

Supplementary information of
***In vitro* evidence for estrogen receptor activity of selected phase II isoflavone metabolites**

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22 **Supplementary Table S1: Multiple reaction monitoring parameters of the UHPLC-MS/MS method.**

Compound Name	Q1 (m/z)	DP (V)	RT (min)	Q3 (m/z)	Ratio ^{*)}	CE (V)	CXP (V)
GEN	268.9	-45	11.1	133.1	-	-44	-15
				159.0	0.42	-42	-21
				131.8	0.36	-48	-11
GEN-4'-glucuronide	444.9	-60	7.8	268.9	-	-34	-17
				113.0	0.39	-22	- 7
				175.0	0.22	-20	-13
GEN-7-glucuronide	444.9	-60	7.2	268.9	-	-34	-17
				113.0	0.52	-22	- 7
				175.0	0.40	-20	-13
GEN-4',7-diglucuronide	620.8	-105	4.5	268.9	-	-56	-15
				444.8	0.88	-28	-29
				112.9	0.27	-30	-17
GEN-7-sulfate	348.8	-15	9.1	269.0	-	-28	-21
				132.9	0.08	-56	-19
				131.9	0.04	-68	-13
GEN-4',7-disulfate	428.8	-40	7.5	268.9	-	-36	-17
				348.9	0.69	-22	-23
GEN-7-glucuronide-4'-sulfate	524.8	-20	5.8	269.1	-	-50	-17
				348.7	0.43	-36	-23
				112.9	0.04	-34	-11
GEN-4'-glucuronide-7-sulfate	524.8	-20	6.3	269.1	-	-50	-17
				445.0	0.66	-28	- 3
				112.9	0.10	-34	-11
DAI	252.8	-95	10.2	132.0	-	-50	-13
				223.8	0.99	-36	-15
				208.0	0.97	-42	-13
DAI-4'-glucuronide	428.9	-40	7.1	253.0	-	-34	-17
				112.9	0.59	-20	-15
				174.9	0.33	-18	-15
DAI-7-glucuronide	428.9	-40	6.4	253.0	-	-34	-17
				112.9	0.69	-20	-15
				174.9	0.62	-18	-15
DAI-4',7-diglucuronide	604.8	-20	3.5	253.0	-	-56	-15
				429.0	0.72	-26	-25
				112.9	0.40	-28	- 7
DAI-4'-sulfate	332.8	-20	8.5 ¹⁾	253.0	-	-30	-21
				116.9	0.22	-52	-19
				134.8	0.06	-44	- 9

24 **Supplementary Table S1: continued**

Compound Name	Q1 (m/z)	DP (V)	RT (min)	Q3 (m/z)	Ratio ^{*)}	CE (V)	CXP (V)
DAI-4',7-disulfate	412.8	-30	6.9	253.1	-	-38	-19
				332.8	0.91	-18	-23
				224.9	0.08	-64	-19
DAI-7-glucuronide-4'-sulfate	508.8	-30	5.2	253.0	-	-52	-17
				332.9	0.65	-34	-21
				174.7	0.01	-42	-15
DAI-4'-glucuronide-7-sulfate	508.8	-30	5.7	253.0	-	-52	-17
				429.0	0.62	-30	-29
				174.7	0.03	-42	-15
EQ	240.9	-80	10.8	121.1	-	-20	- 9
				118.9	0.72	-26	-13
				134.9	0.47	-24	-19
EQ-7-glucuronide	416.9	-70	7.7	113.0	-	-26	-19
				174.9	0.42	-22	-13
				120.9	0.27	-40	-17
EQ-4'-sulfate	320.8	-60	8.9 ²⁾	121.1	-	-36	-21
				119.0	0.79	-40	-17
				241.0	0.68	-28	-15

25 The entrance potential (EP) was set to -10 V for all transitions.

26 Abbreviations: GEN – genistein, DAI – daidzein, EQ – S-equol, Q1 – precursor mass, DP – declustering
27 potential, Q3 – fragment mass, CE – collision energy, CXP – cell exit potential, RT – retention time.

