Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Simultaneous determination of daidzein, its prodrug and major conjugative metabolites in rat plasma and application in a pharmacokinetic study

Authors:

Yingchao Li[1], Xiaoyu Liu[1], Farong Lu[1], Jiaming Zhang[1], Yawei Zhang[1], Wenchao Li[1], Tianhong Zhang[1]*

Affiliation:

[1]Wuya College of Innovation, Shenyang Pharmaceutical University, Shenyang 110016, PR China

*__Correspondence:__

Dr. Tianhong Zhang, Wuya College of Innovation, Shenyang Pharmaceutical University, Shenyang Liaoning Province, 110016, P. R. China

E-mail address: zhangth1058@aliyun.com

Tel: +86-24-23984159. Fax: +86-24-23986321.

Abbreviations: DAN, daidzein; 4'-I-P, 4'-O-L-isoleucyl carbamate prodrug of daidzein; DAN-7-4'-DS, daidzein-7-4'-disulfate; DAN-7-G, daidzein-7-O-glucuronide; LLOQ, lower limit of quantification; CID, collision-induced dissociation; PPT, protein precipitation; SD, Sprague-Dawley; MRM, multiple reaction monitoring; QC, quality control; ESI, electrospray ionization.

Keywords: daidzein; prodrug; metabolites; HPLC–MS/MS; pharmacokinetic study.

