Supplementary Materials

Rapid quantification of 50 fatty acids in small amounts of biological samples for population molecular phenotyping

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Supplementary information notes

Method validation for GC-FID/MS quantification of FAMES

The method for GC-FID/MS quantification of FAMES was validated in accordance with the criteria described in the Food and Drug Administration (FDA) guidelines for validation of bioanalytical methods including linearity, sensitivity, precision, accuracy and stability.

The linearity of detector responses was determined by linear regression of the analyte-to-IS peak ratio as a function of the analyte-to-IS concentration ratio for each FA in a series of mixed FA standards. The limit of detection (LOD) and the lower limit of quantification (LLOQ) were obtained from three replicates as moles on column for each FA with the signal-to-noise (S/N) ratio of 3:1 and 10:1, respectively.

Precision and accuracy were assessed by analyzing the QC working solutions at three concentration levels in plasma samples (n = 5). Whilst precision was expressed as intra- and inter-day coefficient-of-variations (CV) within a day and three consecutive days, respectively; accuracy was assessed with recovery.

To test the analytical stability in the forms of FAMEs, three levels of esterified QC working solutions were analyzed (n = 5). Stability on autosampler was assessed by analysis of the mixed FAMEs over 24 hours at room temperature with an 8-h interval. The storage stability of FAMEs was evaluated at 4 ℃ for 48 hours and –80 ℃ for 72 hours with an interval of 12-h and 24-h, respectively.

LC-MS analysis of residual lipids after transesterification

Remaining residual lipids in the methylated samples were extracted using the reported Matyash method with MTBE/methanol/water (10:3:2.5, *V/V/V*) (Matyash *et al.* 2008). All lipids were analyzed based on methods reported previously (Huang *et al.* 2019; Loo *et al.* 2021) with some minor modification. Here we used an LC-MS based method on a QTRAP 6500 plus mass spectrometer (Sciex, USA) coupled with an ultrahigh performance liquid chromatography system LC30A (Shimazu, Japan). A BEH HILIC column (2.1 * 100 mm, 1.7 μm) and Kinetex C18 column (2.1 * 100 mm, 2.6 μm) were employed, where appropriate, with a solvent flow-rate of 0.5 mL/min and 0.3 mL/min, respectively. Mobile phases were H_2O/ACN (5:95, *V/V*) and H_2O/ACN (1:1, *V/V*) both containing 10 mmol/L NH_4AC for the former whereas

H_2O/MeOH/ACN (1:1:1, *V/V/V*) containing 7 mmol/L NH_4OH and IPA containing 7 mmol/L NH_4AC for the latter. A 12-min gradient elution was employed for both columns.

The derivatization efficiency for each type of lipids was calculated as following:

$$\textbf{Transmethylation efficiency } (\%) = \left(1 - \frac{A_m}{A_0}\right) \times 100\%$$

where A_m and A_0 denote lipid peak areas for methylated and original samples, respectively.

Calculation of no-additives retention index (NARI)

Here, RI for a given analyte *t* was determined by measuring the retention time of this compound and two calibrating peaks from either saturated fatty acids or added alkanes neighboring this compound. The no-additives retention index (NARI) and alkanes-based classical RI was calculated according to Kovats (Kovats 1958) method with some modification as follows:

$$RI_t = RI_m + (RI_n - RI_m) \times \frac{t_{Rt} - t_{Rm}}{t_{Rn} - t_{Rm}}$$

Where t_{Rm}, t_{Rt} and t_{Rn} respectively represented retention time for metabolite *t* and its neighboring two saturated fatty acids (FAs) or alkanes (where $t_{Rm} < t_{Rt} < t_{Rn}$). We calculated three NARI schemes with (a) seven FAs (C8:0, C16:0, C20:0, C22:0, C24:0, C25:0, C30:0), (b) five FAs (C8:0, C16:0, C20:0, C24:0, C30:0), (c) four FAs (C8:0, C18:0 C22:0, C30:0), respectively. The alkanes-based classical RI was calculated from 25 added alkanes (C14–C38) as *m* and *n*. In all cases, RIs for the calibrants *m* and *n* were defined as the number of carbon atoms multiplied by 100.

The above retention indexes were all calculated from retention time values obtained from our optimal method (M_0). Retention time data for all 45 FAMEs were also recorded using other methods having different temperature-gradients (M_1, M_2), flow rates (M_3, 0.5 mL/min; M_4, 1.5 mL/min) from M_0 and acquired from a different batch (M_5) using M_0.

