

SUPPLEMENTARY INFORMATION

Phenyl- γ -valerolactones and phenylvaleric acids, the main colonic metabolites of flavan-3-ols: synthesis, analysis, bioavailability, and bioactivity

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Supplementary Table S1. Summary of analytical methods for the analysis of PVLs and PVAs in biological samples. ET means enzymatic treatment; LD, limits of detection; AS, available standards; GIT, gastrointestinal tract; VL, γ -valerolactone; and VA, valeric acid.

Analytical Techniques	Food Intake	Fluid/Tissue	Separation	ET	Identified Metabolites	LD	AS	Refs.
<i>Animals</i>								
HPLC-colourimetric electrochemical array detection	(-)-Epicatechin at different doses	Urine	WAKOPAC 3C18 column (150 x 4.6 mm, 3 μ m)	Yes	5-(3',4'-dihydroxyphenyl)VL	-	Yes	46
HPLC-MS/MS	Catechin, dimer B3, trimer C2 and mixed polymers of catechin	Urine	Hypersil BDS C18 (150 x 2.1 mm, 5 μ m)	Yes	5-(3',4'-dihydroxyphenyl)VA; 5-(3'-hydroxyphenyl)VA	0.05-6 μ M	Yes	45
HPLC-MS/MS	Soluble cocoa powder	Urine	Luna C18 column (50 x 2.0 mm, 5 μ m)	Yes	5-(3',4'-dihydroxyphenyl)VL; 5-(4'-hydroxyphenyl)VL-3'-methoxy	-	No	49
UHPLC-MS/MS	Nuts-cocoa cream	Large intestine	Acquity HSS T3 column (100 x 2.1 mm, 1.8 μ m)	No	5-(3',4'-dihydroxyphenyl)VL; 5-(hydroxyphenyl)VL	-	No	170
UHPLC-MS/MS	Catechin and procyanidin rich grape extract	Cecum, proximal and distal colon	Acquity HSS T3 column (100 x 2.1 mm, 1.8 μ m)	No	5-(3',4'-dihydroxyphenyl)VL; 4-hydroxy-5-(3',4'-dihydroxyphenyl)VA	-	No	174
UHPLC-TOF-MS/MS	Grape seed proanthocyanidin extract	Plasma	Zorbax SB-Aq Column (150 x 2.1 mm, 3.5 μ m)	No	5-(3',4'-dihydroxyphenyl)VL	14.4 nM	Yes	171
UHPLC-Q-Orbitrap-HRMS	Partially purified cranberry or apple procyanidins	Plasma	Ace Excel 2 C18-PFP column (100 x 2.1 mm, 2 μ m)	No	5-(3',4'-dihydroxyphenyl)VA; 4-hydroxy-5-phenylVA-sulphate; 5-phenylVL-sulphate	-	No	153
HPLC-RC-MS ²	[2- ¹⁴ C](-)-Epicatechin	Tissues from the GIT, plasma, urine and faeces	Luna Phenyl Hexyl Column (250 x 4.6 mm, 5 μ m)	No	5-(3',4'-dihydroxyphenyl)VL; 5-(4'-hydroxyphenyl)VL-3'-sulphate; 4-hydroxy-5-(3',4'-dihydroxyphenyl)VA; 4-hydroxy-5-	-	Yes	93

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Analytical Techniques	Food Intake	Fluid/Tissue	Separation	ET	Identified Metabolites	LD	AS	Refs.
					(3'-hydroxyphenyl)VA-4'-glucuronide; 4-hydroxy-5-(4'-hydroxyphenyl)VA; among other minor derivatives			
UPLC-DAD-ESI-IT-TOF-MS ⁿ	Procyanidin B2	Tissues, plasma and urine	HR-ODS Column (150 x 2.1mm, 3µm)	No	45 metabolites tentatively identified	-	No	173
UHPLC-Orbitrap-HRMS	Red wine proanthocyanidin extract	Urine, plasma and faeces	Kinetex Phenyl-Hexyl 100 A (150 x 4.6 mm, 2.6 µm)	No	5-(3',4'-dihydroxyphenyl)VL; 5-(hydroxyphenyl)VL; 5-phenylVL-3'-sulphate; 5-phenylVL-3'-glucuronide	1 to 670 nM	Yes	65
<i>Humans</i>								
HPLC-coulorimetric electrochemical array detection	Green tea	Plasma and urine	NBS C18 column (150 x 4.6 mm, 5 µm)	Yes	5-(3',4',5'-trihydroxyphenyl)VL; 5-(3',4'-dihydroxyphenyl)VL	-	Yes	43
HPLC-coulorimetric electrochemical array detection	Flavan-3-ol monomers or procyanidins or flavan-3-ols and procyanidins	Urine	Luna C18 (150 x 4.6 mm, 3 µm)	Yes	5-(3',4'-dihydroxyphenyl)VL	4-6 pmol	Yes	104
HPLC-coulorimetric electrochemical array detection	Green tea	Urine and plasma	Alltima C18 column (53 x 7 mm, 5 µ)	Yes	5-(3',4',5'-trihydroxyphenyl)VL; 5-(3',4'-dihydroxyphenyl)VL	-	No	123
HPLC-MS/MS	Green tea	Urine	Gemini C18 column (50 x 2.0 mm, 3 µm)	No	5-(3',4',5'-trihydroxyphenyl)VL; 5-(3',4'-dihydroxyphenyl)VL; glucuronides and sulphates thereof	-	Yes	16
HPLC-MS/MS	Soluble cocoa powder	Urine	Luna C18 column (50 x 2.0 mm, 5 µm)	Yes	5-(3',4'-dihydroxyphenyl)VL; 5-(4'-hydroxyphenyl)VL-3'-methoxy	-	No	49
HPLC-MS/MS	Almond skin extract	Urine and	Luna C18 column	No	PVL conjugates	-	No	17

