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Analysis of active compounds in *Flos Chrysanthemi Indici* by UHPLC Q Exactive HF Hybrid Quadrupole-Orbitrap MS and comprehensive quality assessment of its preparation

Tingting Dai, Guoxiang Sun*

Supplementary Table S1 Information of reference standards

Corresponding compound No.	Name	CAS	Provider
1	Maleic acid	110-16-7	National Institute of Pharmaceutical and Biological Products Control (Beijing, China)
2	D-(-)-Quinic acid	77-95-2	
7	5-Caffeoylquinicacid	906-33-2	
9	Caffeic acid	331-39-5	
10	4-Caffeoylquinicacid	905-99-7	
11	1-Caffeoylquinicacid	1241-87-8	
12	7-Hydroxycoumarine	93-35-6	
13	Clonidine hydrochloride	4205-91-8	Chengdu Puri France Science and Technology Development Co., Ltd. (Chengdu, China)
14	Chlorogenic acid	327-97-9	
15	Hydrochlorothiazide	58-93-5	Shanghai Zheyan Biotech Co., Ltd.
16	D-(+)-Camphor	464-49-3	
19	Quercetin	117-39-5	
23	Quercitrin	522-12-3	
33	4,5-Dicaffeoylquinicacid	32451-88-0	Chengdu Puri France Science and Technology Development Co., Ltd. (Chengdu, China)
36	Rutin	205-814-1	
38	Apigenin7-O-glucuronide	29741-09-1	
40	Glycitein	40957-83-3	
49	Apigenin	520-36-5	National Institute of Pharmaceutical and Biological Products Control (Beijing, China)
51	Linarin	480-36-4	
56	3,4-Dicaffeoylquinicacid	14534-61-3	Chengdu Puri France Science and Technology Development Co., Ltd. (Chengdu, China)
57	Chrysin	480-40-0	
60	3,5-Dicaffeoylquinicacid	2450-53-5	
61	Luteolin	491-70-3	Shanghai Zheyan Biotech Co., Ltd.
66	Handelin	62687-22-3	

Supplementary Table S2 Quality level of SQFM

Quality	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Grade	**best**	**better**	**good**	**fine**	**moderate**	**common**	**defective**	**inferior**
$S_m \geq$	0.95	0.9	0.85	0.8	0.7	0.6	0.5	＜0.50
$P_m \leq$	95~105	90~110	85~115	80~120	70~130	60~140	50~150	0~∞
$\alpha \leq$	0.05	0.1	0.15	0.2	0.3	0.4	0.5	＞0.50

Supplementary Table S3 Identification of the chemical constituents in FCI/FCIP samples by UHPLC Q Exactive HF Hybrid Quadrupole-Orbitrap MS

