ELECTRONIC SUPPLEMENTARY INFORMATION

Bioavailability of red wine and grape seed proanthocyanidins in rats

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1. Identification of Plasma, Fecal and Urinary Metabolites

Targeted identifications of (–)-epicatechin metabolites, phenyl-γ-valerolactones, phenylvaleric acids and other phenolics were achieved as follows: (i) by comparing the exact mass and the retention time with available standards, (ii) in the absence of standards, compounds were tentatively identified by comparing the theoretical exact mass of the molecular ion with the measured accurate mass of the molecular ion. The HPLC-HRMS and UHPLC-ESI-MS^n based identifications of structurally-related epicatechin metabolites (SREMs), and the 5 carbon side chain ring fission metabolites (5C-RFMs) phenyl-γ-valerolactones and phenylvaleric acids in urine, plasma and feces collected 0-24 h after rats were fed with a wine or grape seed proanthocyanidin extract are shown in Tables S1 and S2. Moreover, the HPLC-HRMS-based identifications of phenolic acid catabolites excreted in urine, plasma and feces collected 0-24 h after rats were fed with a wine or grape seed proanthocyanidin extract is shown in Table S3. Details of the identification are presented below:

1.1 Unmetabolized parent flavan-3-ols

Peaks M1, M2 and M3 (Rts 28.3, 21.8 and 29.4 mins) co-chromatographed with and had the same negative accurate mass at *m/z* 289.0708 (0.46 ppm) as (–)-epicatechin and at *m/z* 577.1348 (0.51 ppm) as procyanidin B1 and B2 standards, respectively.

1.2 Structurally-related (–)-(epi)catechin metabolites

Peaks M4 and M5 (19.2 and 23.5 min) had negative accurate masses at *m/z* 465.1024 (–0.75 ppm), which yielded an ion at *m/z* 289.0708 (0.46 ppm) [(–)-epicatechin]. The loss of 176.0316 Da (glucuronide ion) indicated both these metabolites were (epi)catechin-glucuronides derived from (–)-epicatechin and/or (+)-catechin. Borges *et al.*[1] identified (–)-epicatechin-5-glucuronide and (–)-epicatechin-7-glucuronide in plasma of rats obtained after feeding [^{14}C](–)-epicatechin. (–)-Epicatechin was used as reference compound for the quantification of peaks M4 and M5.

Peak M6 (31.8 min) had a negative mass at *m/z* 303.0864 (0.28 ppm), characteristic of a methoxy-(epi)catechin. The Borges *et al*. study[1] previously identified 3'-methoxy-(–)-epicatechin in rat plasma. (–)-Epicatechin was used as reference compound for the quantification of peak M6.

Peak M7 (33.9 min) had a negative accurate mass at *m/z* 383.0430 (–0.33 ppm) which yielded ion at *m/z* 303.0864 (0.28 ppm) (corresponding to methoxy-(epi)catechin) at low collision energy. The losses of 79.9566 Da, indicate cleavage of a sulfate group. This metabolite is a methoxy-(–)-(epi)catechin-sulfate. 3'-Methoxy-(–)-epicatechin-5-sulfate has been identified in plasma after ingestion of [^{14}C](–)-epicatechin by rats.[1] (Epi)catechin was used as reference compound for the quantification of peak M7.

Peaks M8-M9 (29.1 and 32.4 min) had a negative accurate mass at *m/z* 479.1180 (-0.84 ppm), yielding at low collision energy ions at *m/z* 303.0864 (0.28 ppm) (a methylated epicatechin) and at *m/z* 465.1033 (-0.75 ppm) (corresponding to an (–)-epicatechin-glucuronide). In keeping with the losses of 176.0316 Da (glucuronide moiety) and 14.0145 Da (methyl moiety). Both metabolites are, therefore, methoxy-(epi)catechin-glucuronides. 3'-Methoxy-(–)-epicatechin-5-glucuronide and 3'-methoxy-(–)-epicatechin-7-glucuronide are plasma metabolites of (–)-epicatechin in rats.[1] (–)-Epicatechin was used as reference compound for the quantification of peaks M8 and M9.

1.3 *5-(Phenyl)-γ-valerolactones*

Peaks M10 (Rt 29.1 min) had a negative accurate ion at *m/*z 399.0902 (–0.25 ppm) which under low collision energy produced a fragment at *m/z* 223.0601 (0.21 ppm). This indicates the presence of 5-(3',4',5'-trihydroxyphenyl)-γ-valerolactone metabolites derived from a galloylated subunit of prodelphinidin. The loss of 176.0301 Da indicated that this compound was 5-(dihydroxyphenyl)-γ-valerolactone-glucuronide. The MS^n analysis has confirmed this identification. 5-(3',4'-Dihydroxyphenyl)-γ-valerolactone was used as reference compound for the quantification of peak M10.

Peak M11 (Rt 28.8 min) co-chromatographed with and had the same negative accurate mass at *m/z* 207.0649 (–3.38 ppm) as a 5-(3',4'-dihydroxyphenyl)-γ-valerolactone standard. This compound, typical ring fission catabolite of procyanidins, has been identified in human urine after green tea[2] and grape extract[3] consumption.

Peak M12 (Rt 32.2 min) had a negative accurate mass at *m/z* 287.0219 (0.0 ppm) and a fragment ion at *m/z* 207.0651 (3.41 ppm) (corresponding to 5-(3',4'-dihydroxyphenyl)-γ-valerolactone). The

loss of 79.9517 Da, indicative of cleavage of a sulfate group. This metabolite was confirmed by MS^n. This metabolite is, therefore, a 5-(hydroxyphenyl)-γ-valerolactone-sulfate. On the basis the data of Borges et al. (2016) it is probably 5-(4'-hydroxyphenyl)-γ-valerolactone-3'-sulfate. This metabolite has also been identified in human urine after green tea[2] and (–)-epicatechin intake.[4] 5-(3',4'-dihydroxyphenyl)-γ-valerolactone was used as reference compound for the quantification of peak M12.