Temperature gradients for M_1 were as follows:

M_1	Rate (℃/min)	Temperature (℃)	Hold time (min)	Duration (min)
(Initial)		50	0.60	0.60
step1	250	175	0.00	1.10
step2	30	190	0.00	1.60
step3	15	210	0.00	2.93
step4	8	220	0.70	4.88
step5	30	230	4.00	9.22

Temperature-gradients for M_2 was as follows:

M_2	Rate (℃/min)	Temperature (℃)	Hold time (min)	Duration (min)
(Initial)		90	0.00	0.00
step1	250	140	1.00	1.20
step2	70	200	2.60	4.66
step3	80	215	0.50	5.34
step4	250	205	0.40	5.78
step5	200	250	2.50	8.51

Supplementary figures

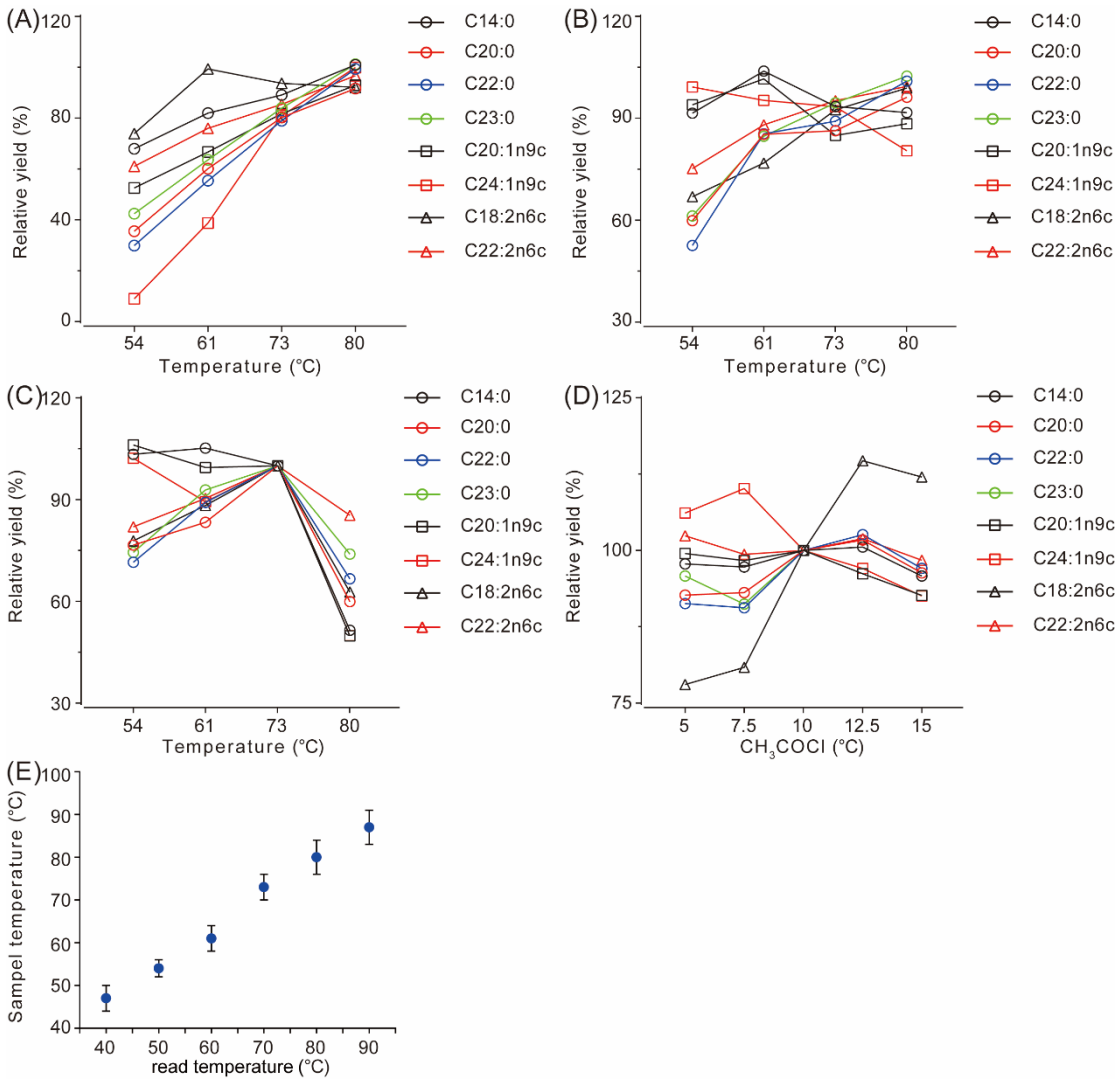

Fig. S1 Relative yields of FAMEs against reaction time, temperature and concentration of CH_3COCl. **A** 1 h with 10% CH_3COCl. **B** 2 h with 10% CH_3COCl. **C** 3 h with 10% CH_3COCl. **D** 73 °C for 3 h. **E** thermomixer's setting and sample temperature

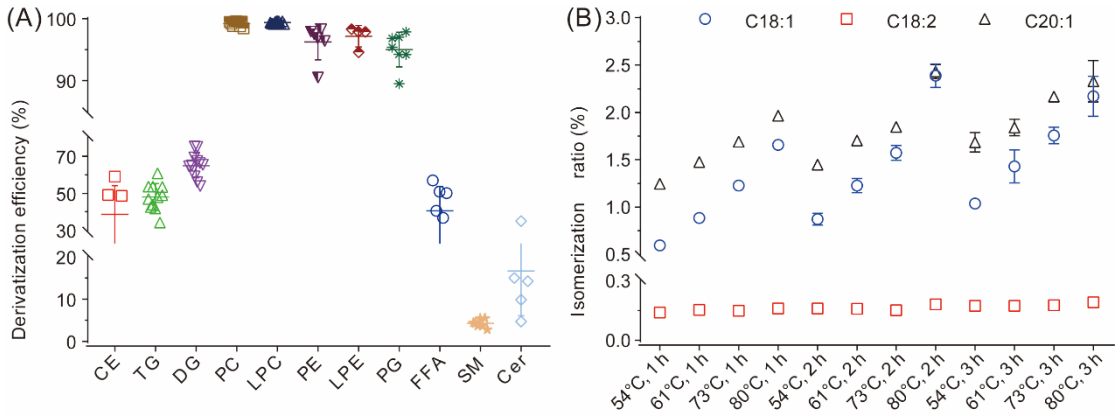

Fig. S2 Transesterification efficiencies for reported method (Tremblay-Franco *et al.* 2015) and isomerization of unsaturated fatty acids (**A**) with current method using 12.5% CH_3COCl (**B**)