No.	RT (min)	Identification	Formula	Ion adduction	Experimental (m/z)	Theoretical (m/z)	Error (ppm)	Fragments (m/z)	Source
1[a]	0.61	Maleic acid	$C_4H_5O_5$	$[M-H]^-$	133.0136	133.0134	1.5	115.0145	FCI
2[a]	0.81	D-(-)-Quinic acid	$C_7H_{12}O_6$	$[M-H]^-$	191.0634	191.0635	-0.5	173.0098, 127.0423	FCI
3	0.90	D-(+)-Proline	$C_5H_9NO_2$	$[M+H]^+$	116.0633	116.0633	0.0	71.0657	FCI/FCIP
4	0.95	L-Pyroglutamic acid	$C_5H_7NO_3$	$[M+H]^+$	130.0499	130.0498	0.8	84.0449, 60.0563	FCI/FCIP
5	1.20	N-Acetyl-L-glutamate	$C_7H_{11}NO_5$	$[M+H]^+$	190.0708	190.0706	1.1	167.5064, 148.0603, 144.0653, 130.0499	FCI/FCIP
6	1.40	Prolylleucine	$C_{11}H_{20}N_2O_3$	$[M+H]^+$	229.1544	229.1547	-1.3	184.0214, 142.0862	FCI/FCIP
7[a]	1.84	5-Caffeoylquinic acid	$C_{16}H_{17}O_9$	$[M-H]^-$	353.0873	353.0872	0.3	191.0377, 179.0178, 173.0964	FCI
8	2.26	L-Phenylalanine	$C_9H_{11}NO_2$	$[M+H]^+$	166.0861	166.0860	0.6	120.0808, 93.0422, 73.0611	FCI/FCIP
9[a]	3.54	Caffeic acid	$C_9H_7O_4$	$[M-H]^-$	179.0344	179.0343	0.6	135.0073	FCI/FCIP
10[a]	4.31	4-Caffeoylquinic acid	$C_{16}H_{17}O_9$	$[M-H]^-$	353.0873	353.0863	2.8	191.1047, 179.0982, 173.0446, 135.0874	FCI
11[a]	4.43	1-Caffeoylquinic acid	$C_{16}H_{18}O_9$	$[M-H]^-$	353.0951	353.0945	1.7	191.0418, 179.0014, 173.0354, 135.0097	FCI
12[a]	4.51	7-Hydroxycoumarine	$C_9H_6O_3$	$[M+H]^+$	163.0318	163.0317	0.6	133.0433, 105.1103	FCI/FCIP
13[a]	4.58	Clonidine	$C_9H_9Cl_2N_3$	$[M-H]^-$	228.0174	228.0172	0.9	-	FCIP
14[a]	5.25	Chlorogenic acid	$C_{16}H_{18}O_9$	$[M-H]^-$	353.0951	353.0949	0.6	191.0556, 179.0614, 173.0031, 135.0715	FCI/FCIP
15[a]	5.42	Hydrochlorothiazide	$C_7H_8ClN_3O_4S_2$	$[M-H]^-$	296.0014	296.0012	0.7	217.0041, 205.0314	FCIP
16[a]	6.09	D-(+)-Camphor	$C_{10}H_{16}O$	$[M-H]^-$	151.1201	151.1200	0.7	136.0845	FCI/FCIP
17	6.12	6,7-Dihydroxy-4-methylcoumarin	$C_{10}H_8O_4$	$[M+H]^+$	193.0493	193.0493	0.0	165.0545, 147.0803, 133.0647, 105.0701, 77.0452	FCI/FCIP
18	6.18	Cyclo (leucylprolyl)	$C_{11}H_{18}N_2O_2$	$[M+H]^+$	211.1439	211.1436	1.4	206.0321, 183.1491, 86.0969	FCI/FCIP
19[a]	6.23	Quercetin	$C_{15}H_{10}O_7$	$[M-H]^-$	301.0427	301.0425	0.7	284.0969, 191.0077, 151.0621,109.0675	FCI/FCIP
20	6.31	Luteolin-7-O-glucuronide	$C_{21}H_{17}O_{12}$	$[M-H]^-$	461.0012	461.0002	2.2	285.0659, 175.1126	FCI/FCIP
21	6.49	Quercetin-3β-D-glucoside	$C_{21}H_{20}O_{12}$	$[M-H]^-$	463.0955	463.0953	0.4	301.0474, 273.3101, 191.03858	FCI/FCIP
22	6.52	Kaempferol-7-O-glucoside	$C_{21}H_{20}O_{11}$	$[M-H]^-$	447.1006	447.1002	0.9	285.0038, 191.0257	FCI
23[a]	6.59	Quercitrin	$C_{21}H_{20}O_{11}$	$[M-H]^-$	447.1006	447.1004	0.4	301.0103, 272.0321, 167.01329	FCI/FCIP
24	6.64	Quercetin-3-O-glucuronide-7-O-glucoside	$C_{29}H_{51}O_{15}$	$[M-H]^-$	639.0429	639.0421	1.3	477.1661, 463.0122, 301.0121	FCI/FCIP
25	6.77	Kaempferol-3-O-rutinoside-7-O-rhamnoside	$C_{33}H_{39}O_{19}$	$[M-H]^-$	739.1041	739.1016	3.4	593.1302, 431.1028, 285.0436,	FCI/FCIP
26	6.84	5,7,2'-Trihydroxyflavone7-glucuronide	$C_{21}H_{18}O_{11}$	$[M-H]^-$	445.0849	445.0846	0.7	269.0347, 152.1022	FCI
27	6.95	6-O-Methylscutellarin	$C_{22}H_{20}O_{12}$	$[M-H]^-$	475.0955	475.0956	-0.2	460.0055, 299.0286, 284.0849	FCI