Peaks M13 and M14 (Rts 28.5 and 29.2 min) both yielded an *m/z* at 383.0970 (–0.52 ppm) which fragmented producing an ion at *m/z* 207.0651 (3.41 ppm) [5-(dihydroxyphenyl)-γ-valerolactone)]. The loss of 176.0319 Da, indicates cleavage of a glucuronide moiety. The fragmentation pattern obtained by UHPLC-ESI-MS^n confirms the results obtained by HPLC-HRMS analysis. The elution order and the findings of Borges *et al.*[1] and Xiao *et al.*[5] are in keeping with M13 being 5-(3'-hydroxyphenyl)-γ-valerolactone-4'-glucuronide and M14 5-(4'-hydroxyphenyl)-γ-valerolactone-3'-glucuronide. 5-(3',4'-Dihydroxyphenyl)-γ-valerolactone was used as reference compound for the quantification of peaks M13 and M14.

Peak M15 (Rt 28.6 min) had a negative accurate mass at *m/z* 463.0540 (0.0 ppm) which yielded a fragment at *m/z* 287.0219 (0.0 ppm) (corresponding to 5-(hydroxyphenyl)-γ-valerolactone-sulfate). The loss of 176.0321 Da and the MS^n analyses demonstrates cleavage of a glucuronide unit, allowed the putative identification of this metabolite as a 5-(phenyl)-γ-valerolactone-glucuronide-sulfate. 5-(3',4'-Dihydroxyphenyl)-γ-valerolactone was used as reference compound for the quantification of peak M15.

Peaks M16 and M17 (Rt 35.1 and 36.5 min) had negative accurate masses at *m/z* 301.0377 (0.33 ppm), in keeping with these metabolites being 5-(methoxyphenyl)-γ-valerolactone-sulfate isomers. The fragmentation pattern obtained by UHPLC-ESI-MS^n confirmed this possibility.

Peaks M18 and M19 (Rt 29.6 and 32.6 min) had negative accurate masses at *m/z* 397.1126 (–0.75 ppm), which yielded a fragment at *m/z* 207.0651 (3.41 ppm) in keeping with these two metabolites being 5-(methoxyphenyl)-γ-valerolactone-glucuronide isomers. It is interesting to note that peaks M16 to M19 were detected previously in rat urine/plasma/feces after ingestion of [2-^{14}C]-(–)-epicatechin.[1] Peaks M16-M19 were quantified by reference to 5-(3',4'-dihydroxyphenyl)-γ-valerolactone.

Peak M20 (Rt 39.5 min) co-chromatographed with and had the same negative accurate mass at *m/z* 191.0702 (2.09 ppm) as a 5-(3'-hydroxyphenyl)-γ-valerolactone standard. This metabolite has been identified in human urine after green tea consumption.[2] Peak M20 was quantified using a 5-(3',4'-dihydroxyphenyl)-γ-valerolactone standard.

Peak M21 (Rt 36.0 min) had a negative accurate mass at *m/z* 271.0277 (0.36 ppm) and in keeping with its mass spectral properties co-chromatographed with a 5-(phenyl)-γ-valerolatone-3'-sulfate standard.

Peaks M22 and M23 (Rts 30.4 and 32.3 min) had negative accurate masses at *m/z* 367.1021 (-0.27 ppm) which yielded a fragment at *m/z* 191.0702 (0.36 ppm). The loss of 176.0319 Da allowed the tentatively identification of these peaks as two 5-(phenyl)-γ-valerolactone-glucuronide isomers. Besides, the fragmentation pattern obtained by UHPLC-ESI-MS^n confirms the results obtained by HPLC-HRMS analysis. In view of the elution order, peak M22 is likely to be 4'-glucuronide and peak M23 3'-glucuronide. The identity of peak M23 was confirmed with a reference compound. Both peaks were quantified based on the calibration curve of 5-(phenyl)-γ-valerolactone-3'-glucuronide.

1.4 *4-Hydroxy-5-(phenyl)valeric acids*

Peak M24 (Rt 18.3 min) produced a negative accurate mass at *m/z* 225.0756 (–0.66 ppm) which yielded a fragment at *m/z* 207.0651. Further, the fragmentation pattern obtained by MS^n analysis allowed us to identify this metabolite as 4-hydroxy-5-(3',4'-dihydroxyphenyl)valeric acid. Peak M24 was quantified by reference to a 5-(3',4'-dihydroxyphenyl)-γ-valerolactone standard.

Peaks M25 (Rt 26.8 min) had negative accurate masses at *m/*z 305.0327 (0.44 ppm), yielding a fragment at *m/z* 225.0756 (–0.66 ppm) (corresponding to a 4-hydroxy-5-(dihydroxyphenyl)valeric acid). The loss of 79.9571 Da indicates that this peak is 4-hydroxy-5-(hydroxyphenyl)valeric acid-sulfate. The fragmentation pattern by MS^n analysis confirmed the identity of this metabolite, which has been identified previously in mice.[5] Peak M25 was quantified based on a 5-(phenyl)-γ-valerolactone-3'-glucuronide reference compound.

Peaks M26 and M27 (Rt 20.3 and 21.7 min) yielded a negative accurate mass at *m/z* 401.1075 (-0.84 ppm) and a fragment ion at *m/*z 225.0756 (–0.66 ppm). The loss of 176.0319 Da, indicative of

cleavage of a glucuronide group, allowed the tentative identification of these peaks as two 4-hydroxy-5-(hydroxyphenyl)valeric acid-glucuronides. In view of the fragmentation pattern by MS^n and the elution order, peak M26 is likely to be the 4'-glucuronide and peak M27 the 3'-glucuronide. Peaks M26 and 27 were quantified based on 5-(phenyl)-γ-valerolactone-3'-glucuronide reference compound.

Peak M28 (Rt 32.7 min) had negative accurate masses at *m/z* 289.0378 (0.51 ppm) and, with the loss of a 79.9572 Da sulfate moiety, producing a fragment ion at *m/z* 209.0806 (–1.12 ppm). The MS^n fragmentation allowed to the tentative identification of this metabolites as 4-hydroxy-5-(phenyl)valeric acid-sulfate. 5-(Phenyl)-γ-valerolactone-3'-glucuronide was used for quantification of peak M28.