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Analytical Techniques	Food Intake	Fluid/Tissue	Separation	ET	Identified Metabolites	LD	AS	Refs.
HPLC-MS/MS and GC-MS/MS	Pure epicatechin, procyanidin B1 and polymeric procyanidin fraction from cocoa	Plasma, urine and faeces	(50 x 2.0 mm, 5 µm) PerfectSIL Target ODS-3 column (250 x 3mm, 3µm) and DB-5MS column (30m x 0.25 mm i.d., 0.25 µm)	Yes	5-(3',4'-dihydroxyphenyl)VL; 5-(hydroxyphenyl)VL-methoxy; 4-hydroxy-5-(3',4'-dihydroxyphenyl)VA	-	No	107
HPLC-FTMS ⁿ and HPLC-TOF-MS-NMR	Green tea	Urine	Luna C18 column (150 x 2.0 mm, 3 µm)	No	48 PVL and PVA conjugates	-	No	54
UHPLC-MS/MS	Green tea	Urine	Kinetex EVO C18 column (100x 2.1 mm, 2.6 µm)	No	12 PVL derivatives (aglycones, glucuronides and sulphates)	0.2 to 1113 nM	Yes	63
UHPLC-Q-Orbitrap-HRMS	Grape pomace	Urine	Luna C18 column (50 x 2.0 mm, 5 µm)	No	5-(3',4'-dihydroxyphenyl)VL; 4-hydroxy-5-(3',4'-dihydroxyphenyl)VA	-	No	116
UHPLC-TOF-MS/MS	Cranberry juice	Plasma and urine	Zorbax Eclipse Plus RRHD column (50 x 2.1mm, 1.8 µm)	No	5-(3'-hydroxyphenyl)VL-4'-sulphate	0.07 nM	Yes	172
HPLC-MS/MS and HPLC-RC-MS/MS	[2- ¹⁴ C](-)-Epicatechin	Plasma, urine and faeces	Luna Phenyl Hexyl Column (250 x 4.6 mm, 5 µm)	No	3 5-(hydroxyphenyl)VLs; 3 5-(dihydroxyphenyl)VLs; 1 4-hydroxy-5-(hydroxyphenyl)VA; 3 4-hydroxy-5-(dihydroxyphenyl)Vas. All phase II conjugates	-	Yes	2
UHPLC-Q-Orbitrap-HRMS	Cranberry juice	Plasma	Ace Excel 2 C18-PFP column (100 x 2.1mm, 2µm)	No	5-(3',4',5'-trihydroxyphenyl)VL	-	No	130