No.	RT (min)	Identification	Formula	Ion adduction	Experimental (m/z)	Theoretical (m/z)	Error (ppm)	Fragments (m/z)	Source
28	7.01	Nictoflorin	$C_{27}H_{30}O_{15}$	$[M-H]^-$	593.1585	593.1605	-3.4	285.0368, 284.1021, 266.0121	FCI/FCIP
29	7.08	Luteolin-7-O-β-D-glucoside	$C_{21}H_{19}O_{11}$	$[M-H]^-$	447.1020	447.1021	-0.2	285.0674, 175.0544, 152.0178, 134.2101	FCI/FCIP
30	7.18	Baicalin	$C_{21}H_{18}O_{11}$	$[M-H]^-$	445.0849	445.0861	-2.7	269.0547, 192.1201, 167.3107	FCI
31	7.23	Scutellarin	$C_{21}H_{18}O_{12}$	$[M-H]^-$	461.0798	461.0801	-0.7	285.0398, 257.0143, 192.0352	FCI
32	7.46	Luteolin-7-O-rutinoside	$C_{27}H_{29}O_{15}$	$[M-H]^-$	593.1045	593.1056	-1.9	285.1024, 257.0386, 151.0343	FCI/FCIP
33[a]	7.69	4,5-Dicaffeoylquinicacid	$C_{25}H_{24}O_{12}$	$[M-H]^-$	515.1268	515.1267	0.2	353.0124, 191.0365, 179.0406, 173.0108, 135.0741	FCI/FCIP
34	7.88	Neodiosmin	$C_{28}H_{32}O_{15}$	$[M-H]^-$	607.1741	607.1739	0.3	592.1179, 460.1214, 299.0716	FCI/FCIP
35	8.01	Esculetin	$C_9H_6O_4$	$[M+H]^+$	179.0266	179.0266	0.0	151.0327, 133.0689, 105.0986	FCI/FCIP
36[a]	8.26	Rutin	$C_{27}H_{30}O_{16}$	$[M-H]^-$	609.1534	609.1541	-1.1	301.0144, 300.0106, 167.0124	FCI/FCIP
37	8.55	Apigenin7-(6-methylglucuronide)	$C_{22}H_{20}O_{11}$	$[M-H]^-$	459.1006	459.1001	1.1	270.0582, 177.0257, 153.6568	FCI/FCIP
38[a]	8.98	Apigenin7-O-glucuronide	$C_{21}H_{18}O_{11}$	$[M-H]^-$	445.0849	445.0858	-2.0	269.0203, 241.3189, 175.0694, 150.0659	FCI
39	9.35	Genistin	$C_{21}H_{20}O_{10}$	$[M-H]^-$	431.1057	431.1056	0.2	269.0475, 175.0658	FCI
40[a]	9.89	Glycitein	$C_{16}H_{12}O_5$	$[M-H]^-$	283.0685	283.0680	1.8	268.0415, 251.0112, 189.0177, 145.0641	FCI
41	10.21	Apigetrin	$C_{21}H_{20}O_{10}$	$[M-H]^-$	431.1057	431.1046	2.6	269.0145, 175.1254, 151.0141	FCI/FCIP
42	10.45	Maritimein	$C_{21}H_{20}O_{11}$	$[M-H]^-$	448.1006	448.1002	0.9	286.0426, 177.0423	FCI/FCIP
43	10.69	Demethyltexasin	$C_{15}H_{10}O_5$	$[M-H]^-$	269.0143	269.0139	1.5	241.0684, 176.1645, 152.0688, 117.0882	FCI
44	11.65	Maritimetin	$C_{15}H_{10}O_6$	$[M-H]^-$	285.0477	285.0467	3.5	268.0215, 251.0121, 213.0506, 175.2144	FCI/FCIP
45	11.87	Kaempferol	$C_{15}H_{10}O_6$	$[M-H]^-$	285.0473	285.0478	-1.8	257.0132, 151.0081, 191.0633, 133.0966	FCI/FCIP
46	12.36	Daidzein	$C_{15}H_{10}O_4$	$[M-H]^-$	253.0156	253.0150	2.4	160.0188, 136.0504, 117.1009	FCI
47	12.58	Tricin5-O-β-D-glucoside	$C_{23}H_{24}O_{12}$	$[M-H]^-$	491.1268	491.1262	1.2	329.0335, 314.0857, 299.0411	FCI
48	12.86	BiochaninA	$C_{16}H_{12}O_5$	$[M-H]^-$	283.0685	283.0694	-3.2	268.0034, 175.0636, 151.3637, 131.0744	FCI
49[a]	13.64	Apigenin	$C_{15}H_{10}O_5$	$[M-H]^-$	269.0524	269.0522	0.7	251.0301, 175.0126, 151.1037, 117.0435 93.0058	FCI/FCIP
50	13.89	Diosmetin-7-O-rutinoside	$C_{28}H_{31}O_{15}$	$[M-H]^-$	607.0614	607.0628	-2.3	592.1501, 445.1201, 299.1021,	FCI/FCIP
51[a]	14.02	Linarin	$C_{28}H_{32}O_{14}$	$[M-H]^-$	591.1893	591.1880	2.2	576.0187, 445.0024, 283.0719, 268.0146, 151.1057, 131.0239	FCI/FCIP
52	14.21	Isorhamnetin	$C_{16}H_{12}O_7$	$[M-H]^-$	315.0578	315.0585	-2.2	300.0108, 287.0044, 272.0764, 151.0641	FCI
53	14.38	Sulfuretin	$C_{15}H_{10}O_5$	$[M-H]^-$	269.0528	269.0521	2.6	252.0324, 213.0960, 159.0645	FCI
54	14.86	5,7,8-Trihydroxyflavone	$C_{15}H_{10}O_5$	$[M-H]^-$	269.0524	269.0521	1.1	192.0323, 167.0021, 101.0319, 76.0021	FCI/FCIP
55	15.04	Formononetin	$C_{16}H_{12}O_4$	$[M-H]^-$	267.0736	267.0735	0.4	252.0108, 235.0641, 223.0507, 159.0062, 135.0674	FCI/FCIP