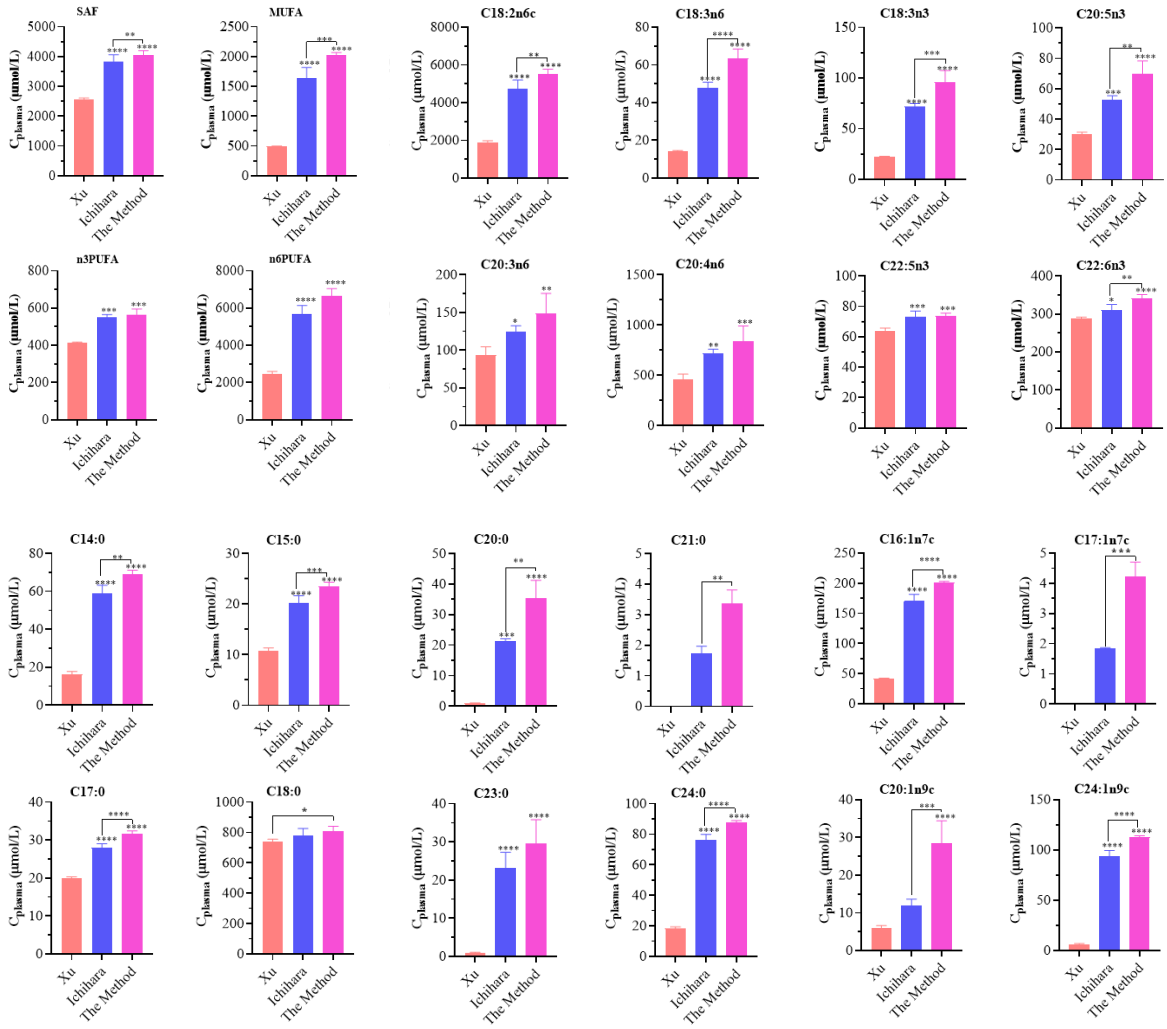

Fig. S3 Comparison of this method with two widely used esterification methods using CH_3COCl/MeOH including Xu method (Xu *et al.* 2010) and Ichihara method (Ichihara and Fukubayashi 2010)

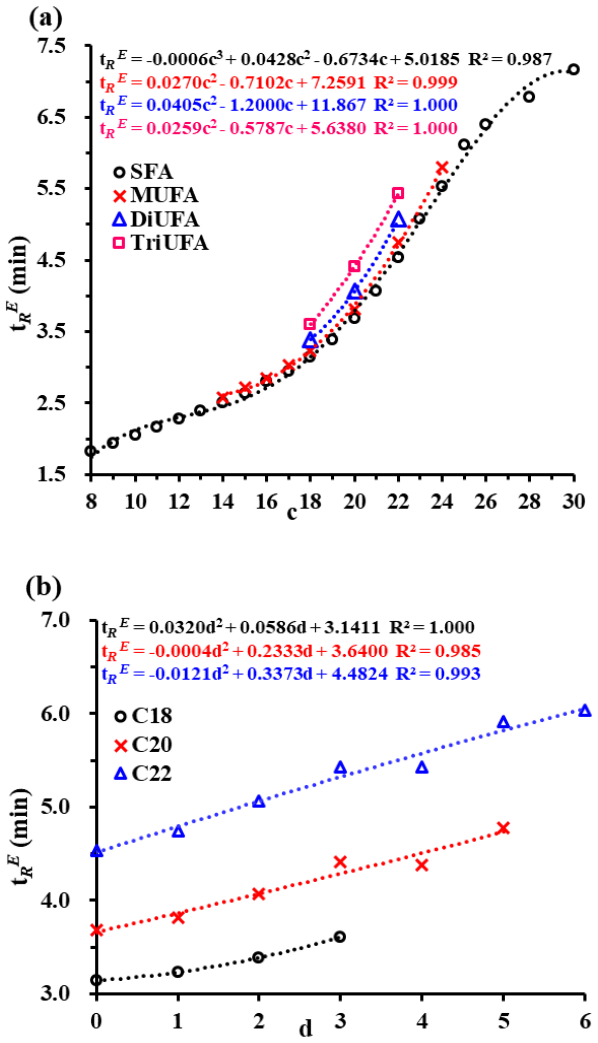

Fig. S4 Dependence of experimental t_R (t_R^E) on chain-length c (**a**), double-bond number d (**b**)

