Electronic supplementary material (ESI)

Automated high-throughput quantification of phenyl-γ-valerolactones and creatinine in urine by laser diode thermal desorption

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Supplemental Table S1. Food and beverage containing flavan-3-ols participants were asked to not consume during study B

Category	Item
Fruits	**All the fruits except :**
	Citrus
	Pineapple
	Watermelon
Vegetables	Indian squash
	Rhubarb
Legumes	All beans
	Lentil
	Cowpea
	Carob
Cereals	Soy (including tofu, etc.)
	Barley
	Buckwheat
	Sorghum
	Millet
	Rice
Beverages	Tea
	Coffee
	Juice of the fruits to avoid
	Wine
	Beer
Others	Chocolate
	Nuts and peanut
	Avocado
	Cinnamon
	Curry

Supplemental Table S2. Daily dose of (poly)phenols provided by cranberry extract used in study B

Class	Compound	Daily dose (mg)		
Flavan-3-ols	Catechin	0.1293	±	0.0017
	Epicatechin	1.865	±	0.017
	Procyanidin A2	4.46	±	0.04
	Procyanidin B2	*Non detected*		
	Flavan-3-ols total*	**82.3**	**±**	**0.2**
Phenolic acids	Coumaric acid	2.315	±	0.017
	Coumaroyl-hexoside - 1	0.415	±	0.004
	Coumaroyl-hexoside - 2	0.1760	±	0.0008
	Coumaroyl-monotropein	0.337	±	0.003
	Coumaroyl-dihydromonotropein	0.716	±	0.005
	Chlorogenic acid	1.795	±	0.015
	Caffeoyl-hexoside	0.399	±	0.004
	Sinapoyl-hexoside	0.403	±	0.003
	Vanilloyl-hexoside	0.0676	±	0.0008
	Feruloyl-hexoside	0.254	±	0.002
	3,4-dihydroxybenzoic acid	*Non detected*		
	Dihydroxybenzoic acid - 1	0.1285	±	0.0014
	Dihydroxybenzoic acid - 2	0.0621	±	0.0007
	Dihydroxybenzoyl-hexoside	0.0451	±	0.0006
	Hydroxybenzoyl-hexosyl-hexoside	0.1388	±	0.0013
	Phenolic acids and derivatives total	**7.25**	**±**	**0.05**
Flavonols	Quercetin	4.75	±	0.06
	Quercetin-3-galactoside	1.897	±	0.016
	Quercetin-3-rhamnoside	0.996	±	0.009
	Quercetin-pentoside - 1	0.662	±	0.005
	Quercetin-pentoside - 2	0.421	±	0.003
	Quercetin-pentoside - 3	0.394	±	0.003
	Quercetin-deoxyhexoside	0.1125	±	0.0010
	Quercetin-hydroxybenzoyl-hexoside	0.385	±	0.004
	Isorhamnetin	0.329	±	0.003
	Myricetin	2.18	±	0.02
	Myricetin-pentoside 1	0.1036	±	0.0012
	Myricetin-pentoside - 2	0.171	±	0.003
	Myricetin-pentoside - 3	0.1449	±	0.0007
	Myricetin-hexoside	1.137	±	0.008
	Myricetin methyl	0.220	±	0.002
	Syringetin-hexoside	0.285	±	0.002
	Flavonols total	**14.18**	**±**	**0.12**
Anthocyanins	Cyanidin 3-galactoside	1.06	±	0.03
	Cyanidin 3-glucoside	0.106	±	0.002
	Cyanidin 3-arabinoside	1.70	±	0.04
	Peonidin 3-galactoside	1.43	±	0.03
	Peonidin 3-glucoside	0.052	±	0.004
	Peonidin 3-arabinoside	1.16	±	0.02
	Anthocyanins total	**5.50**	**±**	**0.13**