No.	RT (min)	Identification	Formula	Ion adduction	Experimental (m/z)	Theoretical (m/z)	Error (ppm)	Fragments (m/z)	Source
56[a]	15.29	3,4-Dicaffeoylquinicacid	$C_{25}H_{23}O_{12}$	$[M-H]^-$	515.1087	515.1103	-3.1	353.0614, 191.2413, 179.0021, 173.0175, 161.0206, 135.0651	FCI/FCIP
57[a]	15.31	Chrysin	$C_{15}H_{10}O_4$	$[M-H]^-$	253.0579	253.0578	0.4	151.0243, 101.0031, 176.0548, 76.0319	FCI/FCIP
58	15.76	5-O-Methylgenistein	$C_{16}H_{12}O_5$	$[M-H]^-$	283.0685	283.0682	1.1	268.0314, 189.3304, 166.0877	FCI/FCIP
59	16.01	5,4′-Dihydroxy-7-methoxyflavone	$C_{16}H_{11}O_5$	$[M-H]^-$	283.0912	283.0914	-0.7	268.0473, 189.0457, 165.0511, 117.0098, 93.0822	FCI
60[a]	16.24	3,5-Dicaffeoylquinicacid	$C_{25}H_{24}O_{12}$	$[M-H]^-$	515.1268	515.1254	2.7	353.0987, 191.0132, 179.1874, 173.0187	FCI
61[a]	16.54	Luteolin	$C_{15}H_9O_6$	$[M-H]^-$	285.0512	285.0506	2.1	257.0106, 199.0137, 175.0188, 151.0108, 133.0289, 109.0956	FCI/FCIP
62	16.79	Eupatolitin	$C_{17}H_{13}O_8$	$[M-H]^-$	345.0109	345.0098	3.2	330.0305, 315.0339, 235.0644, 195.0647	FCI
63	17.01	5,2'-Dihydroxy-6,7,8,6'-tetramethoxyflavone	$C_{19}H_{18}O_8$	$[M-H]^-$	373.0994	373.0991	0.8	358.0301, 343.0494, 328.0511, 249.0119, 123.1306	FCI
64	17.31	Chrysoeriol	$C_{16}H_{11}O_6$	$[M-H]^-$	299.0812	299.0806	2.0	284.0039, 175.0058, 150.0066, 148.0614	FCI/FCIP
65	17.66	Jaceosidin	$C_{17}H_{13}O_7$	$[M-H]^-$	329.0745	329.0745	0.1	314.0169, 299.0078, 180.0069, 148.0582	FCI
66[a]	18.01	Handelin	$C_{32}H_{39}O_8$	$[M+HCOOH+H]^+$	597.2706	597.2692	2.3	-	FCI

Supplementary Table S4 Linear regression equations, LOD, LOQ and linear range for quantitative analysis by HPLC.

