## Versatile UHPLC-MSMS method for simultaneous quantification of various alcohol intake related compounds in human urine and blood

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Time (min.)	Flow (mL min <sup>-1</sup> )	A:B	Curve
0	0.55	98:2	6
1	0.55	98:2	6
1.5	0.43	75:25	6
1.9	0.43	50:50	6
4.1	0.6	20:80	6
4.5	0.6	0:100	3
4.9	0.6	0:100	6
5.1	0.55	98:2	3
6	0.55	98:2	6

 Table S-1. Table overview of LC gradient.

Compound	RT	RT window	<b>Primary</b> <b>transition</b> (quantifier)	Secondary transition (qualifier)	Cone voltage	Collision E
L– (+)–tartaric acid	0.56	0-0.85	149.1→87.1	149.1→73	25	<sup>a</sup> 10 / <sup>b</sup> 15
L– (+)–tartaric acid–d <sub>2</sub>	0.55	0-0.85	151.1→74	151.1→88.1	25	15
ethyl sulphate	0.80	0.65-1.15	125→97	125→80	25	20
ethyl sulphate–d <sub>5</sub>	0.79	0.65-1.15	130→98	130→80	25	20
ethyl-β-D-glucuronide	1.39	1–1.65	221.2→85.1	221.2→75.1	25	15
ethyl-β-D-glucuronide–d <sub>5</sub>	1.34	1–1.65	226.2→85.1	226.2→75.1	25	15
indoxyl sulphate	2.54	2.35-2.75	212→80	212→132	30	20
indoxyl sulphate-d <sub>4</sub>	2.53	2.35-2.75	216→80	216→136	30	20
<i>p</i> –cresol sulphate	2.68	2.65-3	186.7→107	186.7→80	30	20
<i>p</i> –cresol sulphate–d <sub>7</sub>	2.80	2.25-2.8	194.1→114.1	194.1→80	30	20
resveratrol	°3.04/ 3.26	2.85-3.55	227.1→143.2	227.1→185	30	<sup>a</sup> 25 / <sup>b</sup> 20
resveratrol-13C <sub>6</sub>	°3.04/ 3.25	2.85-3.55	233.2→149.2	233.2→191.2	35	<sup>a</sup> 25 / <sup>b</sup> 20
estrone 3–sulphate	3.75	3.6-4	349.2→269.4	349.2→80	35	30
estrone-d <sub>4</sub> - 3-sulphate	3.76	3.6-4	353.2→273.4	353.2→80	35	30
DHEAS	4.12	3.9-4.45	367.2→97	_	30	30
DHEAS-d <sub>5</sub>	4.13	3.9-4.45	372.2→98	-	30	30

**Table S–2.** Overview of mass spectrometry parameters of target compounds with related internal standards (span =  $\pm 0.2$ ; dwell = 0.100 s).

RT – retention time; <sup>a</sup>value for primary transition; <sup>b</sup>value for secondary transition; <sup>c</sup>commercial resveratrol is a mixture of *cis*– and *trans*–resveratrol

	0.55																		2. MRW 012 Charmers E3- 149.1 > 87.1 (Tartaric acid) 3.82e4
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 2: MRM of 2 Channels ES- 149.1 > 73 (Tartaric acid) 3.13e4
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 3: MRM of 2 Channels ES- 125 > 97 (Ethyl sulfate) 2.17e5
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 3: MRM of 2 Channels ES- 125 > 80 (Ethyl sulfate) 8.62e4
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 1: MRM of 2 Channels ES- 151.1 > 88.1 (Tartaric acid-d2) 2.50e4
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 1: MRM of 2 Channels ES- 151.1 > 74 (Tartaric acid-d2) 3.36e4
40	0.60	0.80 0.79	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 4: MRM of 2 Channels ES- 130 > 98 (Ethyl sulfate-d5) 2.05e5
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 4: MRM of 2 Channels ES- 130 > 80 (Ethyl sulfate-d5 6.83e4
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00 226.2	4.20 4.40 5: MRM of 2 Channels ES- 2 > 85.1 (Ethyl glucuronide-d5) 2.63e4
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00 226.2	4.20 4.40 5: MRM of 2 Channels ES- 2 > 75.1 (Ethyl glucuronide-d5 2.86e4
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40

Figure S-1. Illustrative chromatograms of target compounds and related internal standards.

