

***In vitro* production of (+)-catechin and (–)-epicatechin glucuronides to better understand the *in vivo* metabolism of a polyphenol-rich extract (Memophenol™)**

Lisa Texier^{a,b}, Adeline Vignault^b, Arnaud Courtois^{a,c}, Grégory Da Costa^a, Line Pourtau^b, Benjamin Moras^b, Tristan Richard^a, Stéphanie Krisa^{a,*}

^aUniv. Bordeaux, INRAE, Bordeaux INP, Bordeaux Sciences Agro, UMR 1366, OENO, ISVV, 33140 Villenave d'Ornon, France

^bActiv'Inside, ZA du Grand Cazau, 33750 Beychac et Caillau, France

^cCentre Antipoison de Nouvelle Aquitaine, CHU de Bordeaux, 33076 Bordeaux, France

*** Corresponding author:**

Stéphanie Krisa

stephanie.krisa@u-bordeaux.fr

ISVV, 210 Chemin de Leysotte, 33140 Villenave d'Ornon, France

+33 5 57 57 59 54

Table S1. Sensitivity and linearity of the analytical method for the quantification of (+)-catechin and (–)-epicatechin monoglucuronides. C5G = (+)-catechin-5-O- β -glucuronide. C7G = (+)-catechin-7-O- β -glucuronide. C3'G = (+)-catechin-3'-O- β -glucuronide. C4'G = (+)-catechin-4'-O- β -glucuronide. E5G = (–)-epicatechin-5-O- β -glucuronide. E7G = (–)-epicatechin-7-O- β -glucuronide. E3'G = (–)-epicatechin-3'-O- β -glucuronide. E4'G = (–)-epicatechin-4'-O- β -glucuronide.

	Linearity		Sensitivity	
	Linear equation	Regression coefficient (R ²)	LOD (μ M)	LOQ (μ M)
C5G	Expressed as C7G equivalent		0.005	0.014
C7G	y = 1125.2063x – 65.1439	0.9982	0.005	0.015
C3'G	Expressed as C7G equivalent		0.004	0.011
C4'G	Expressed as C7G equivalent		0.003	0.009
E5G	Expressed as E7G equivalent		0.006	0.018
E7G	y = 1247.1199x – 60.0756	0.9992	0.003	0.008
E3'G	Expressed as E7G equivalent		0.004	0.011
E4'G	Expressed as E7G equivalent		0.006	0.017

Table S2. Quantification parameters of polyphenol metabolites in human plasma after Memophenol™ consumption. Metabolites were quantified by UHPLC-DAD-MS/MS in negative SRM mode. CE: collision energy. RT: retention time.