Analyte	Regression equation	R^2	Linear range (μg/mL)	Precision[a] （RSD%）	Repeatability[b] （RSD%）	Recovery （%）	LOD (ng)	LOQ (ng)
Hydrochlorothiazide (Peak 6)	y = 7.1372x - 7.71	0.9998	23.8-713.2	1.01	1.36	98.70%	0.523	1.264
Chlorogenic acid (Peak 8)	y = 14.65x - 0.7567	0.9998	0.7-21.0	1.37	1.63	97.10%	0.129	0.414
Rutin (Peak 16)	y = 1.8344x - 5.5383	1.0000	83.7-2512.0	0.87	1.48	99.10%	1.012	4.256
Linarin (Peak 23)	y = 18.05x - 9.6231	0.9999	3.3-100.0	1.64	1.87	97.30%	0.151	0.383

[a,b] Precision and repeatability of the quantitative method were expressed as the RSD of the peak areas of 6 injections of each analyte.

Supplementary Table S5 Weighting factor of three parameters by CVWM algorithm for three types of fingerprints

Para.	Fusion HPLC-FP			UV-FP			DSC-FP		
	$S_{m\text{-}HPLC}$	$P_{m\text{-}HPLC}$	$\alpha_{\text{-}HPLC}$	$S_{m\text{-}UV}$	$P_{m\text{-}UV}$	$\alpha_{\text{-}UV}$	$S_{m\text{-}DSC}$	$P_{m\text{-}DSC}$	$\alpha_{\text{-}DSC}$
Mean	0.956	97.2	0.047	0.998	95.5	0.017	0.957	98.4	0.042
SD	0.027	12.205	0.030	0.001	9.263	0.011	0.012	4.581	0.012
CV	2.84	12.56	64.44	0.12	9.70	68.74	1.27	4.66	29.70
f_w	0.67	0.47	0.40	0.03	0.36	0.42	0.30	0.17	0.18

Supplementary Table S6 Overview of the experimental and predicted values for total antioxidant activity of ABTS test obtained by PLS model

No.	Experimental data (μg/ml)	Predicted Data (μg/ml)	RE[c] (%)	No.	Experimental data (μg/ml)	Predicted Data (μg/ml)	RE[c] (%)
S1[a]	56.98	58.77	3.14	**S14**[a]	50.69	51.33	1.26
S2[a]	58.79	59.78	1.68	**S15**[a]	59.67	58.81	-1.46
S3[a]	59.65	59.40	-0.41	**S16**[a]	64.41	64.81	0.61
S4[a]	49.21	50.65	2.92	**S17**[b]	68.21	65.46	-4.03
S5[a]	71.01	71.96	1.33	**S18**[a]	64.07	64.11	0.06
S6[b]	58.38	59.59	2.07	**S19**[a]	65.67	65.25	-0.64
S7[a]	56.71	58.59	3.32	**S20**[b]	69.64	67.21	-3.49
S8[a]	63.46	64.02	0.87	**S21**[b]	68.85	66.19	-3.86
S9[a]	62.32	61.42	-1.44	**S22**[a]	64.49	65.14	1.02
S10[b]	49.45	47.89	-3.15	**S23**[a]	72.35	70.12	-3.08
S11[a]	51.23	48.92	-4.51	**S24**[a]	66.87	66.42	-0.67
S12[a]	48.57	47.82	-1.55	**S25**[a]	63.98	63.64	-0.53
S13[a]	53.11	54.81	3.21				