1.5 *5-(Phenyl)valeric acids*

Peak M29 (Rt 36.5 min) presented a negative accurate mass at *m/z* 209.0806 (–1.12 ppm) in keeping with the presence of a 4-hydroxy-5-(hydroxyphenyl)valeric acid or 5-(3',4'-dihydroxyphenyl)valeric acid. In view of the MS^n fragmentation pattern and the elution order, peak M29 is likely to be 5-(3',4'-dihydroxyphenyl)valeric acid. 5-(Phenyl)-γ-valerolactone-3'-glucuronide was used as a reference compound for quantification of peak M29.

Peaks M30 (Rt 35.8 min) had negative accurate masses at *m/z* 289.0378 (0.51 ppm) and, with the loss of a 79.9572 Da sulfate moiety, producing a fragment ion at *m/z* 209.0806 (–1.12 ppm). Together with the MS^n fragmentation, this indicates this metabolite is 5-(hydroxyphenyl)valeric acid-sulfate. 5-(Phenyl)-γ-valerolactone-3'-glucuronide was used for quantification of peak M30.

Peak M31 (Rt 30.4 min) had a negative accurate mass at *m/z* 385.1127 (–0.57 ppm) which with the loss of a 176.0321 Da glucuronide unit yielded a fragment at *m/z* 209.0806 (–1.12 ppm). This is in keeping with the MS^n fragmentation being a 5-(hydroxyphenyl)valeric acid-glucuronide. 5-(Phenyl)-γ-valerolactone-3'-glucuronide was used for quantification of peak M31.

Peak M32 (Rt 40.2 mins) presented negative accurate masses at *m/z* 399.1284 (-0.43 ppm). Upon low collision energy this peak gave a fragment at *m/z* 223.0969 (–0.57ppm) and 209.0807 (–1.12 ppm) which corresponded to a 5-(hydroxy-methoxyphenyl)valeric acid and 5-(dihydroxyphenyl)valeric acid. The loss of 14.0094 Da (methyl group) and 176.0383 Da (glucuronic

moiety) and the MS^n fragmentation pattern partially identified this peak as a 5-(methoxyphenyl)valeric acid-glucuronide. 5-(Phenyl)-γ-valerolactone-3'-glucuronide was used for quantification of peak M32.

Peak M33 (Rt 58.0 min) had a negative accurate mass at *m/z* 193.0860 (0.41 ppm) which is indicative of it being a 5-(hydroxyphenyl)valeric acid. *Peaks M34 and M35* (Rts 50.1 and 46.1 mins) had negative accurate masses at *m/z* 273.0430 (0.96 ppm) and 369.1180 (−0.02 ppm), respectively. Both peaks yielded upon low collision energy fragments at *m/z* 193.0860 (0.41 ppm). The losses of 79.9570 Da and 176.0320 Da indicated sulfate and glucuronic moieties. In keeping with the MS^n fragmentation pattern, peaks M34 and M35 were tentatively identified as a 5-(phenyl)valeric acid-sulfate and a 5-(phenyl)valeric acid-glucuronide, respectively. 5-(Phenyl)-γ-valerolactone-3'-glucuronide was used as a reference compound for quantification of peaks M33-35.

1.6 Cinnamic acids

Peaks C1-C9. Two were free acids, 3'-hydroxycinnamic acid (C1) and 4'-hydroxy-3'-methoxycinnamic acid (C6) and the remaining seven were phase II metabolites. All were identified by comparing the exact mass and the retention time with authentic standards, except peaks C3 and C4 which presented negative accurate masses at *m/z* 242.9952 (2.40 ppm). Both these metabolites yielded low collision energy fragments at *m/z* 163.0386 (2.1 ppm) (corresponding with a hydroxycinnamic acid). The loss of 79.9566 Da (sulfate group) suggested these two peaks were cinnamic acid-sulfate isomers. In view of the elution order peak C3 is tentatively identified as the 4'-sulfate and peak C4 the 3'-sulfate.

1.7 Phenylpropanoic acids

Peaks C10-C21. Among them, five free acids and seven glucuronide and sulfate metabolites. Peaks *C10-C15* and *C17-C21* were identified based on comparisons with authentic standards. Peak C16 had a negative accurate mass at *m/z* 275.0218 (0.72 ppm), which yielded an ion at *m/z* 195.0652 (0.89 ppm) (corresponding to a standard of 3-(3',4'-dihydroxyphenyl)propanoic acid) upon low collision energy. The loss of 79.9566 Da (sulfate ion) tentatively identified this catabolite as a 3-(methoxyphenyl)propanoic acid-sulfate.

1.8 Phenylacetic acids

Peaks C22-C30. Peaks *C22*, *C23*, *C26* and *C27* were identified by reference to standards and comprised a range of free phenolic acids. Peak *C2*4 had a negative accurate mass at *m/z* 357.0805 (3.14 ppm), yielding a fragment ion at *m/z* 181.0492 (2.56 ppm) (methoxy-hydroxyphenylacetic acid moiety). The loss of 176.0313 Da indicates that this catabolite as a methoxyphenylacetic acid-glucuronide with the glucuronide moiety at either the 3'- or 4'-position. Peak *C25* had a negative exact mass at *m/z* 261.0062 (0.57 ppm) and a fragment at *m/z* 181.0491 (2.56 ppm), was a further loss of 79.9571 Da, indicating that this compound is a methoxyphenylacetic acid-sulfate. *Peak C28* had a negative accurate mass at *m/z* 327.0699 (3.36 ppm) which yield a fragment at *m/z* 151.0384 (3.97 ppm) (corresponding to an hydroxyphenylacetic acid structure). The loss of a glucuronic acid moiety (176.0315 Da) indicates that this metabolite is a phenylacetic acid-glucuronide. *Peaks C29* and *C3*0 had negative accurate masses at *m/z* 230.9956 (0.43 ppm) giving a fragment ion at *m/z* 151.0385 (3.97 ppm) (corresponding to an hydroxyphenylacetic acid) upon low collision energy. The loss of 79.9571 Da, is cleavage of a sulfate group, indicating that these two metabolites are phenylacetic acid-sulfate isomers. In view of the elution order, peak *C29* is likely to be the 4'-sulfate and peak *C30* the 3'-sulfate.