Compound	Parent ion (m/z)	Tube Lens	Quantifier		Qualifier		RT (min)	Calibration Compound
			Product ion (m/z)	CE (V)	Product ion (m/z)	CE (V)		
(Epi)catechin-glucuronide-sulfate	545	98	369	27	289	27	12	Epicatechin
(Epi)catechin-sulfate (isomer 1)	369	98	289	27	245	16	12	Epicatechin
(Epi)catechin-sulfate (isomer 2)	369	98	289	27	245	16	12	Epicatechin
2,3-dihydroxybenzoic acid	153	52	109	12	65	16	6.34	2,3-dihydroxybenzoic acid
2,4-dihydroxybenzoic acid	153	52	109	12	65	16	6.3	2,3-dihydroxybenzoic acid
3-hydroxybenzoic acid	137	49	93	11	65	25	6.37	3-hydroxybenzoic acid
3-hydroxyphenylacetic acid	151	58	107	5	65	23	6.57	3-hydroxyphenylacetic acid
3- <i>O</i> -methylgallic acid	183	68	168	13	124	18	5.42	3- <i>O</i> -methylgallic acid
3,4-dihydroxyphenylacetic acid	167	63	123	5	95	19	4.52	3,4-dihydroxyphenylacetic acid
4-hydroxy-5-(hydroxyphenyl)valeric acid-glucuronide (isomers)	401	95	225	20	207	30	12	Epicatechin
4-hydroxy-5-(hydroxyphenyl)valeric acid-sulfate (isomer 1)	305	95	225	20	207	30	12	Epicatechin
4-hydroxy-5-(hydroxyphenyl)valeric acid-sulfate (isomer 2)	305	95	225	20	207	30	12	Epicatechin
4-hydroxybenzoic acid	137	46	93	13	65	27	5.26	4-hydroxybenzoic acid
4-hydroxyphenylacetic acid	151	72	107	5	65	22	6	3-hydroxyphenylacetic acid
4- <i>O</i> -methylgallic acid	183	65	168	10	124	18	6.11	3- <i>O</i> -methylgallic acid
5-(3'-hydroxyphenyl)-gamma-valerolactone	191	70	147	20	106	31	12	5-(3'-hydroxyphenyl)-gamma- valerolactone
5-(3',4'-dihydroxyphenyl)-gamma-valerolactone	207	75	163	20	123	25	12	5-(3'-hydroxyphenyl)-gamma- valerolactone
5-(3',4',5'-trihydroxyphenyl)-gamma-valerolactone	223	78	179	21	138	26	18.21	5-(3'-hydroxyphenyl)-gamma- valerolactone
5-(3',5'-dihydroxyphenyl)-gamma-valerolactone	207	75	163	18	123	20	12	5-(3'-hydroxyphenyl)-gamma- valerolactone
5-(4'-hydroxyphenyl)-gamma-valerolactone	191	70	147	13	106	31	12	5-(3'-hydroxyphenyl)-gamma- valerolactone
5-(x-hydroxyphenyl)-gamma-valerolactone- <i>O</i> - glucuronide	383	87	207	27	163	40	12.14	5-(3'-hydroxyphenyl)-gamma- valerolactone
5-(x-hydroxyphenyl)-gamma-valerolactone- <i>O</i> -sulfate	287	96	207	23	163	34	6.96	5-(3'-hydroxyphenyl)-gamma- valerolactone
5-phenyl-gamma-valerolactone-3'- <i>O</i> -glucuronide	367	93	191	25	147	43	12	5-(3'-hydroxyphenyl)-gamma- valerolactone

5-phenyl-gamma-valerolactone-3',4'- <i>O</i> -disulfate	367	52	287	12	207	33	12	5-(3'-hydroxyphenyl)-gamma-valerolactone
5-phenyl-gamma-valerolactone-4'- <i>O</i> -glucuronide	367	93	191	25	147	43	12	5-(3'-hydroxyphenyl)-gamma-valerolactone
5-phenyl-gamma-valerolactone-glucuronide-sulfate	463	87	287	27	207	40	14.62	5-(3'-hydroxyphenyl)-gamma-valerolactone
5-phenyl-gamma-valerolactone- <i>O</i> -sulfate	271	93	191	23	147	48	7.67	5-(3'-hydroxyphenyl)-gamma-valerolactone
5-phenyl-gamma-valerolactone-sulfate-methoxy	301	96	221	23	206	40	17.19	5-(3'-hydroxyphenyl)-gamma-valerolactone
5-phenyl-valeric acid-sulphate-glucuronide	465	98	289	27	209	30	12	5-(3'-hydroxyphenyl)-gamma-valerolactone
B2-gallate	729	60	577	30	151	30	9.18	Epicatechin
Benzoic acid-4-sulfate	217	71	137	18	93	32	12	Benzoic acid
Caffeic acid-glucuronide	355	70	179	20			12	Caffeic acid
Caffeic acid-sulfate	259	70	179	15			12	Caffeic acid
Catechin-7-glucuronide	465	98	289	27	245	16	5.19	Catechin
Catechin-4'-glucuronide	465	98	289	27	245	16	5.62	Catechin
Catechin-5-glucuronide	465	98	289	27	245	16	5.97	Catechin
Catechin-3'-glucuronide	465	98	289	27	245	16	6.64	Catechin
Catechol-sulfate	189	70	109	20	81	20	2.46	Catechin
Dihydrocaffeic acid-glucuronide	357	70	191	20			12	Dihydrocaffeic acid
Dihydrocaffeic acid-sulphate	261	96	181	20	137	25	5.49	Dihydrocaffeic acid
Dihydroferulic acid-glucuronide	371	70	195	20			12	Dihydroferulic acid
Dihydroferulic acid-sulphate	275	75	195	31	135	17	12	Dihydroferulic acid
Dimers (epi)catechin-(epi)gallo catechin	593	60	411	30	303	30	8	Epicatechin
ϵ -viniferin	453	60	435	20	359	20	16.01	<i>trans</i> -Resveratrol
Epicatechin-3- <i>O</i> -gallate	441	70	289	20	151	20	10.2	Epicatechin-3- <i>O</i> -gallate
Epicatechin-7-glucuronide	465	98	289	27	245	16	6.9	Epicatechin
Epicatechin-5-glucuronide	465	98	289	27	245	16	7.03	Epicatechin
Epicatechin-4'-glucuronide	465	98	289	27	245	16	7.95	Epicatechin
Epicatechin-3'-glucuronide	465	98	289	27	245	16	8.01	Epicatechin
Epigallocatechin-glucuronide	481	70	305	20			12	Epigallocatechin
Ferulic acid 4-glucuronide	369	91	193	18	178	32	6.96	Ferulic acid
Ferulic acid 4-sulphate	273	92	193	18	178	28	10.07	Ferulic acid
Gallic acid-sulfate	249	70	169	15			9.43	Gallic acid
Hydroxyphenylacetic acid-sulfate	231	70	151	15			12	3-hydroxyphenylacetic acid
Hydroxytyrosol-glucuronide	329	70	153	20			12	Hydroxytyrosol
Hydroxytyrosol-sulfate	233	70	153	15			8.91	Hydroxytyrosol