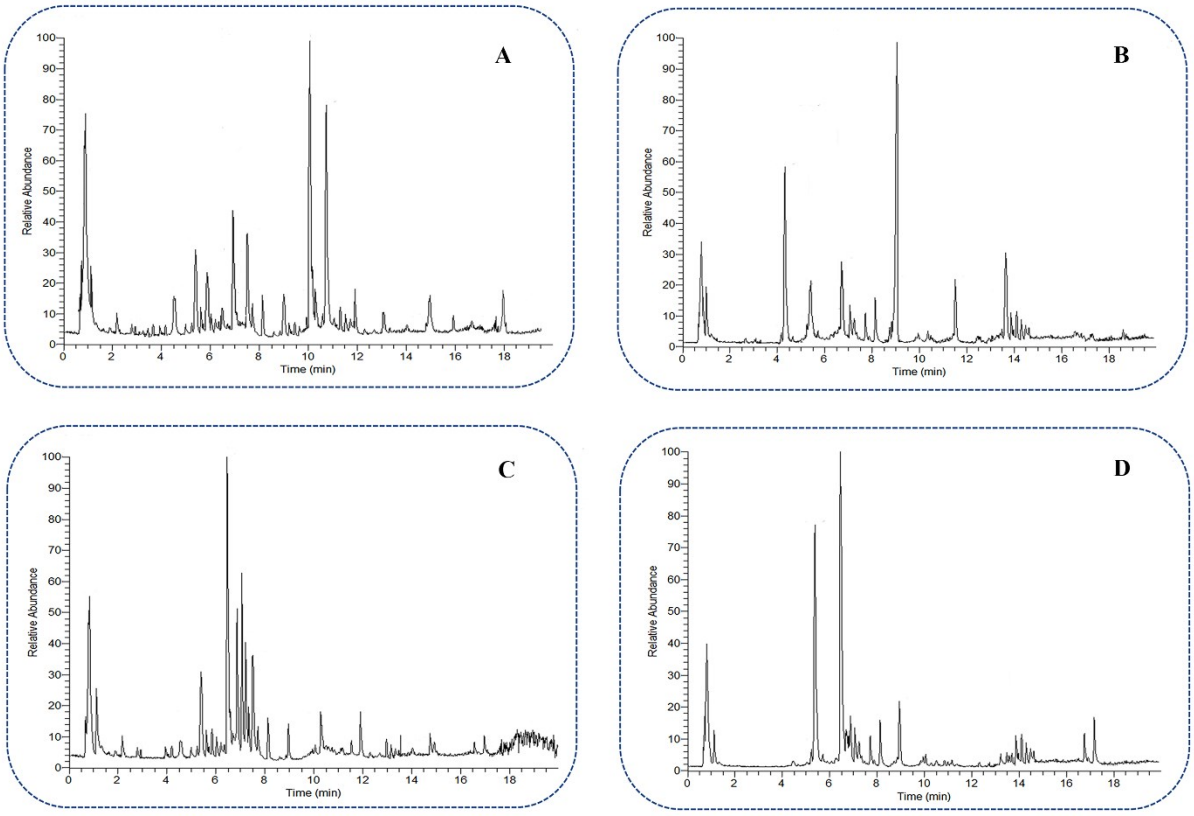
[a] Used for the calibration model.

[b] Used for the prediction model.