					1.39														6: MRM of 2 Channels ES- 221.2 > 85.1 (Ethyl glucuronide) 5.65e4
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 6: MRM of 2 Channels ES- 221.2 > 75.1 (Ethyl glucuronide) 5.70e4
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 7: MRM of 2 Channels ES- 216 > 136 (Indoxyl sulfate-d4) 9.45e4
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 7: MRM of 2 Channels ES- 216 > 80 (Indoxyl sulfate-d4) 1.33e5
40	0.60	0.80	1.00	1.20	<mark>1</mark> .40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 8: MRM of 2 Channels ES- 212 > 132 (Indoxyl sulfate) 1.43e6
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 8: MRM of 2 Channels ES- 212 > 80 (Indoxyl sulfate) 2.25e6
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00 1	4.20 4.40 9: MRM of 2 Channels ES- 94.1 > 114.1 (Cresol sulfate-d7) 4.22e5
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 9: MRM of 2 Channels ES- 194.1 > 80 (Cresol sulfate-d7) 6.57e4
40	0.60	0.80	1.00	1.20	<mark>1</mark> .40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 10: MRM of 2 Channels ES- 186.7 > 107 (Cresol sulfate) 2.81e5
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 10: MRM of 2 Channels ES- 186.7 > 80 (Cresol sulfate) 1.09e6
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40

Figure S-1. Illustrative chromatograms of target compounds and related internal standards.

													3.03	3.26					12: MRM of 2 Channels ES- 227.1 > 185.2 (Resveratrol) 6.28e4
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 12: MRM of 2 Channels ES- 227.1 > 143.2 (Resveratrol) 7.11e4
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00 23	4.20 4.40 13: MRM of 2 Channels ES- 33.2 > 191.2 (Resveratrol-13C6) 6.72e4
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00 23	4.20 4.40 13: MRM of 2 Channels ES- 33.2 > 149.2 (Resveratrol-13C6) 7.25e4
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00 35	4.20 4.40 15: MRM of 2 Channels ES- 3.2 > 273.4 (Estrone sulfate-d4) 8.45e4
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 15: MRM of 2 Channels ES- 53.2 > 80.2 (Estrone sulfate-d4) 1.90e4
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 16: MRM of 2 Channels ES- 349.2 > 269.4 (Estrone sulfate) 4.36e5
40 40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 16: MRM of 2 Channels ES- 349.2 > 80.2 (Estrone sulfate) 8.69e4
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 18: MRM of 1 Channel ES- 4.16 367.2 > 97 (DHEAS) 4.02e6
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40 19: MRM of 1 Channel ES- 4.14 372.2 > 98 (DHEAS-d5) 7.87e4
40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00	4.20 4.40

Figure S-1. Illustrative chromatograms of target compounds and related internal standards.

Analyte		Repeatability		Intermediate precision					
	Standard solution	Urine	Plasma	Standard solution	Urine	Plasma			
L-(+)-tartaric acid	1.7	1.4	2.0	3.5	2.0	3.3			
ethyl sulphate	8.7	1.7	11	12	8.2	26			
ethyl-β-D- glucuronide	3.3	1.6	2.1	3.9	3.2	2.5			
indoxyl sulphate	2.5	2.1	2.7	3.4	1.8	1.9			
<i>p</i> -cresol sulphate	4.2	2.1	3.8	4.5	2.3	19			
resveratrol	2.1	1.7	1.6	7.5	3.3	3.7			
estrone 3- sulphate	1.5	1.8	1.1	2.5	2.0	4.5			
DHEAS	0.51	0.97	1.1	0.90	1.3	1.7			

 Table S-3. Coefficients of variation (%) of repeatability (n=6) and intermediate precision (n=9).



Figure S–2a. Illustrative chromatograms of isoxanthohumol and isocohumulone in 10× diluted pilsner beer.

225.1 > 181.1 (3-nitro-tyrosine) 7.28e4
5.00 5.20 5.40 5.60 5.80 3: MRM of 2 Channels ES- 225.1 > 163.1 (3-nitro-tyrosine) 1.56e5
5.00 5.20 5.40 5.60 5.80
7: MRM of 2 Channels ES- 225.1 > 181.1 (3-nitro-tyrosine) 1.69e4
4.00 4.10 4.20 4.30 4.40 4.50 4.60 4.70 4.80 4.00 4.10 4.20 4.30 4.40 4.50 4.60 4.70 4.80 7. MRM of 2 Channels ES- 225.1 > 163.1 (3-nitro-tyrosing) 1.05e3

Figure S-2b. Illustrative chromatograms of 3-nitro-tyrosine in a standard solution (1 µg mL<sup>-1</sup>) and an unknown isomer in pooled sample (n=80).



Figure S–2c. Illustrative chromatogram of indole–3–acetic acid in a standard solution.



Figure S–2d. Illustrative chromatograms of cortisol and cortisol sulphate in standard solution.