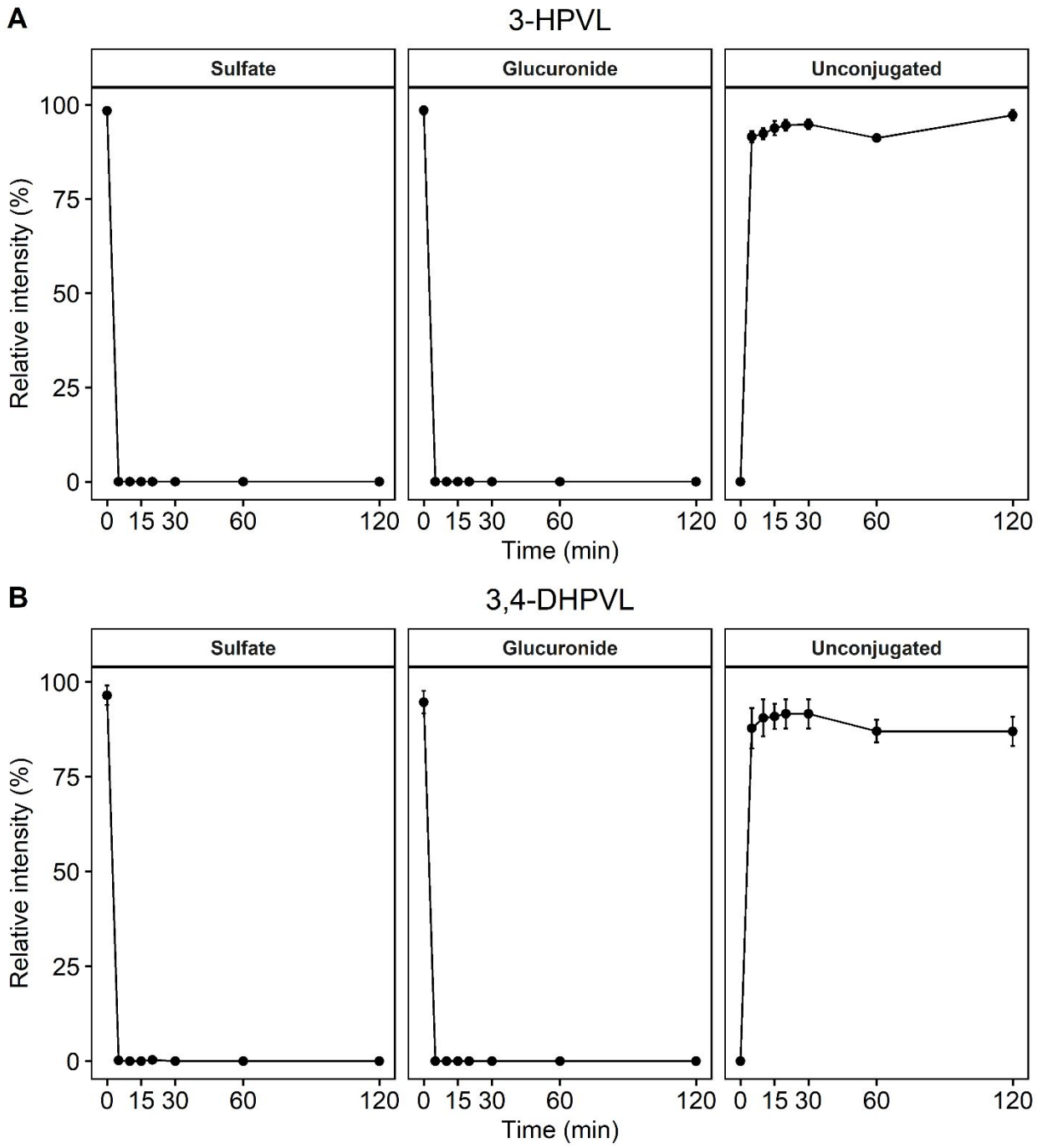
* Determined by phloroglucinolysis with a mean degree of polymerization of 5.

Supplemental Table S3. Concentration of the working solutions

Working solution	Concentration (mg/L)		
	3-HPVL	**3,4-DHPVL**	**Creatinine**
CS #1	0,5	1	1000
CS #2	1	2	2000
CS #3	5	10	4000
CS #4	10	20	8000
CS #5	15	30	16000
CS #6	20	40	24000
CS #7	25	50	32000
CS #8	30	60	40000
QC-L	1,5	3	3000
QC-M	12,5	25	12000
QC-H	22,5	45	30000

Supplemental Table S4. Transitions used for quantification by UPLC-MS/MS

Analyte	Transition	CE (V)	Transition type
3-HPVL	191 → 106	-22	Quantitative
	191 → 147	-18	Confirmation
3,4-DHPVL	207 → 163	-18	Quantitative
	207 → 122	-24	Confirmation
Acetominophen-d_4	154 → 111	-18	Quantitative & confirmation
Creatinine	114 → 86	10	Quantitative
	114 → 72	14	Confirmation
Creatinine-d_3	117 → 89	10	Quantitative
	117 → 75	14	Confirmation



Supplemental Figure S1: Kinetic of degradation of conjugated PVLs and release of unconjugated PVLs during enzymatic hydrolysis. Results are expressed as mean of triplicate ± standard error of mean.