28 ^{*)} The ratio always refers to the first provided transition for each metabolite which was also the one with
29 the highest intensity.

30 ¹⁾ The obtained standard does not seem to be as pure as stated since a double peak was visible, see Figure
31 11. Since no baseline separation was achieved and both compounds were present in the standard the sum
32 of the peak areas was taken into consideration for all the evaluations.

33 ²⁾ No standard for EQ-7-sulfate was available, no clear second peak was visible in the samples, but both
34 isoforms might be present.

Supplementary Table S2: Mean values and standard deviations of recoveries and matrix effects for the four different spiking solutions performed in triplicate.

	Compound	Recovery	Matrix effects
Isoflavones	GEN	90 ± 9	88 ± 3
	DAI	82 ± 8	85 ± 1
	EQ	81 ± 6	88 ± 3
GEN-metabolites	GEN-4'-glucuronide	86 ± 13	88 ± 5
	GEN-7-glucuronide	91 ± 10	93 ± 3
	GEN-4',7-diglucuronide	79 ± 25	68 ± 10
	GEN-7-sulfate	79 ± 18	91 ± 6
	GEN-4',7-disulfate	86 ± 5	97 ± 9
	GEN-7-glucuronide-4'-sulfate	100 ± 15	98 ± 9
	GEN-4'-glucuronide-7-sulfate	97 ± 12	94 ± 4
DAI-metabolites	DAI-4'-glucuronide	86 ± 17	90 ± 7
	DAI-7-glucuronide	97 ± 24	90 ± 2
	DAI-4',7-diglucuronide	<LOQ	<LOQ
	DAI-4'-sulfate	106 ± 7	105 ± 9
	DAI-4',7-disulfate	85 ± 15	72 ± 3
	DAI-7-glucuronide-4'-sulfate	82 ± 25	82 ± 3
	DAI-4'-glucuronide-7-sulfate	87 ± 24	84 ± 3
EQ-metabolites	EQ-7-glucuronide	91 ± 1	85 ± 2
	EQ-4'-sulfate	98 ± 18	100 ± 4

Abbreviations: GEN – genistein, DAI – daidzein, EQ – S-equol, < LOQ – below the limit of quantification

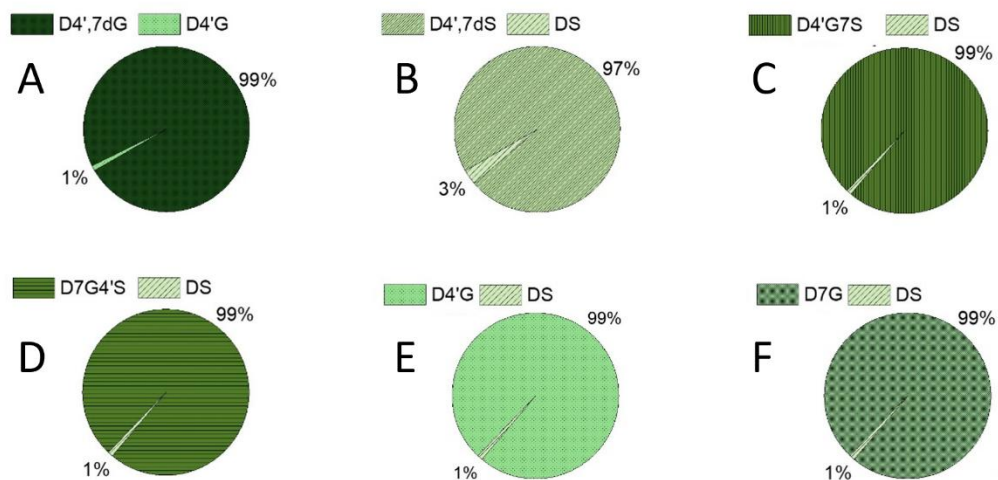
Supplementary Table S3: Determined concentrations of the different metabolites after 48 h of incubation (all concentrations provided in nmol/80 µL).

