) Standard solution; (b) Sample of Guizhou; (c) Sample of Gansu; (d) Sample of Xizang. Peaks 1, 2, 3, 4, 5, 6 represented Gastrodin, *p*-Hydroxybenzyl alcohol, Parisin E, Parisin B, Parisin C, Parisin A.

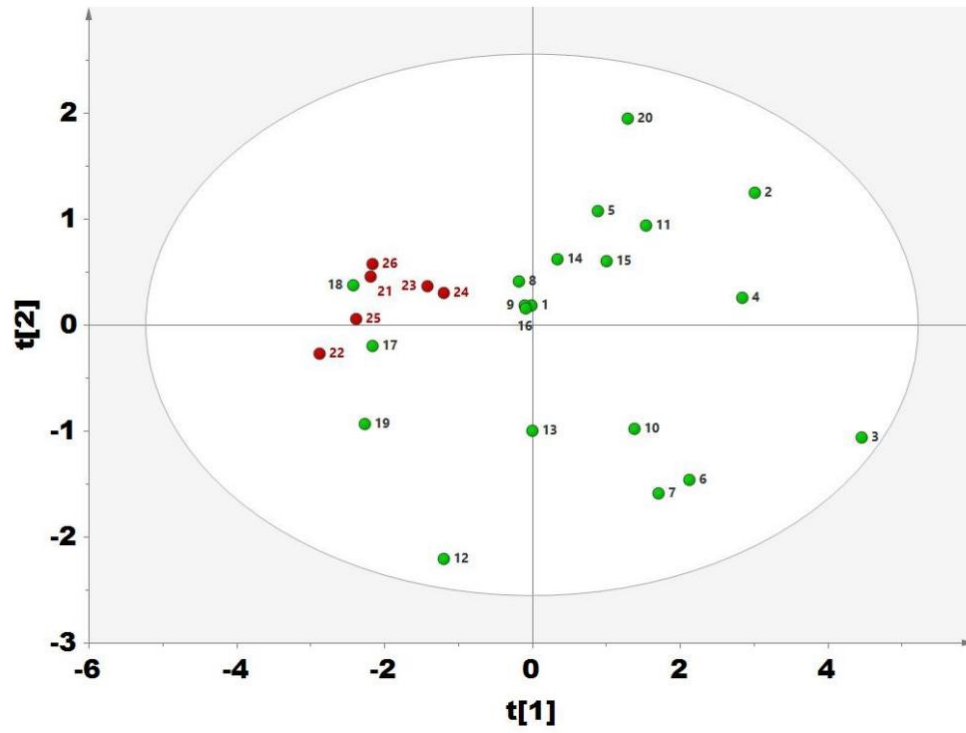


Figure S5 Score plot of PCA of the wild and cultivated *Gastrodia elata*. Green-hued points represent wild cultivated samples, those hued red represent cultivated samples.