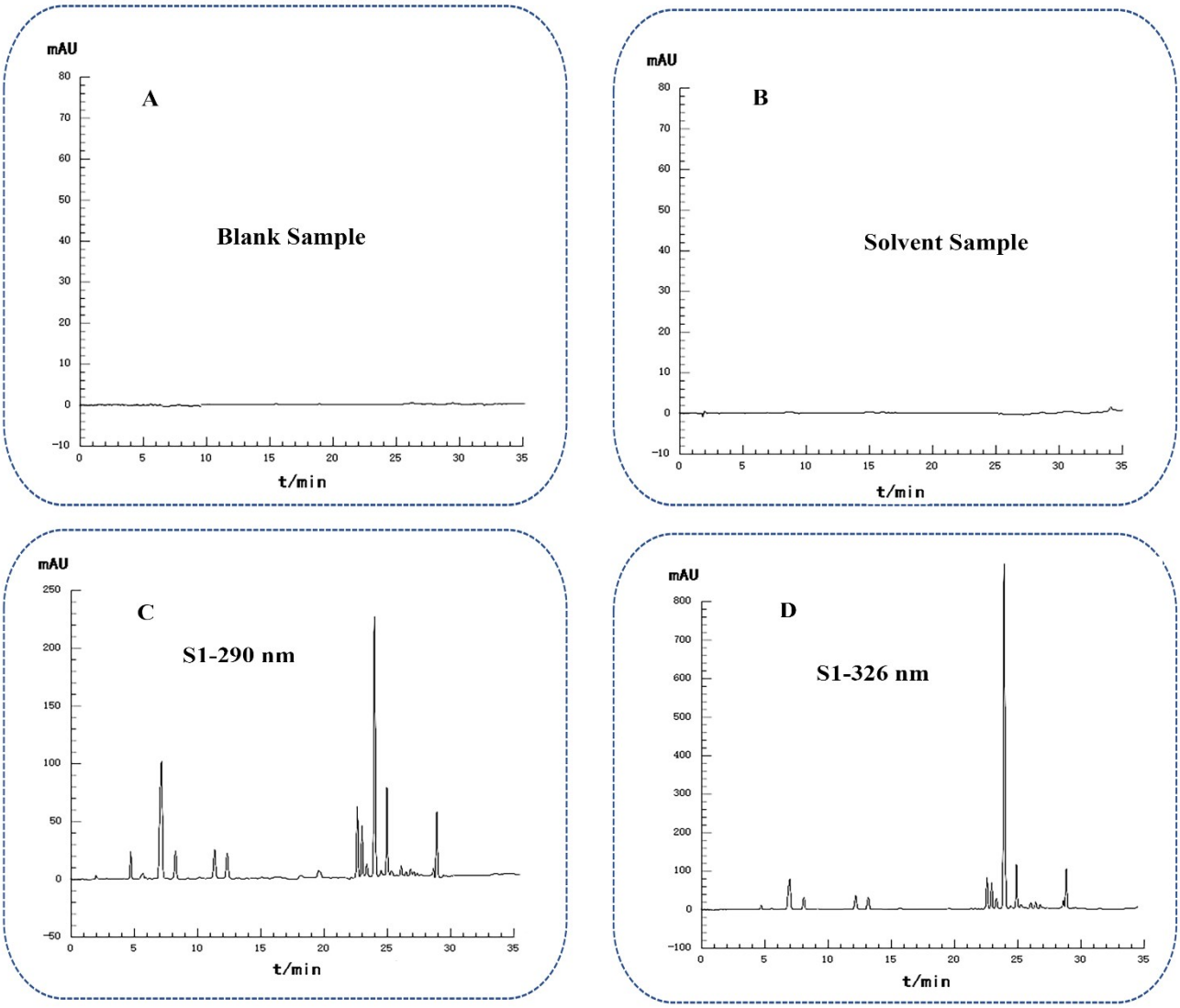
[c] RE: relative error.

Supplementary Table S7 Values of Enthalpy-1 and Enthalpy-2 of DSC curve for 25 FCIP samples