Kaempferol-glucuronide	461	70	285	20			12	Kaempferol
Methylcatechol-sulphate	203	70	123	20	108	26	5.74	Catechin
Methyl-(epi)catechin-sulfate (isomer 1)	383	98	303	20	245	16	12	Epicatechin
Methyl-(epi)catechin-sulfate (isomer 2)	383	98	303	20	245	16	12	Epicatechin
Methylcatechin	303	70	289	15	245	15	5.66	Epicatechin
Methylcatechol	123	61	108	17	105	18	7.08	Catechin
Methylgallic acid-sulfate	263	70	183	15			12	Gallic acid
Methylpyrogallol-sulphate	219	68	139	20	124	24	12	Pyrogallol
<i>p</i> -coumaric acid	163	49	119	14	93	30	13.95	<i>p</i> -coumaric acid
<i>p</i> -coumaric acid-glucuronide	339	70	163	20			12	<i>p</i> -coumaric acid
<i>p</i> -coumaric acid-sulfate	243	70	163	15			11.11	<i>p</i> -coumaric acid
Piceid-glucuronide	564	70	403	20	227	20	12	<i>trans</i> -Resveratrol
Piceid-sulfate	469	70	389	20	227	20	12	<i>trans</i> -Resveratrol
Protocatechuic acid-3-glucuronide	329	64	153	20	123	25	3.76	Protocatechuic acid
Protocatechuic acid-3-sulfate	233	77	153	19	109	33	12	Protocatechuic acid
Pyrogallol-glucuronide	301	70	125	20			12	Pyrogallol
Pyrogallol-sulfate	205	70	125	15			12	Pyrogallol
Quercetin-3-glucuronide	477	87	301	20	179	31	11.59	Quercetin-3-glucuronide
Resveratrol-glucuronide	403	70	228	20			12	<i>trans</i> -Resveratrol
Resveratrol-sulfate	307	70	228	20			12	<i>trans</i> -Resveratrol
Sinapic acid-glucuronide	399	70	224	20			12	Sinapic acid
Sinapic acid-sulphate	303	63	223	30	208	30	12	Sinapic acid
<i>trans</i> -Resveratrol	227	60	185	20	143	20	14.83	<i>trans</i> -Resveratrol
Tyrosol-glucuronide	313	70	137	20			12	Tyrosol
Tyrosol-sulfate	218	70	138	15			12	Tyrosol
Vanillic acid-4-glucuronide	343	92	167	23	152	40	4.65	Vanillic acid
Vanillic acid-4-sulfate	247	74	167	17	152	27	5.53	Vanillic acid
B1 dimer	577	60	411	30	289	30	6.45	Epicatechin
B2 dimer	577	60	411	30	289	30	7.77	Epicatechin
B4 dimer	577	60	411	30	289	30	7.46	Epicatechin
Benzoic acid	121	73	93	10	77	11	9.34	Benzoic acid
Caffeic acid	179	66	135	14	107	21	7.05	Caffeic acid
Catechin	289	72	245	13	203	18	7.41	Catechin
Chlorogenic acid	353	75	191	15	85	39	7.25	Chlorogenic acid
Cinnamic acid	147	58	103	10	77	22	13.23	Cinnamic acid
Dihydrocaffeic acid	181	72	137	10	109	14	6.12	Dihydrocaffeic acid
Dihydroferulic acid	181	70	59	32			6.89	Dihydroferulic acid
Epicatechin	289	72	245	13	203	18	8.78	Epicatechin