1.9 Benzoic acids

Peaks C31-C36. Peaks *C32* and *C33* were free acids, respectively, 4-hydroxybenzoic acid and 3-hydroxybenzic acid, which were identified based on a comparison with authentic standards. Peak *C31* with a negative accurate mass at *m/z* 232.9743 (3.00 ppm), which yielded a fragment at *m/z* 153.0177 (loss of 79.9566 Da) corresponding to a standard of 3,4-dihydroxybenzoic acid and partially identifying peak *C31* as a benzoic acid-sulfate. Peaks *C34* and *C35* had negative exact masses at *m/z* 216.9799 (0.92 ppm) and at *m/z* 313.0542 (3.83 ppm), respectively, yielding fragments at *m/z* 137.0230 (4.37 ppm) (corresponding to an hydroxybenzoic acid), with the loss of 79.9569 Da (sulfate group) and 176.0312 Da (glucuronide group), facilitating the tentative identification of peaks *C34-C35*, respectively, as a benzoic acid-sulfate and a benzoic acid-glucuronide. Likewise, peak *C36* was tentatively identified as a methoxybenzoic acid-sulfate on the basis of its negative accurate mass at *m/z* 246.9906 (−0.27 ppm).

1.10 Hydroxycarboxylic acids and benzene derivatives

Peaks C37 and *C38* co-chromatographed with and, respectively, had the same mass spectrum as 4'-hydroxymandelic acid and 1,3,5-trihydroxybenzene (phloroglucinol). The remaining peaks, *C39-C48*, were partially identified on the basis of their fragmentation patterns as sulfated and glucuronidated hydroxybenzene and dihydroxybenzene catabolites.

1.11 Benzoylglycine derivatives

Peaks C49 and *C50* co-chromatographed with and, respectively, had the same mass spectra as 4'-hydroxyhippuric acid and hippuric acid.

2. Quantification of metabolites and catabolites

4'-Hydroxycinnamic acid-3'-glucuronide, 3'-methoxycinnamic acid-4'-sulfate, 3'-methoxycinnamic acid-4'-glucuronide, 4'-methoxycinnamic acid-3'-glucuronide, 3-(3'-methoxy-4'-hydroxyphenyl)propanoic acid, 3-(3'-methoxyphenyl)propanoic acid-4'-sulfate, 3-(3'-methoxyphenyl)propanoic acid-4'-glucuronide, 3-(4'-methoxyphenyl)propanoic acid-3-sulfate, 3-(3'-hydroxyphenyl)propanoic acid, 3-(4'-hydroxyphenyl)propanoic acid, 3'-hydroxyphenylacetic acid and 4'-hydroxyphenylacetic acid were quantified using calibration curves prepared with reference compounds.

3-(Phenyl)propanoic acid-3'-sulfate, phenylacetic acid-4'-sulfate, phenylacetic acid-3'-sulfate, hydroxybenzene-4-sulfate and hydroxybenzene-3-sulfate were quantified using 3-(4'-hydroxyphenyl)propanoic acid-3'-sulfate. Dihydroxybenzene-sulfate was quantified using 3-(3'-hydroxyphenyl)propanoic acid-4'-sulfate. Methoxyphenylacetic acid-sulfate was quantified using 3-(3'-methoxyphenyl)propanoic acid-4'-sulfate and methoxyphenylacetic acid-glucuronide was quantified by reference to 3-(3'-methoxyphenyl)propanoic acid-4'-glucuronide. Phenylacetic acid-glucuronide was quantified using 3-(4'-hydroxyphenyl)propionic acid-3'-glucuronide.

References

1 G. Borges, J.J.J. van der Hooft, A. Crozier, comprehensive evaluation of the [2-^{14}C](−)-epicatechin metabolome in rats. Free Radic. Biol. Med. 99 (2016) 128−138.

2 N. Brindani, P. Mena, L. Calani, I. Benzie,

S.W. Choi, F. Brighenti, F. Zanardi, C. Curti, D. Del Rio, Synthetic and analytical strategies for the quantification of phenyl-γ-valerolactone conjugated metabolites in human urine, *Mol. Nutr. Food Res.,* 2017, **61** doi: org/10.1002/mnfr.201700077.

3 G. Sasot, M. Martínez-Huélamo, A. Vallverdú-Queralt, M. Mercader-Marti, R. Estruch, R.M. Lamuela-Raventós, Identification of phenolic metabolites in human urine after the intake of a functional food made from grape extract by a high resolution LTQ-Orbitrap-MS approach. *Food Res. Int.,* 2017, **100**, 435–444.

4 J. I. Ottaviani, G. Borges, T. Y. Momma, J. P. E. Spencer, C. L. Keen, A. Crozier, H. Schroeter, The metabolome of [2-^{14}C](−)-epicatechin in humans: implications for the assessment of efficacy, safety, and mechanisms of action of polyphenolic bioactives*. Sci. Rep*., 2016, **6**, 29034.

5 Y. Xiao, Z. Hu, Z. Yin, Y. Zhou, T. Liu, X. Zhou, D. Chang, Profiling and distribution of metabolites of procyanidin B2 in mice by UPLC-DAD-ESI-IT-TOF-MS^n technique. *Front. Pharmacol*.. 2017, **8**, 231 (doi: 10.3389/fphar.2017.00231).

Table S1. UHPLC-HRMS-based identifications of structurally-related epicatechin metabolites (SREMs), and the 5 carbon side chain ring fission metabolites (5C-RFMs), phenyl-γ-valerolactones and phenyl-4-hydroxyvaleric acids in urine, plasma and feces collected 0-24 h after rats were fed a grape seed proanthocyanidins extract.