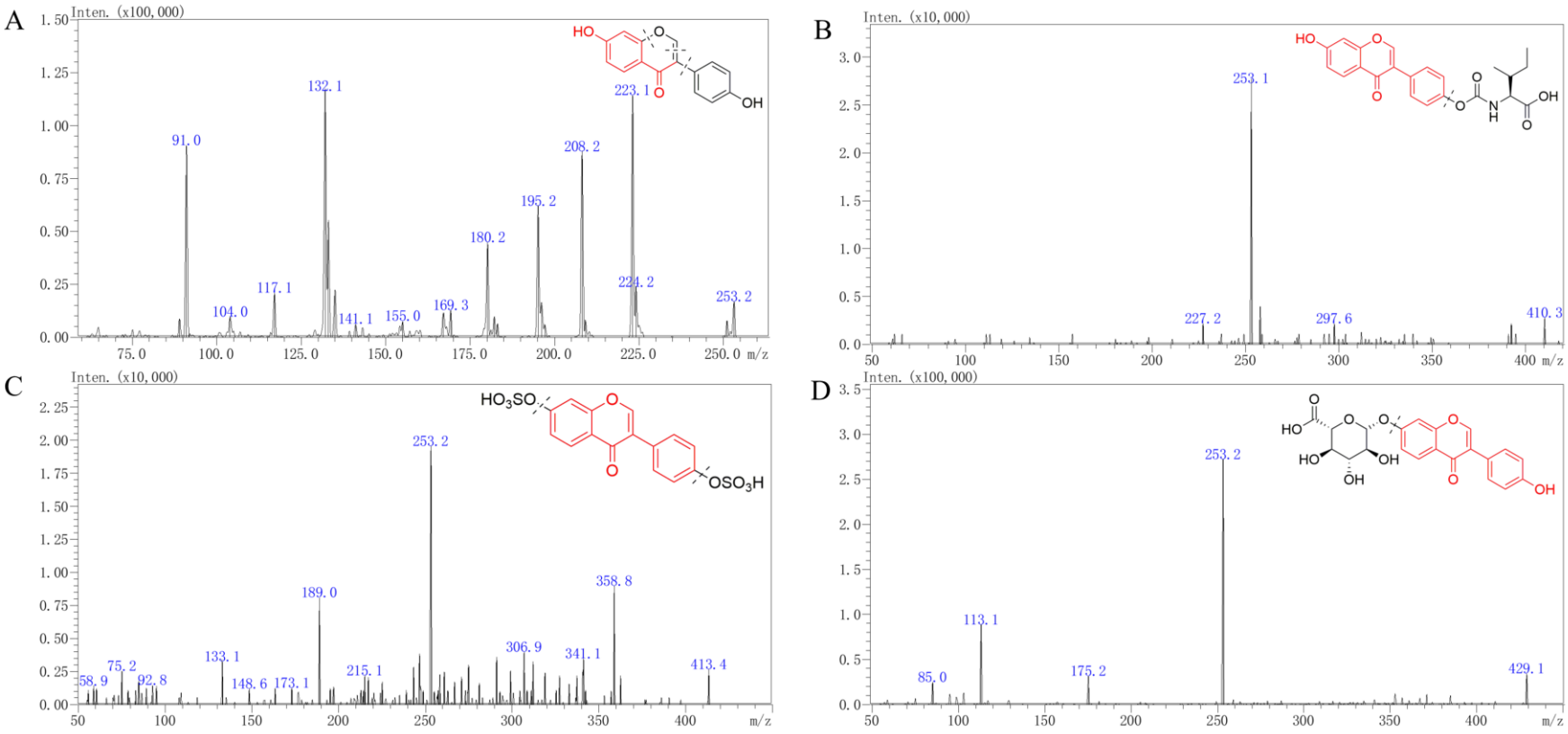

Fig. S1 Full scan product ion mass spectrum of (A) DAN, (B) 4'-I-P, (C) DAN-7-4'-DS and (D) DAN-7-G in negative mode.

Table S1 Gradient time table for HPLC-MS/MS method. Mobile phase A was water containing 5 mM ammonium acetate adjusted to pH 4.2 with acetic acid. Mobile phase B was acetonitrile.

Time (min)	Flow rate (mL/min)	A %	B %
0.00	0.3	90	10
4.50	0.3	18	82
4.70	0.3	90	10
6.20	0.3	90	10

Table S2 The precision and trueness values of the analytes for dilution integrity (mean ± SD., n = 6).

Analytes	Added concentration (ng/mL)	Dilution times	Found concentration (ng/mL)	Accuracy RSD (%)	Precision RE (%)
DAN	2000	2.5	750.6 ± 19.5	2.7	-6.2
	4000	5	770.7 ± 34.4	4.5	-3.7
4'-I-P	10000	2.5	4096 ± 122	3.0	2.5
	20000	5	4156 ± 214	5.2	4.0
D-7-4'-DS	10000	2.5	4298 ± 156	3.7	7.5
	20000	5	4152 ± 69	1.7	3.8
D-7-G	10000	2.5	4206 ± 282	6.8	5.2
	20000	5	4062 ± 211	5.2	1.6

Table S3 Stability of DAN, 4'-I-P, DAN-7-4'-DS and D-7-G in rat plasma under different storage conditions (mean ± SD., n = 3).

Analytes	Nominal (ng/mL)	Room temperature for 4 h		Pretreatment for 8 h		three freeze–thaw cycles		-80°C frozen storage 30 days	
		Measured (ng/mL)	RSD (%)	Measured (ng/mL)	RSD (%)	Measured (ng/mL)	RSD (%)	Measured (ng/mL)	RSD (%)
DAN	1.600	1.638 ± 0.106	6.5	1.600 ± 0.092	5.8	1.649 ± 0.104	3.3	1.538 ± 0.160	11
	40.00	36.47 ± 0.93	2.6	36.52 ± 0.94	3.6	37.07 ± 0.96	2.9	37.34 ± 1.40	3.8
	800	711.8 ± 14.0	2.0	724.7 ±10.46	1.5	706.1 ± 17.0	2.5	737.7 ± 19.0	2.6
4'-I-P	8.00	7.917 ± 0.620	7.9	7.842 ± 0.355	4.6	8.31 ± 0.56	6.8	8.07 ± 0.40	5.1
	200.0	183.3 ± 10.5	5.8	187.3 ± 11.74	6.3	200.6 ± 15.6	7.9	214.6 ± 7.05	3.3
	4000	4132 ± 175	4.3	4218 ± 316	7.5	3893 ± 333	9	4182 ± 225	5.4
D-7-4'-DS	8.00	7.727 ± 0.371	4.9	8.64 ± 0.159	1.9	7.837 ± 0.886	12	7.879 ± 0.700	9
	200.0	202.7 ± 5.6	2.8	213.7 ± 6.2	2.9	201.2 ± 15.8	7.9	199.5 ± 9.9	5.0
	4000	4369 ± 189	4.4	4132 ± 299	7.3	4396 ± 197	4.5	4346 ± 143	3.3
D-7-G	8.00	8.20 ± 0.34	4.2	7.401 ± 0.449	6.1	8.38 ± 0.66	7.9	8.01 ± 0.62	7.8
	200.0	183.1 ± 8.0	4.4	180.6 ± 8.4	4.7	176.2 ± 2.6	1.5	183.3 ± 11.1	6.1
	4000	4297 ± 172	4.1	3862 ± 242	6.3	4284 ± 193	4.6	4253 ± 304	7.2