Epigallocatechin	305	80	289	15	137	15	7.14	Epigallocatechin
Ferulic acid	193	54	178	12	134	15	9.7	Ferulic acid
Gallic acid	169	68	125	14	79	22	1.73	Gallic acid
Gallocatechin	305	80	289	15	137	15	6.42	Epigallocatechin
Gentisic acid	153	63	108	20			5.21	2,3-dihydroxybenzoic acid
Hippuric acid	178	53	134	10	77	16	6.05	Hippuric acid
Homovanillic acid	181	66	137	6	112	6	6.91	Vanillic acid
Hydroxytyrosol	153.05	53	123	13	95	20	3.7	Hydroxytyrosol
Kaempferol	285	88	239	26	185	26	16.85	Kaempferol
Oleuropein	539	67	307	19	275	20	12.67	Oleuropein
Phenylacetic acid	135	58	91	55			9.01	Phenylacetic acid
Piceatannol	243	60	225	20	173	20	10.77	<i>trans</i> -Resveratrol
Piceid	389	60	227	20	185	20	10.28	<i>trans</i> -Resveratrol
Protocatechuic acid	153	57	109	13	65	16	3.65	Protocatechuic acid
Pyrogallol	125	69	81	14	79	18	1.89	Pyrogallol
Quercetin	301	78	179	17	151	20	15.08	Quercetin
Quercetin sulfate	381	78	301		179		12	Quercetin
Salicylic acid	137	45	93	15	65	27	9.75	3-hydroxybenzoic acid
Sinapic acid	223	58	208	12	164	15	10.15	Sinapic acid
Syringic acid	197	62	181	12	153	11	7.68	Syringic acid
Taxifolin	303	70	285	10	125	19	10.6	Catechin
Trimers	685	60	577	40	291	40	8	Catechin
Tyrosol	137	69	118	21	106	15	5.22	Tyrosol
Vanillic acid	167	58	152	13	123	10	6.78	Vanillic acid

Table S3. Yield of the bioproduction of (+)-catechin and (–)-epicatechin monoglucuronides. C5G = (+)-catechin-5-O- β -glucuronide. C7G = (+)-catechin-7-O- β -glucuronide. C3'G = (+)-catechin-3'-O- β -glucuronide. C4'G = (+)-catechin-4'-O- β -glucuronide. E5G = (–)-epicatechin-5-O- β -glucuronide. E7G = (–)-epicatechin-7-O- β -glucuronide. E3'G = (–)-epicatechin-3'-O- β -glucuronide. E4'G = (–)-epicatechin-4'-O- β -glucuronide. ND = Not determined.

Parent compound	Total starting material (mg)	Monoglucuronide	Yield (mg)	Purity (%)
(+)–catechin	36.3	C5G	0.9	69
		C7G	5.1	97
		C3'G	ND	ND
		C4'G	ND	ND
(–)-epicatechin	39.9	E5G	0.5	92
		E7G	8.3	99
		E3'G	0.3	69
		E4'G	ND	ND

Table S4. Statistical analyzes after quantification of polyphenol metabolites in human plasma following Memophenol™ consumption. Metabolites were quantified by UHPLC-DAD-MS/MS in negative SRM mode. P-value derived from linear mixed model on log10 (metabolite+1000) followed by Tukey post-hoc comparison when the interaction time*product <0,05. SE: Standard error.