Peak	Rt (min)	Flavan3-ols and Metabolites	Chemical Formula [m/z]-	Experimental mass [m/z]-	δ (ppm)	Fragments low collision energy [*m/z*]-	Location[a]	MSI MI level[b]
		Parent flavan-3-ols						
M1	28.3	(–)-Epicatechin	C15H13O6	289.0708	0.46	245.0808	U,P,F	1
M2	21.8	Procyanidin B1	C30H25O12	577.1348	0.51	425.0889; 289.0725	U,P	1
M3	29.4	Procyanidin B2	C30H25O12	577.1348	0.51	425.0889; 289.0725	U,P	1
		SREMs						
M4	19.2	(Epi)catechin-5-glucuronide	C21H21O12	465.1024	–0.75	289.0708	U,P	2
M5	23.5	(Epi)catechin-7-glucuronide	C21H21O12	465.1024	–0.75	289.0708	U,P,F	2
M6	31.8	3'-Methoxy-(epi)catechin	C16H15O6	303.0864	0.28	No fragment	U,F	2
M7	33.9	3'-Methoxy-(epi)catechin-5-sulfate	C16H15O9S	383.0430	–0.33	303.0863	U,P,F	2
M8	29.1	3'-Methoxy-(epi)catechin-5-glucuronide	C22H23O12	479.1180	–0.84	465.1024; 303.0863	U,P,F	2
M9	32.4	3'-Methoxy-(epi)catechin-7-glucuronide	C22H23O12	479.1180	–0.84	465.1024; 303.0863	U,P	2
		5C-RFMs						
		5-(Phenyl)-γ-valerolactones						
M10	29.1	5-(Dihydroxyphenyl)-γ-valerolactone-glucuronide	C17H19O11	399.0920	–0.25	223.0598	U	2
M11	28.8	5-(3',4'-Dihydroxyphenyl)-γ-valerolactone	C11H11O4	207.0649	–3.38	No fragment	F	1
M12	32.2	5-(4'-Hydroxyphenyl)-γ-valerolactone-3'-sulfate	C11H11O7S	287.0219	0.0	207.0651	U,P,F	2
M13	28.5	5-(3'-Hydroxyphenyl)-γ-valerolactone-4'-glucuronide	C17H19O10	383.0970	–0.52	207.0651	U,P,F	2
M14	29.2	5-(4'-Hydroxyphenyl)-γ-valerolactone-3'-glucuronide	C17H19O10	383.0970	–0.52	207.0651	U	2
M15	28.6	5-(Phenyl)-γ-valerolactone-glucuronide-sulfate	C17H19O13S	463.0540	0.0	287.0219	U,P	2
M16	35.1	5-(Methoxyphenyl)-γ-valerolactone-sulfate-I	C12H13O7S	301.0377	0.33	No fragment	U,F	2
M17	36.5	5-(Methoxyphenyl)-γ-valerolactone-sulfate-II	C12H13O7S	301.0377	0.33	No fragment	U	2
M18	29.6	5-(Methoxyphenyl)-γ-valerolactone-glucuronide-I	C18H21O10	397.1126	–0.75	207.0651	U	2
M19	32.6	5-(Methoxyphenyl)-γ-valerolactone-glucuronide-II	C18H21O10	397.1126	–0.75	207.0651	U	2
M20	39.5	5-(3'-Hydroxyphenyl)-γ-valerolactone	C11H11O3	191.0702	2.09	No fragment	F	1
M21	36.0	5-(Phenyl)-γ-valerolactone-3'-sulfate	C11H11O6S	271.0271	0.36	191.0702	U,P,F	1
M22	30.4	5-(Phenyl)-γ-valerolactone-4'-glucuronide	C17H19O9	367.1021	–0.27	191.0702	U	2
M23	32.3	5-(Phenyl)-γ-valerolactone-3'-glucuronide	C17H19O9	367.1021	–0.27	191.0702	U,P	1
		4-Hydroxy-5-(phenyl)valeric acids						

M24	25.1	4-Hydroxy-5-(3',4'-dihydroxyphenyl)valeric acid	C11H13O5	225.0756	–0.66	207.0651	U,F	2
M25	26.8	4-Hydroxy-5-(hydroxyphenyl)valeric acid-sulfate	C11H13O8S	305.0327	0.44	225.0756	U,F	2
M26	20.3	4-Hydroxy-5-(3'-hydroxyphenyl)valeric acid-4'-glucuronide	C17H21O11	401.1075	–0.84	225.0756	U,P,F	2
M27	21.7	4-Hydroxy-5-(4'-hydroxyphenyl)valeric acid-3'-glucuronide	C17H21O11	401.1075	–0.84	225.0756	U,P,F	2
M28	27.3	4-Hydroxy-5-(phenyl)valeric acid-sulfate	C11H13O7S	289.0378	0.51	209.0806	U,P,F	2
		5-(Phenyl)valeric acids						
M29	36.5	5-(3',4'-Dihydroxyphenyl)valeric acid	C11H13O4	209.0806	–1.12	191.0702	U,F	2
M30	35.8	5-(Hydroxyphenyl)valeric acid-sulfate	C11H13O7S	289.0378	0.51	209.0806	U,P,F	2
M31	30.4	5-(Hydroxyphenyl)valeric acid-glucuronide	C17H21O10	385.1127	–0.57	209.0806	U,P,F	2
M32	40.2	5-(Methoxyphenyl)valeric acid-glucuronide	C18H23O10	399.1284	–0.43	223.0969; 209.0807	U,P,F	2
M33	58.0	5-(Hydroxyphenyl)valeric acid	C11H13O3	193.0860	0.41	No fragment	U,P	2
M34	50.1	5-(Phenyl)valeric acid-sulfate	C11H13O6S	273.0430	0.96	193.0860	U,P,F	2
M35	46.1	5-(Phenyl)valeric acid-glucuronide	C17H21O9	369.1180	–0.02	193.0860	U,P,F	2

[a] U, urine; F, fecal; P, plasma

[b] Metabolite standards initiative (MSI) metabolite identification (MI) levels[1]. Reference compounds were available for all compounds identified at MSI MI level 1.

[1] L. W. Summer, A. Amberg, D. Barrett, M. H. Beale, R. Beger, C. A. Daykin, T. W. Fan, O. Fiehn, R. Goldagre, J. L. Griffin, Proposed minimum reporting standards for chemical analysis. Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics*, 2007, **3**, 211–221

Table S2. UHPLC-ESI-MS^n-based identifications of the 5 carbon side chain ring fission metabolites (5C-RFMs) phenyl-γ-valerolactones and phenylvaleric acids detected in urine, plasma and feces collected 0-24 h after rats were fed a grape seed proanthocyanidin extract.