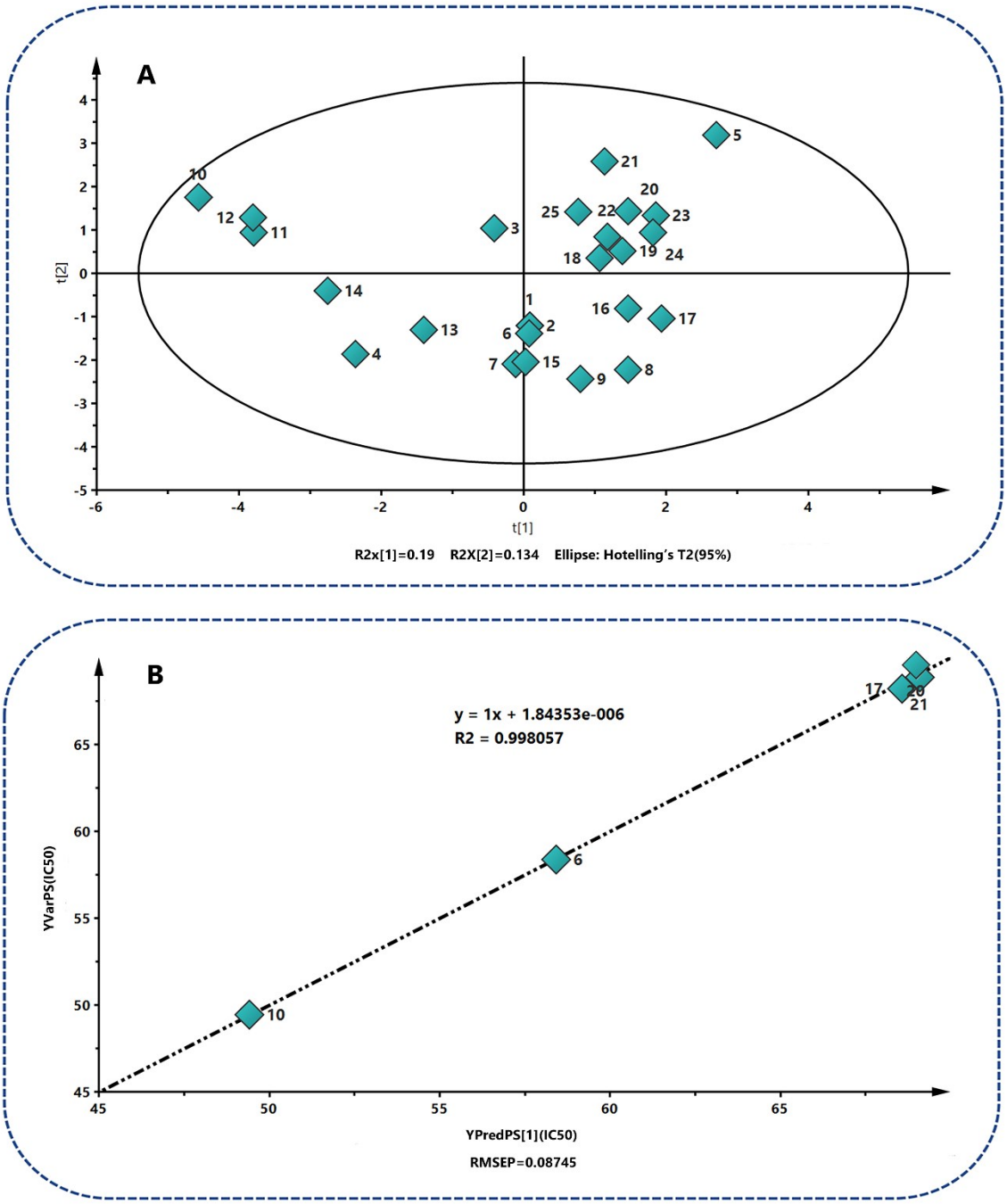
No.	Enthalpy-1 (J/g)	Enthalpy-2 (J/g)	No.	Enthalpy-1 (J/g)	Enthalpy-2 (J/g)	No.	Enthalpy-1 (J/g)	Enthalpy-2 (J/g)
S1	418.90	2987.63	**S10**	443.88	2847.16	**S19**	285.13	2574.29
S2	390.74	2949.38	**S11**	383.63	3071.80	**S20**	260.71	2252.48
S3	316.14	2634.00	**S12**	401.08	3035.11	**S21**	281.62	2015.08
S4	417.73	2892.60	**S13**	431.50	3043.60	**S22**	343.96	2289.46
S5	248.43	2265.60	**S14**	417.41	3370.48	**S23**	240.47	2152.00
S6	390.04	2750.97	**S15**	326.57	2738.05	**S24**	290.03	2222.20
S7	402.68	2773.70	**S16**	299.49	2244.49	**S25**	299.93	2826.95
S8	307.03	2648.55	**S17**	347.28	2012.43			
S9	330.10	2740.20	**S18**	306.07	2483.12			



Supplementary Fig. S1 Total ion chromatogram of FCI in negative ion mode (A), FCI in positive ion mode (B), FCIP (S1) in negative ion mode (C), FCIP (S1) in positive ion mode



Supplementary Fig. S2 Specificity of HPLC quantitative method. Chromatogram of blank sample (A), solvent sample (B), S1 sample at 290 nm (C), S1 sample at 326 nm (D).



Supplementary Fig. S3 Scores scatter plot (A) and Y observed versus Y predicted plot for the prediction model (B) of the partial least squares (PLS) model between the fusion fingerprints and the antioxidant activities