																If Time*Product effect < 0,05			If Product effect < 0,05		
Compound	Placebo				Memophenol™ 300 mg				Memophenol™ 600 mg							Control		300 mg	Control		300 mg
	T1		T2		T1		T2		T1		T2		P-value*			300 mg	600 mg	600 mg	300 mg	600 mg	600 mg
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Time	Product	Time* Product	Tukey post-hoc at T2			Tukey post-hoc		
3-hydroxyphenylacetic acid	274.70	73.93	236.22	72.28	271.71	69.53	166.51	44.43	280.53	71.48	238.36	60.27	0.01	0.3	0.4						
4-hydroxyphenylacetic acid	295.30	146.03	217.80	113.14	181.06	84.73	138.82	64.92	207.75	95.79	142.29	64.63	0.04	0.06	0.9						
3-O-methylgallic acid	1.07	1.07	3.94	2.71	1.16	1.00	11.45	5.89	2.81	2.81	14.94	12.07	0.07	0.5	0.7						
4-O-methylgallic acid	0.00	0.00	0.00	0.00	0.00	0.00	3.24	2.29	0.00	0.00	12.00	6.06	0.02	0.06	0.06						
Benzoic acid-4-sulfate	2744.07	939.04	2974.21	1473.89	2046.77	776.91	2616.18	1127.28	1923.48	714.74	1638.73	762.37	0.3	0.5	0.7						
Caffeic acid	13.65	6.62	5.73	2.84	8.93	4.16	4.50	2.24	14.76	6.53	6.43	2.72	0.02	0.5	0.8						
Catechin	0.00	0.00	0.52	0.52	2.56	2.56	0.00	0.00	0.00	0.00	4.40	2.63	0.5	0.4	0.07						
Chlorogenic acid	0.95	0.72	0.68	0.48	0.94	0.94	0.74	0.74	1.38	1.38	0.70	0.70	0.2	0.8	0.8						
Dihydrocaffeic acid-sulfate	67.84	21.85	49.27	17.49	31.25	7.79	20.82	7.10	114.70	46.66	61.82	20.43	0.07	0.004	0.6				0.1	0.3	0.002
Epicatechin	1.04	0.76	2.53	1.60	4.81	3.18	5.43	2.22	0.66	0.66	5.23	3.52	0.1	0.2	0.6						
Epicatechin-3'-glucuronide	0.00	0.00	16.91	16.91	0.00	0.00	72.27	17.99	0.00	0.00	124.09	23.37	<0.0001	0.0003	0.0003	0.02	<0.0001	0.07			
Epigallocatechine-glucuronide	348.52	37.46	340.43	32.78	371.31	36.43	386.83	61.51	509.29	165.49	479.96	156.27	0.8	0.2	0.9						
Ferulic acid 4-sulfate	34518.52	11903.24	25343.37	7071.60	24334.07	8646.40	21967.93	7041.83	23139.68	5710.33	18394.55	3852.80	0.2	0.7	0.8						
Hippuric acid	933.48	770.64	476.53	343.81	581.66	436.55	205.06	205.06	1632.68	706.54	196.87	196.87	0.04	0.4	0.2						
Hydroxyphenylacetic acid-sulfate	500.02	500.02	0.00	0.00	0.00	0.00	0.00	0.00	624.38	624.38	312.65	312.65	0.3	0.2	0.6						
Methylcatechol-sulfate	0.00	0.00	0.00	0.00	0.00	0.00	829.44	829.44	0.00	0.00	0.00	0.00	0.3	0.4	0.4						
Salicylic acid	2486.05	932.04	1703.32	401.24	2051.09	483.26	1910.63	569.52	1924.56	430.64	1450.87	359.65	0.05	0.4	0.8						
5-(3',4',5'-trihydroxyphenyl)-gamma-valerolactone	3649.49	2503.85	0.00	0.00	5054.00	3487.99	8121.40	4573.90	1944.25	1944.25	5335.54	3741.39	0.9	0.3	0.2						
5-(x-hydroxyphenyl)-gamma-valerolactone-O-sulfate	5757.99	2079.37	2938.06	1106.79	7745.26	3401.62	4822.79	2448.50	10222.33	5270.14	5950.76	2725.73	0.07	0.9	0.9						
5-phenyl-gamma-valerolactone-O-sulfate	472.14	338.14	102.34	70.51	1393.98	911.34	1035.86	705.47	856.79	752.05	467.01	415.42	0.3	0.1	0.9						