Peak	RT HPLC (min)	RT UHPLC (min)	Flavan-3-ols and Metabolites	[M-H]$^-$ *m/z*	MS^2 Fragmentation Pattern								MS^3 Fragmentation Pattern						MSI MI Level[a]
5C-RFMs																			
5-(Phenyl)-γ-valerolactones																			
M10	29.1	3.76	5-(Dihydroxyphenyl)-γ-valerolactone-glucuronide	399	223								399 --> 223	179					2
M11	28.8	3.11	5-(3',4'-Dihydroxyphenyl)-γ-valerolactone	207	163	122													1
M12	32.2	3.41	5-(4'-Hydroxyphenyl)-γ-valerolactone-3'-sulfate	287	207	161	163	121					287 --> 207	163	122	109	145	177	1
M13	28.5	3.22	5-(3'-Hydroxyphenyl)-γ-valerolactone-4'-glucuronide	383	207	175	163	157	113				383 --> 207	163	122				1
M14	29.2	3.32	5-(4'-Hydroxyphenyl)-γ-valerolactone-3'-glucuronide	383	207	175	113	147	193	339	365		383 --> 207	163					1
M15	28.6	2.62	5-(Phenyl)-γ-valerolactone-glucuronide-sulfate	463	383	287	447						463 --> 383	207					2
M16	35.1	3.59	5-(Methoxyphenyl)-γ-valerolactone-sulfate-I	301	221	206	259	283					301 --> 221	206	162	177	203		2
M17	36.5	3.79	5-(Methoxyphenyl)-γ-valerolactone-sulfate-II	301	221	206	187	181	165	259	283		301 --> 221	206	145				2
M18	29.6	3.48	5-(Methoxyphenyl)-γ-valerolactone-glucuronide-I	397	175	221	206	113	379	157			397 --> 221	206	162				2
M19	32.6	3.54	5-(Methoxyphenyl)-γ-valerolactone-glucuronide-II	397	175	221	379						397 --> 221	206					2
M20	39.5	4.50	5-(3'-Hydroxyphenyl)-γ-valerolactone	191	111	147	173	176											1
M21	36.0	3.65	5-(Phenyl)-γ-valerolactone-3'-sulfate	271	191	207	173	163	147				271 --> 191	147	173	107	145	161	1
M22	30.4	3.95	5-(Phenyl)-γ-valerolactone-4'-glucuronide	367	191	147							367 --> 191	147	106				2
M23	32.3	4.03	5-(Phenyl)-γ-valerolactone-3'-glucuronide	367	191	147							367 --> 191	147	106				2
4-Hydroxy-5-(phenyl)valeric acids																			
M24	25.1		4-Hydroxy-5-(3',4'-dihydroxyphenyl)valeric acid	225															
M25	26.8	2.69	4-Hydroxy-5-(3'-hydroxyphenyl)valeric acid-4'-sulfate	305	225	223	207	163	287				305 --> 225	207	101	163	123		
M26	20.3	2.41	4-Hydroxy-5-(3'-hydroxyphenyl)valeric acid-4'-glucuronide	401	225	175	165						401 --> 225	207	163	101			2
M27	21.7	2.47	4-Hydroxy-5-(4'-hydroxyphenyl)valeric acid-3'-glucuronide	401	225	175	163	207	123				401 --> 225	207	163	101	123		2
M28	27.3	2.96	4-Hydroxy-5-(phenyl)valeric acid-sulfate	289	209	271	191	207					289 --> 209	191	147	101	107		2

5-(Phenyl)valeric acids																			
M29	36.5	4.12	5-(3',4'-hydroxyphenyl)valeric acid	209	191	151	149	165	163	123		209 --> 191	147	149	173	123		2
M30	35.8	3.57	5-(Hydroxyphenyl)valeric acid-sulfate	289	209	207	163	191	175			289 --> 209	191	151	149	165	147	2
M31	30.4	3.36	5-(Hydroxyphenyl)valeric acid-glucuronide	385	209	175	113	209	367	191	149	385 --> 209	191	151	149	165	123	2
M32	40.2	3.27	5-(Methoxyphenyl)valeric acid-3'-glucuronide	399	223	208	175	193				399 --> 223	208	179	164			
M33	58.0	5.50	5-(Hydroxyphenyl)valeric acid	193	175	147	149	135	103									2
M34	50.1	4.34	5-(Phenyl)valeric acid-sulfate	273	193							273 --> 193	175	149				2
M35	46.1	4.26	5-(Phenyl)valeric acid-glucuronide	369	175	193	113	351				369 --> 193	175	149	133			2

[a] Metabolite standards initiative (MSI) metabolite identification (MI) levels[1]. Reference compounds were available for all compounds identified at MSI MI level 1.

[1] L. W. Summer, A. Amberg, D. Barrett, M. H. Beale, R. Beger, C. A. Daykin, T. W. Fan, O. Fiehn, R. Goldagre, J. L. Griffin, Proposed minimum reporting standards for chemical analysis. Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics*, 2007, **3**, 211–221

Table S3. UHPLC-HRMS-based identifications of phenolic catabolites in urine, plasma and feces collected 0-24 h after rats were a grape seed proanthocyanidin extract.

