

Supporting information

Table S1. Optimized MS/MS conditions for the detection of microbial phenolic acids.

#	Compound	Q1 [M-H] ⁻ (m/z)	Q3 (m/z)	Cone voltage (V)	Collision energy (V)
1	3,4-Dihydroxymandelic acid	183	137	32	14
2	Phloroglucinol	125	83	33	12
3	4-Hydroxymandelic acid	167	123	30	12
4	Gallic acid	169	125	33	15
5	Pyrogallol	125	79	36	16
6	4-Hydroxy-3-methoxymandelic acid	197	137	33	20
7	3-Hydroxymandelic acid	167	121	30	16
8	3,5-Dihydroxybenzoic acid	153	109	30	12
9	Protocatechuic acid	153	109	30	14
10	3-Hydroxy-4-methoxymandelic acid	197	137	30	24
11	4-Hydroxyhippuric acid	194	100	27	11
12	Catechol/Pyrocatechol	109	81	44	12
13	3,4-Dihydroxyphenylacetic acid	167	123	20	12
14	3-O-methylgallic acid	183	168	33	12
15	4-Hydroxybenzoic acid	137	93	27	12
16	4-O-methylgallic acid	183	168	30	12
17	Mandelic acid	151	107	27	8
18	4-Hydroxyphenylacetic acid	151	107	24	6
19	3-(3,4-Dihydroxyphenyl)-propionic acid	181	137	32	12
20	3-Hydroxybenzoic acid	137	93	30	18
21	Hippuric acid	178	134	30	10
22	Caffeic acid	179	135	35	16
23	Vanillic acid	167	152	30	12
24	3-Hydroxyphenylacetic acid	151	107	25	16
25	Syringic acid	197	182	30	12
26	4-Hydroxy-3-methoxyphenylacetic acid	181	137	27	8
27	4-Methylcatechol	123	108	35	14
28	3-(4-Hydroxyphenyl)-propionic acid	165	121	33	12
29	<i>p</i> -Coumaric acid	163	119	30	14
30	3-(3-Hydroxyphenyl)-propionic acid	165	121	32	12
31	Ferulic acid	193	134	30	16
32	<i>m</i> -Coumaric acid	163	119	30	18
33	3,4-Dimethoxybenzoic acid	181	107	30	30
34	Benzoic acid	121	77	27	12
35	Isoferulic acid	193	134	27	14
36	Phenylacetic acid	135	91	21	18
37	Salicylic acid	137	93	27	17
38	3,4-Dimethoxyphenylacetic acid	195	136	25	14
39	4-Methoxyphenylacetic acid	165	106	30	12
40	4-Methoxybenzoic acid	151	107	32	12
41	4-Ethylcatechol	137	122	30	16
42	3,4,5-Trimethoxybenzoic acid	211	167	30	12
43	3-Methoxybenzoic acid	151	107	30	12
44	3-(3,4-Dimethoxyphenyl)-propionic	209	150	36	12
45	Phenylpropionic acid	149	105	33	10
46	3,4,5-Trimethoxycinnamic acid	237	103	30	18
47	<i>t</i> -Cinnamic acid	147	103	27	12
IS	4-Hydroxybenzoic 2,3,5,6 d ₄ acid	141	97	30	12

Table S2. Concentration range ($\mu\text{g/mL}$) of each calibration level of the pool standard solution.

Dilution	Group A	Group B	Group C	Group D	Group E
1:1	25	50	100	200	500
1:2	12.5	25	50	100	250
1:5	5	10	20	40	100
1:10	2.5	5	10	20	50
1:20	1.25	2.5	5	10	25
1:50	0.5	1	2	4	10
1:100	0.25	0.5	1	2	5
1:500	0.05	0.1	0.2	0.4	1
1:1000	0.025	0.05	0.1	0.2	0.5
1:5000	0.005	0.01	0.02	0.04	0.1
1:10000	0.0025	0.005	0.01	0.02	0.05

Table S3. Application of external and internal calibration methods for the quantification of microbial phenolic acids in human faeces.