Products of GEN	1 µM ¹⁾	10 µM ²⁾	Products of DAI	1 µM ¹⁾	10 µM ²⁾
GEN	0.02 ± 0.01	0.75 ± 0.44	DAI	0.06 ± 0.01	0.56 ± 0.29
GS	0.07 ± 0.01	0.05 ± 0.03	DS	0.24 ± 0.07	0.26 ± 0.17
Sum	0.10 ± 0.02	0.80 ± 0.44	Sum	0.29 ± 0.07	0.83 ± 0.34
Products of G4'G	1 µM	10 µM ²⁾	Products of D4'G	1 µM	10 µM ²⁾
G4'G	n.t.	1.95 ± 1.32	D4'G	n.t.	3.13 ± 0.94
			DS		0.02 ± 0.01
			Sum		3.15 ± 0.94
Products of G7G	1 µM ¹⁾	10 µM ²⁾	Products of D7G	1 µM	10 µM ²⁾
G7G	0.14 ± 0.4	0.84 ± 0.13	D7G	n.t.	2.22 ± 0.24
GS	0.0007 ± 0.0003	-	DS		0.02 ± 0.01
Sum	0.14 ± 0.4	-	Sum		2.25 ± 0.24
Products of G4',7dG	1 µM ¹⁾	10 µM ²⁾	Products of D4',7dG	1 µM	10 µM ²⁾
G4',7dG	0.13 ± 0.05	1.22 ± 0.26	D4',7dG	n.t.	0.97 ± 0.31
G4'G	-	0.03 ± 0.01	D4'G		0.009 ± 0.002
G7G	-	0.02 ± 0.01			
Sum	0.13 ± 0.05	1.27 ± 0.26	Sum		0.98 ± 0.31
Products of G7S	1 µM	10 µM ²⁾	Products of D4'S	1 µM ¹⁾	10 µM ²⁾
G7S	0.07 ± 0.05	1.36 ± 0.14	DS	0.18 ± 0.11	0.67 ± 0.25
Products of G4'7dS	1 µM	10 µM ²⁾	Products of D4',7dS	1 µM	10 µM ²⁾
G4',7dS	n.t.	1.53 ± 0.29	D4',7dS	n.t.	1.62 ± 0.21
			DS		0.06 ± 0.02
			Sum		1.68 ± 0.21
Products of G7G4'S	1 µM	10 µM ²⁾	Products of D7G4'S	1 µM	10 µM ²⁾
G7G4'S	n.t.	1.90 ± 0.97	D7G4'S	n.t.	1.06 ± 0.22
			DS		0.02 ± 0.01
			Sum		1.08 ± 0.22
Products of G4'G7S	1 µM	10 µM ²⁾	Products of D4'G7S	1 µM ¹⁾	10 µM ²⁾
G4'G7S	n.t.	1.99 ± 1.11	D4'G7S	n.t.	1.28 ± 0.25
			DS		0.008 ± 0.004
			Sum		1.29 ± 0.25
Products of EQ	1 µM ¹⁾	10 µM ²⁾	Products of E7G	1 µM ¹⁾	10 µM ²⁾
EQ	0.02 ± 0.01	0.28 ± 0.22	E7G	n.t.	0.82 ± 0.19
ES	0.02 ± 0.01	0.05 ± 0.04	Products of E4'S	1 µM	10 µM
Sum	0.04 ± 0.02	0.33 ± 0.22	[nmol/80 µL]	[nmol/80 µL]	
			E4'S	0.09 ± 0.05	0.26 ± 0.12

n.t. – not tested

1) theoretical concentration 0.08 nmol/80 µL

2) theoretical concentration 0.8 nmol/80 µL



Supplementary Figure S1: Pie charts representing the DAI-metabolites present after incubating Ishikawa cells for 48 h with A - D4',7dG; B – D4',7dS; C – D4'G7S, D – D7G4'S; E – D4'G; F – D7G