Peak	Rt (min)	Catabolites	Chemical Formula $[m/z]^-$	Experimental mass $[m/z]^-$	δ (ppm)	Fragment (m/z)	Location[a]	MSI MI level[b]
		Cinnamic acids						
C1	37.7	3'-Hydroxycinnamic acid	C9H7O3	163.0378	4.18	119.0481	U	1
C2	28.2	Cinnamic acid-3'-glucuronide	C15H15O9	339.0712	–0.41	163.0386	U,F	1
C3	28.8	Cinnamic acid-4'-sulfate	C9H7O6S	242.9952	2.40	163.0385	U,P	2
C4	33.5	Cinnamic acid-3'-sulfate	C9H7O6S	242.9952	2.40	163.0382	U,P	2
C5	26.5	4'-Hydroxycinnamic acid-3'-glucuronide	C15H15O10	355.0659	0.20	179.0339	U	1
C6	38.0	4'-Hydroxy-3'-methoxycinnamic acid	C10H9O4	193.0492	1.73	134.0354	U,F	1
C7	30.8	3'-Methoxycinnamic acid-4'-sulfate	C10H9O7S	273.0064	–0.18	193.0494	U,P,F	1
C8	26.3	3'-Methoxycinnamic acid-4'-glucuronide	C16H17O10	369.0812	1.14	193.0495	U,F	1
C9	33.3	4'-Methoxycinnamic acid-3'-glucuronide	C16H17O10	369.0812	1.14	193.0494	U	1
		Phenylpropanoic acids						
C10	33.7	3-(4'-Hydroxy-3'-methoxyphenyl)propanoic acid	C10H11O4	195.0648	1.97	136.0512	U,F	1
C11	28.8	3-(3'-Methoxyphenyl)propanoic acid-4'-sulfate	C10H11O7S	275.0218	0.72	195.0652	U,P,F	1
C12	27.4	3-(3'-Methoxyphenyl)propanoic acid-4'-glucuronide	C16H19O10	371.0963	2.62	195.0652	U,F	1
C13	37.6	3-(3'-Hydroxy-4'-methoxyphenyl)propanoic acid	C10H11O4	195.0648	1.97	136.0516	U,F	1
C14	22.4	3-(3'-Hydroxyphenyl)propanoic acid-4'-glucuronide	C15H17O10	357.0816	–0.31	181.0491	U,F	1
C15	23.7	3-(4'-Hydroxyphenyl)propanoic acid-3'-glucuronide	C15H17O10	357.0816	–0.31	181.0491	U,F	1
C16	26.5	3-(4'-Methoxyphenyl)propanoic acid-3'-sulfate	C10H11O7S	275.0218	0.72	195.0652	U,P,F	2
C17	32.1	3-(3'-Hydroxyphenyl)propanoic acid	C9H9O3	165.0536	4.18	121.0639	U,P	1
C18	28.4	3-(4'-Hydroxyphenyl)propanoic acid	C9H9O3	165.0536	4.18	121.0639	U,P	1
C19	28.2	3-(Phenyl)propanoic acid-3'-sulfate	C9H9O6S	245.0108	2.59	165.0540	U,P	1
C20	26.6	3-(Phenyl)propanoic acid-3'-glucuronide	C15H17O9	341.0869	–0.56	165.0542	U,P	1
C21	30.0	2-Hydroxy-3-(Phenyl)propanoic acid	C9H10O3	165.0536	4.18	119.0483	U,P	1
		Phenylacetic acids						
C22	20.7	3'-Hydroxy-4'-methoxyphenylacetic acid	C9H9O4	181.0490	2.95	137.0593	U,F	1
C23	25.3	4'-Hydroxy-3'-methoxyphenylacetic acid	C9H9O4	181.0490	2.95	137.0593	U,F	1
C24	14.2	Methoxyphenylacetic acid-glucuronide	C15H17O10	357.0805	3.14	137.0594; 181.0492	U,P,F	2
C25	17.9	Methoxyphenylacetic acid-sulfate	C9H9O7S	261.0062	0.57	137.0591; 181.0491	U,P,F	2

C26	24.2	3'-Hydroxyphenylacetic acid	C8H7O3	151.0383	3.97	107.0486	U,P,F	1
C27	20.2	4'-Hydroxyphenylacetic acid	C8H7O3	151.0384	3.31	108.0438	U,P,F	1
C28	15.0	Phenylacetic acid-glucuronide	C14H15O9	327.0699	3.36	151.0384	U,P	2
C29	13.8	Phenylacetic acid-4'-sulfate	C8H7O6S	230.9956	0.43	151.0385	U,P,F	2
C30	18.6	Phenylacetic acid-3'-sulfate	C8H7O6S	230.9956	0.43	151.0385	U	2
		Benzoic acids						
C31	12.9	Hydroxybenzoic acid-sulfate	C7H5O7S	232.9743	3.00	153.0177	U	2
C32	18.3	4-Hydroxybenzoic acid	C7H5O3	137.0227	4.37	93.0327	U,P,F	1
C33	22.5	3-Hydroxybenzoic acid	C7H5O3	137.0227	4.37	93.0327	P	1
C34	16.5	Benzoic acid-sulfate	C7H5O6S	216.9799	0.92	137.0230	U,P,F	2
C35	13.6	Benzoic acid-glucuronide	C13H13O9	313.0542	3.83	137.0230	U,P	2
C36	18.9	Methoxybenzoic acid-sulfate	C8H7O7S	246.9906	–0.27	No fragment	U	2
		Hydroxycarboxylic acid derivatives						
C37	6.9	4'-Hydroxymandelic acid	C8H7O4	167.0333	2.99	121.0279	U,P,F	1
		Benzene derivatives						
C38	7.6	Benzene-1,3,5-triol (phloroglucinol)	C6H5O3	125.0228	–3.70	No fragment	U	1
C39	8.5	Hydroxybenzene-4-sulfate	C6H5O5S	188.9846	3.17	109.0277	U	2
C40	15.6	Hydroxybenzene-3-sulfate	C6H5O5S	188.9846	3.17	109.0277	U,P	2
C41	6.5	Hydroxybenzene-glucuronide-I	C12H13O8	285.0589	5.26	109.0277	U	2
C42	10.1	Hydroxybenzene-glucuronide-II	C12H13O8	285.0589	5.26	109.0277	U	2
C43	19.7	Hydroxybenzene-glucuronide-III	C12H13O8	285.0589	5.26	109.0277	U	2
C44	9.9	Dihydroxybenzene-sulfate-I	C6H5O6S	204.9799	0.97	125.0228	U,P,F	2
C45	13.6	Dihydroxybenzene-sulfate-II	C6H5O6S	204.9799	0.97	125.0228	U,F	2
C46	17.9	Dihydroxybenzene-sulfate-III	C6H5O6S	204.9799	0.98	125.0228	U	2
C47	10.4	Dihydroxybenzene-glucuronide-I	C12H13O9	301.0542	3.98	125.0228	U	2
C48	12.8	Dihydroxybenzene-glucuronide-II	C12H13O9	301.0542	3.98	125.0228	U	2
		Benzoylglycine derivatives						
C49	12.8	4'-Hydroxyhippuric acid	C9H8NO4	194.0442	2.57	No fragment	U,P,F	1
C50	21.7	Hippuric acid	C9H8NO3	178.0489	5.05	No fragment	U,P	1

[a] U, urine; F, fecal; P, plasma

[b] Metabolite standards initiative (MSI) metabolite identification (MI) levels[1]. Reference compounds were available for all compounds identified at MSI MI level 1.