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Analytical Techniques	Food Intake	Fluid/Tissue	Separation	ET	Identified Metabolites	LD	AS	Refs.
HPLC-qTOF-MS	Grape skin extract	Urine	Luna RP 18 (50 x 2.0 mm, 5 µm)	No	7 PVL and PVA phase II conjugates	-	No	127
<i>Faeces</i>								
HPLC-MS/MS	(-)-Epicatechin and (+)-catechin	Faecal incubates	Nova-Pak C18 column (250 x 4.6 mm, 4 µm)	No	5-(3',4'-dihydroxyphenyl)VL; 5-phenylVL	-	No	147
HPLC-LCT-TOF-ESI	Procyanidin dimer	Faecal incubates	Inertsil ODS-3 (4.6 x 150 mm, 5 µm)	No	5-(3',4'-dihydroxyphenyl)VL; 5-(hydroxyphenyl)VL; 5-(3',4'-dihydroxyphenyl)VA; 5-(hydroxyphenyl)VA	-	No	48
UHPLC-MS/MS	Catechin, epicatechin, epicatechin gallate, epigallocatechin gallate and dimer B2	Faecal incubates	Acquity HSS T3 column (100 x 2.1 mm, 1.8 µm)	No	5-(3',4'-dihydroxyphenyl)VL; 5-(hydroxyphenyl)VL	-	No	170
UPLC-TQ MS	Two grape seed flavan-3-ol fractions	Faecal incubates	BEH C18 column (100 x 2.1 mm, 1.7 µm)	No	5-(3',4'-dihydroxyphenyl)VL; 4-hydroxy-5-(3',4'-dihydroxyphenyl)VA	-	No	118
UPLC-TQ MS	Grape seed polyphenols	Faecal incubates	BEH C18 column (100 x 2.1 mm, 1.7 µm)	No	5-(3',4'-dihydroxyphenyl)VL; 4-hydroxy-5-(3',4'-dihydroxyphenyl)VA; 5-(3'-hydroxyphenyl)VL; 4-hydroxy-5-(3'-hydroxyphenyl)VA; 4-hydroxy-5-phenylVA	0.005-0.09 µg/mL	Yes	141
UPLC-TQ MS	Grape seed polyphenols	Faecal incubates	BEH C18 column (100 x 2.1 mm, 1.7 µm)	No	5-(3',4'-dihydroxyphenyl)VL	0.003 µg/mL	Yes	53

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Supplementary Table S2. Mass spectrometric characteristics of PVL reference standards as assessed by using validated LC-MS methods in negative mode. Frag. Pat. means Fragmentation Pattern; No frag., no fragment available.

Metabolite	Molecular Formula	Triple quadrupole (MS/MS)					HRMS		Refs.
		Parent Ion (<i>m/z</i>)	Quantifier		Qualifier		Accurate Mass [<i>m/z</i>] ⁻	Frag. Pat.	
			Product ion (<i>m/z</i>)	CE (V)	Product ion (<i>m/z</i>)	CE (V)			
<i>5-(Trihydroxyphenyl)-γ-valerolactone derivatives</i>									
5-(3',4',5'-Trihydroxyphenyl)-γ-valerolactone	C11H12O5	223	179	21	138	26			63
<i>5-(Dihydroxyphenyl)-γ-valerolactone derivatives</i>									
5-(3',4'-Dihydroxyphenyl)-γ-valerolactone	C11H12O4	207	163	20	122	25	207.0651	No frag.	53,63,65,116
5-(3',5'-Dihydroxyphenyl)-γ-valerolactone	C11H12O4	207	163	18	123	20	207.0662		63,116
5-(3'-Hydroxyphenyl)-γ-valerolactone-4'-sulphate	C11H12O7S	287	207	23	163	34	287.0231		63,172
5-(4'-Hydroxyphenyl)-γ-valerolactone-3'-sulphate	C11H12O7S	287	207	23	163	35	287.0219	207.0651	63,65,173
5-(5'-Hydroxyphenyl)-γ-valerolactone-3'-sulphate	C11H12O7S	287	207	23	163	35			63,173
5-(5'-Hydroxyphenyl)-γ-valerolactone-3'-glucuronide	C17H20O10	383	207	24	163	40	383.097	207.0651	63,65
5-(3'-Hydroxyphenyl)-γ-valerolactone-4'-glucuronide	C17H20O10	383					383.097	207.0651	65
5-Phenyl-γ-valerolactone-3',4'-disulphate	C11H12O10S2	367	287	12	207	33			63
<i>5-(Hydroxyphenyl)-γ-valerolactone derivatives</i>									
5-(3'-Hydroxyphenyl)-γ-valerolactone	C11H12O3	191	147	20	106	31	191.0702	No frag.	63,65
5-(4'-Hydroxyphenyl)-γ-valerolactone	C11H12O3	191	147	13	106	31			63
5-Phenyl-γ-valerolactone-3'-sulphate	C11H12O6S	271	191	23	106	48	271.027	191.0702	65
5-Phenyl-γ-valerolactone-4'-sulphate	C11H12O6S	271	191	23	147	35			63
5-Phenyl-γ-valerolactone-3'-glucuronide	C17H20O9	367	191	25	147	43	367.1023	191.0702	63,65
5-Phenyl-γ-valerolactone-4'-glucuronide	C17H20O9	367					367.1023	191.0702	65