#	Compound (µg/g)	#1		#2		#3		#4		#5		#6	
		EC ^a	IC	EC	IC	EC	IC	EC	IC	EC	IC	EC	IC
8	3,5-Dihydroxybenzoic acid	0.271 ^b	0.271	0.207	0.226	0.221	0.281	0.170	0.180	0.057	0.084	0.425	0.464
		±	±	±	±	±	±	±	±	±	±	±	±
		0.055	0.014	0.053	0.032	0.053	0.058	0.026	0.017	0.013	0.014	0.063	0.092
9	Protocatechuic acid	0.398	0.407	0.163	0.155	0.737*	0.954	1.06	1.09	0.881*	1.04	0.536	0.600
		±	±	±	±	±	±	±	±	±	±	±	±
		0.343	0.380	0.147	0.160	0.006*	0.047	0.10	0.16	0.005*	0.04	0.250	0.273
18	4-Hydroxyphenylacetic acid	3.41	4.16	5.99	6.12	5.15	6.33	1.72	1.65	1.47	1.67	4.16	4.47
		±	±	±	±	±	±	±	±	±	±	±	±
		0.24	0.21	1.07	0.67	0.62	0.57	0.16	0.09	0.15	0.12	0.02	0.27
24	3-Hydroxyphenylacetic acid	2.50	2.69	3.42	3.65	2.74	3.46	0.130	0.105	0.153	0.187	12.6	13.9
		±	±	±	±	±	±	±	±	±	±	±	±
		0.54	0.37	0.51	0.14	0.63	0.91	0.094	0.118	0.093	0.129	1.4	1.2
25	Syringic acid	0.366	0.405	0.096	0.076	0.549	0.711	0.846	0.881	0.507	0.608	0.627	0.721
		±	±	±	±	±	±	±	±	±	±	±	±
		0.054	0.075	0.065	0.096	0.097	0.102	0.118	0.163	0.103	0.141	0.058	0.081
29	<i>p</i> -Coumaric acid	0.241	0.252	0.228	0.237	0.156	0.170	0.596	0.616	0.553*	0.669	0.191	0.205
		±	±	±	±	±	±	±	±	±	±	±	±
		0.051	0.087	0.042	0.074	0.054	0.056	0.118	0.141	0.017	0.015	0.046	0.020
30	3-(3-Hydroxyphenyl)-propionic acid	0.472	0.524	1.98	2.03	1.01	1.25	9.31	8.84	8.52	9.41	3.94	4.18
		±	±	±	±	±	±	±	±	±	±	±	±
		0.116	0.123	0.22	0.19	0.16	0.16	1.01	0.73	0.70	0.60	0.30	0.21
34	Benzoic acid	0.539	0.803	0.470	0.761	1.80*	2.51	nd ^c	nd	5.39	6.10	2.82	3.02
		±	±	±	±	±	±			±	±	±	±
		0.041	0.191	0.323	0.354	0.20	0.20			0.70	0.77	0.55	0.36
36	Phenylacetic acid	149	155	142	147	189	232	211	200	164	182	58	62
		±	±	±	±	±	±	±	±	±	±	±	±
		16	7	11	5	24	17	16	6	15	13	6	2
45	Phenylpropionic acid	49.0	50.6	58.6	59.9	56.9	69.6	45.4	42.7	32.9	36.2	88.6	92.7
		±	±	±	±	±	±	±	±	±	±	±	±
		6.5	7.0	8.6	9.4	2.9	8.5	4.2	5.9	1.4	2.6	15.3	16.2

^a EC: External calibration; IC: Internal calibration.^b Mean ($n=3$) ± standard deviation (SD)^c nd: not detected.* Mean values between EC and IC significantly differed at $p<0.05$.

Figure S1