Pseudo-code for automated sample preparation

PVLs

Liquid handler initial state

- One empty deep-well for liquid-liquid extraction (DW1)
- One deep-well with one row prefilled with working solution and acetonitrile (DW2)
- One empty deep-well for final dilution (DW3)
- One empty Lazwell plate
- One reservoir containing enzyme solution.
- One reservoir containing the internal standards solution.
- One reservoir containing the NaCl saturated solution.
- One reservoir containing the desorption solution.
- One sample holder (carousel or deep-well) loaded with urine samples.
- Two boxes of 300 µL tips
- Two boxes of 20 µL tips.

Step 1: Transfer 25 µl of enzyme to an empty deep-well #1

Enzyme transfer parameters

- Aspiration volume = 45 µL
- Dispense volume = 25 µL
- Conditioning volume = 50 µL
- Aspiration position = 2 mm above bottom of well
- Dispensing position = 1 mm above bottom of well

Pick up 300 µL tips.

Condition tip with conditioning volume.

Transfer loop:

- Go to aspiration position in reservoir,
- Aspirate with aspiration volume,
- Go to dispensing position in deep-well and deliver dispense volume,
- Repeat as needed to fill required well in DW1.

Blow out remaining volume in reservoir.

Drop tip into waste.

Step 2: Transfer 50 µL of urine sample to deep-well #1

Sample transfer parameters

- Aspiration volume = 50 µL
- Conditioning volume = 100 µL
- Aspiration position = 2 mm above bottom of well

- Dispensing position = 2 mm above bottom of well

Pick up 300 µL tips

Condition tip with conditioning volume

Transfer loop:

- Go to aspiration position in sample holder,
- Aspirate with aspiration volume,
- Go to dispensing position in DW1,
- Drop tips into waste,
- Repeat as needed to transfer every sample in DW1.

Step 3: Vortex deep-well #1

Vortex DW1 for 30 seconds at 1000 rpm.

Step 4: Hydrolyze urine samples

Wait for 300 seconds.

Step 5: Transfer internal standards to deep-well #1

Internal standards transfer parameters

- Aspiration volume = 295 µL
- Conditioning volume = 300 µL
- Aspiration position = 2 mm below solution level
- Dispensing position = 25 mm above bottom of well

Pick up 300 µL tips,

Condition tips with conditioning volume,

Transfer loop:

- Go to aspiration position in reservoir,
- Aspirate with aspiration volume,
- Go to dispensing position in deep-well and deliver dispense volume,
- Repeat as needed to add to every used well in DW1,

Drop tips into waste.

Step 6: Vortex deep-well

Vortex deep-well for 30 seconds at 1000 rpm.

Step 7: Add working solution (or acetonitrile) to deep-well

Working solution transfer parameters

- Aspiration volume = 5 µL

- Conditioning volume = 10 µL
- Aspiration position = 2 mm above bottom of well
- Dispensing position = 5 mm above bottom of well

Pick up 20 µL tips,

Condition tips with conditioning volume,

Transfer step:

- Go to aspiration position in DW2,
- Aspirate with aspiration volume,
- Go to dispensing position in DW1 and deliver dispense volume,
- Drop tip into waste,
- Repeat as needed.

Step 8: Vortex deep-well #1

Vortex deep-well for 30 seconds at 1000 rpm.

Step 9: Transfer NaCl saturated solution to deep-well #1

NaCl solution transfer parameters

- Aspiration volume = 75 µL
- Conditioning volume = 100 µL
- Aspiration position = 2 mm above bottom of well
- Dispensing position = 25 mm above bottom of well

Pick up 300 µL tips,

Condition tip with conditioning volume,

Transfer loop:

- Go to aspiration position in reservoir,
- Aspirate with aspiration volume,
- Go to dispensing position in deep-well and deliver dispense volume,
- Repeat as needed to add to every used well in DW1.

Drop tips into waste.

Step 10: Vortex deep-well

Vortex deep-well for 30 seconds at 1000 rpm.