[1] L. W. Summer, A. Amberg, D. Barrett, M. H. Beale, R. Beger, C. A. Daykin, T. W. Fan, O. Fiehn, R. Goldagre, J. L. Griffin, Proposed minimum reporting standards for chemical analysis. Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics*, 2007, **3**, 211–221

Table S4 Diol HPLC-HRMS characteristics and quantities (μmol) of flavan-3-ols in 50 mg of wine and grape seed proanthocyanidin extracts.

Rt (min)	Flavan-3-ols	Chemical Formula [*m/z*]-	Experimental mass [*m/z*]-	δ (ppm)	Wine Extract	Grape Seed Extract
4.8	(+)-Catechin[a]	C15H14O6	289.0716	3.5	0.7 ± 0.1	0.9 ± 0.1
	(–)-Epicatechin[a]	C15H14O6	289.0716	3.5	0.8 ± 0.1	0.8 ± 0.1
				Total monomers	***1.5 ± 0.2***	***1.7 ± 0.2***
8.4	B-type procyanidin dimers	C30H26O12	577.1346	1.0	5.0 ± 0.21	2.4 ± 0.1
				Total dimers	***5.0 ± 0.21***	***2.4 ± 0.1***
13.9	B-type procyanidin trimer	C45H38O18	865.1986	1.4	0.45 ± 0.11	0.38 ± 0.11
				Total trimers	***0.45 ± 0.11***	***0.38 ± 0.11***
20.5	B-type procyanidin tetramers	C60H50O24	1153.262	1.0	0.15 ± 0.1	0.22 ± 0.01
26.4	B-type procyanidin pentamers	C75H62O30	1441.3236	–0.4	0.07 ± 0.01	0.15 ± 0.01
31.4	B-type procyanidin hexamers	C90H74O36	1729.3945	4.0	0.02 ± 0.01	0.08 ± 0.01
33.8	Heptamers	C105H86O42	–	–	0.01 ± 0.01	0.030 ± 0.003
36.2	Octamers	C120H98O48	–	–	0.008 ± 0.002	0.016 ± 0.006
38.4	Nonamers	C135H110O54	–	–	0.008 ± 0.002	0.016 ± 0.004
40.6	Decamers	C150H122O60	–	–	0.003 ± 0.001	0.003 ± 0.001
				Total tetramers–decamers	***0.27 ± 0.15***	***0.50 ± 0.06***
				Total dimers-decamers	***5.7 ± 0.31***	***3.3 ± 0.27***
				Total monomers-decamers	**7.2 ± 0.8**	**5.0 ± 0.6**

[a] Analysis of (+)-catechin and (–)-epicatechin is based on C18 reverse phase HPLC, which, unlike diol HPLC, is able to separate these compounds.
– not detected

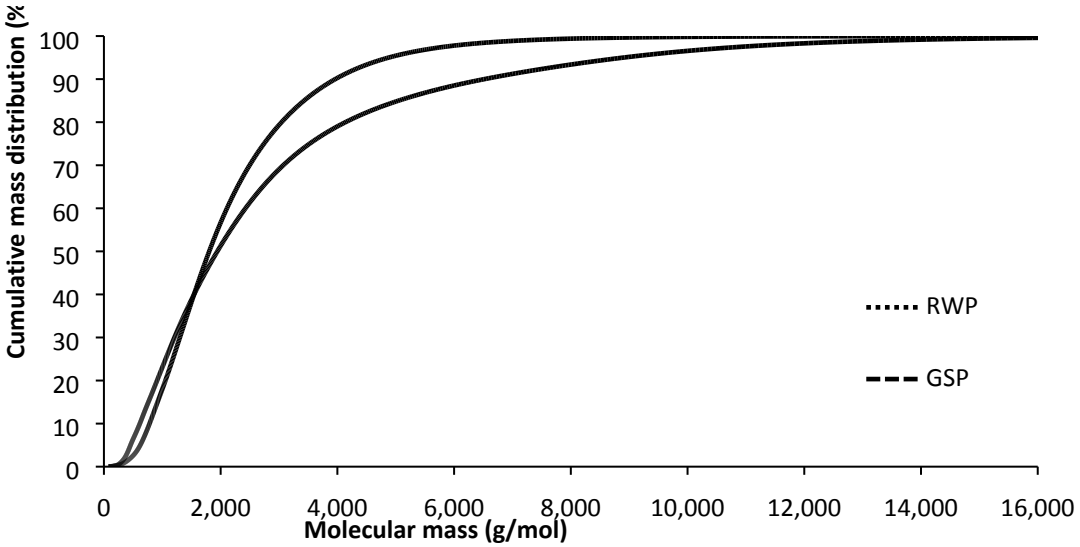

Fig. S1. Cumulative molecular mass distribution of wine (RWP) and seed (GSP) proanthocyanidins versus molecular mass for purified skin-derived and grape seed-derived proanthocyanidin standards. Gel permeation chromatography (GPC) consisted of two PLgel (30 x 7.5 mm, 5µm, 500 by 100A columns (Amherst, MA, USA) connected in series. The column was eluted isocratically with a mobile phase consisting of *N,N*-dimethylformamide containing 1% (v/v) glacial acetic acid, 5% (v/v) water and 0.15 M lithium chloride at a flow rate of 1mL/min. The column temperature was 60°C. The column elate was monitored at 280 nm (Kennedy and Taylor 2003; Bindon et al. 2011).

J. A. Kennedy, A. W. Taylor, Analysis of proanthocyanidins by high-performance gel permeation chromatography. *J. Chromatogr.* A, 2003, **99**, 99–107.

K. A. Bindon, J. A. Kennedy, Ripening-induced changes in grape skin proanthocyanidins modify their interaction with cell walls. *J. Agric. Food Chem*., 2011, **5**9, 2696–2707.