Step 11: Mix upper layer with desorption solution in deep-well #3 and transfer to Lazwell

Desorption solution transfer parameters

- Aspiration volume = 20 µL
- Conditioning volume = 20 µL

- Aspiration position = 1 mm above bottom of well
- Dispensing position = 2 mm above bottom of well

Upper layer transfer parameters

- Aspiration volume = 20 µL
- Mixing volume = 20 µL
- Aspiration position = 8 mm above bottom of well
- Dispensing position = 2 mm above bottom of well

Dilution transfer to Lazwell parameters

- Aspiration volume = 4 µL
- Aspiration position = 2 mm above bottom of well
- Dispensing position = 1 mm above bottom of well

Transfer loop:

- Pick up 20 µL tips
- Condition tip with conditioning volume of desorption solution
- Aspirate with desorption solution aspiration volume
- Go to dispensing position in DW3 and deliver desorption solution
- Go to aspiration position in DW1
- Aspirate with upper layer aspiration volume
- Go to dispensing position in DW3 and deliver solution
- Mix in DW3 by pipetting with mixing volume two times
- Aspirate 3 µL air gap
- Go to aspiration position in DW3
- Aspirate with dilution aspiration volume
- Go to dispensing position in LazWell and dispense 7 µL
- After dispense, touch tip at the bottom of the LazWell
- Drop tip into waste
- Repeat as needed to dilute and transfer every sample

Creatinine

Liquid handler initial state

- One empty deep-well for sample preparation (DW1)
- One empty deep-well for sample dilution (DW2)
- One Lazwell plate
- Two 300 uL tips rack
- Two 20 uL tips rack
- One reservoir containing dilution solution
- One reservoir containing internal standard solution
- One sample holder (carousel or deep-well) loaded with urine samples.

Step 1: Transfer internal standard to deep-well #1

Internal standard transfer parameters

- Aspiration volume = 300 µL
- Conditioning volume = 200 µL
- Aspiration position = 2 mm below solution level
- Dispensing position = 10 mm above bottom of well

Pick up 300 µL tips

Condition tip with conditioning volume

Transfer loop:

- Go to aspiration position in internal standard solution reservoir
- Aspirate with aspiration volume
- Go to appropriate dispensing position in DW1 and deliver dispense volume,
- Repeat as needed to fill required well in DW1.

Drop tips into waste

Step 2: Transfer samples to deep-well #1

Samples transfer parameters

- Aspiration volume = 40 µL
- Mixing volume = 200 µL
- Aspiration position = 2 mm above bottom of well
- Dispensing position = 2 mm above bottom of well
- Mixing position = 5 mm above bottom of well

Transfer loop:

- Pick up 300 µL tips
- Go to aspiration position in sample container (carousel or sample deep-well)
- Condition tip with mixing volume

- Aspirate with aspiration volume
- Go to appropriate dispensing position in DW1 and deliver dispense volume
- Mix with mixing volume six times
- Drop tips into waste
- Repeat as needed to transfer every sample in DW1

Step 3: Dilution solution transfer to deep-well #2

Dilution solution transfer parameters

- Aspiration volume = 300 µL
- Conditioning volume = 200 µL
- Aspiration position = 2 mm below solution level
- Dispensing position = 5 mm above bottom of well

Pick up 300 µL tips

Condition tips with conditioning volume

Transfer loop:

- Go to aspiration position in dilution solution reservoir,
- Aspirate with aspiration volume
- Go to appropriate dispensing position in DW2 and deliver dispense volume,
- Repeat as needed to fill required well in DW2.

Drop tips into waste

Step 4: Transfer prepared urine sample to deep-well #2

Prepared urine transfer parameters

- Aspiration volume = 3 µL
- Mixing volume = 10 µL
- Aspiration position = 5 mm above bottom of well
- Dispensing position = 5 mm above bottom of well
- Mixing position = 5 mm above bottom of well

Transfer loop:

- Pick up 20 µL tips
- Go to aspiration position in DW1
- Aspirate with aspiration volume
- Go to dispensing position in DW2 and deliver dispense volume
- Drop tip into waste
- Repeat as needed to transfer every prepared urine sample

Step 5: Vortex deep-well

Vortex deep-well for 30 seconds at 1000 rpm.

Step 6: Transfer diluted urine samples to Lazwell

Transfer to Lazwell parameters

- Aspiration volume = 4 µL
- Conditioning volume = 4 µL
- Aspiration position = 5 mm above bottom of well
- Dispensing position = 1 mm above bottom of well

Transfer loop:

- Pick up 20 µL tips
- Move to aspiration position in DW2
- Condition tip with conditioning volume
- Aspirate 3 µL air gap
- Aspirate with aspiration volume
- Go to dispensing position in LazWell and dispense 7 µL
- After dispense, touch tip at the bottom of the LazWell
- Drop tip into waste
- Repeat as needed to transfer every diluted urine sample