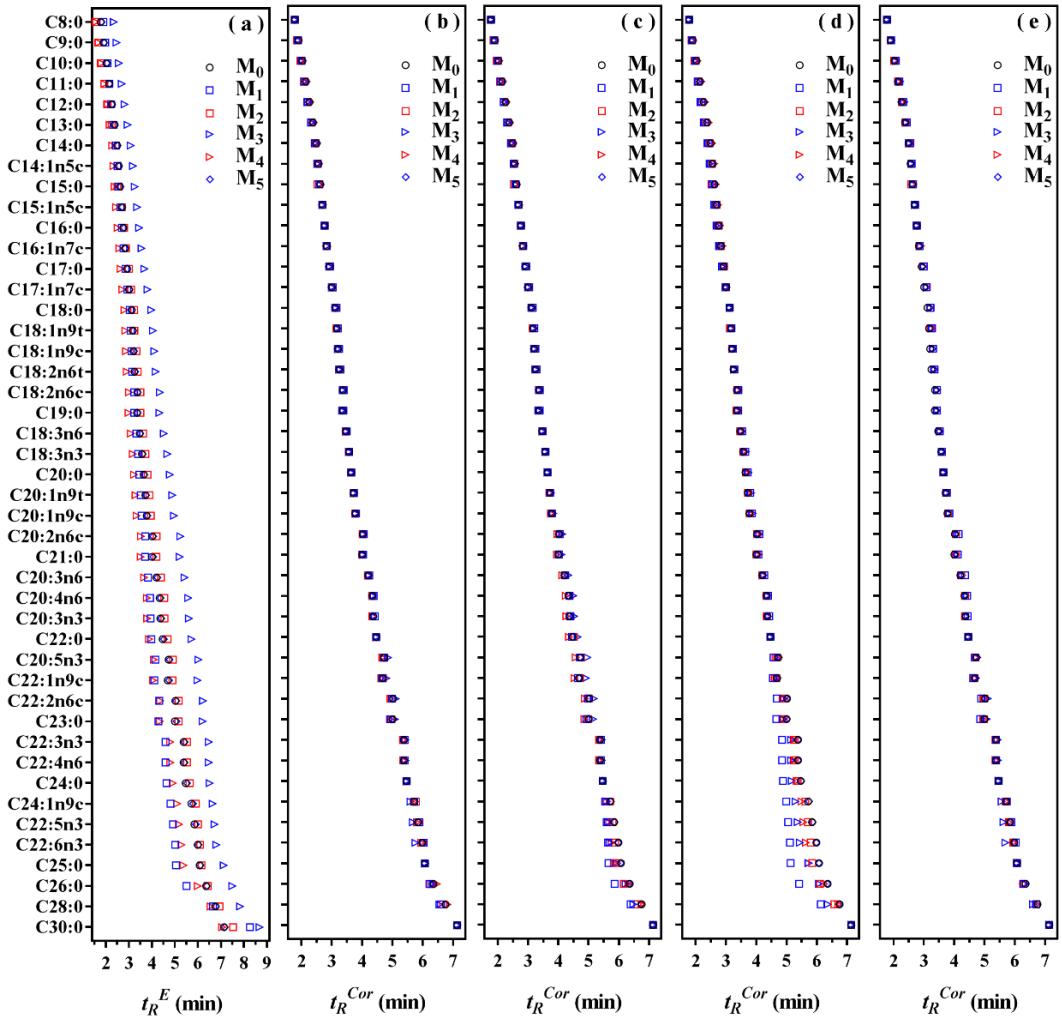

Fig. S5 No-additives reference index (NARI) to enable comparing experimental retention time (t_R^E) for 45 FAMEs obtained from the optimized method (M_0) and other ones with different temperature-gradients (M_1, M_2), flow rates (M_3, M_4) and from another acquisition batch (M_5). These include (**a**) t_R^E without correction and (**b**) NARI-corrected data (t_R^{Cor}) from NARI schemes based on endogenous FAMEs C8:0, C16:0, C20:0, C22:0, C24:0, C25:0 and C30:0, (**c**) C8:0, C16:0, C20:0, C24:0 and C30:0, (**d**) C8:0, C18:0, C22:0 and C30:0 together with (**e**) the alkanes-based RI

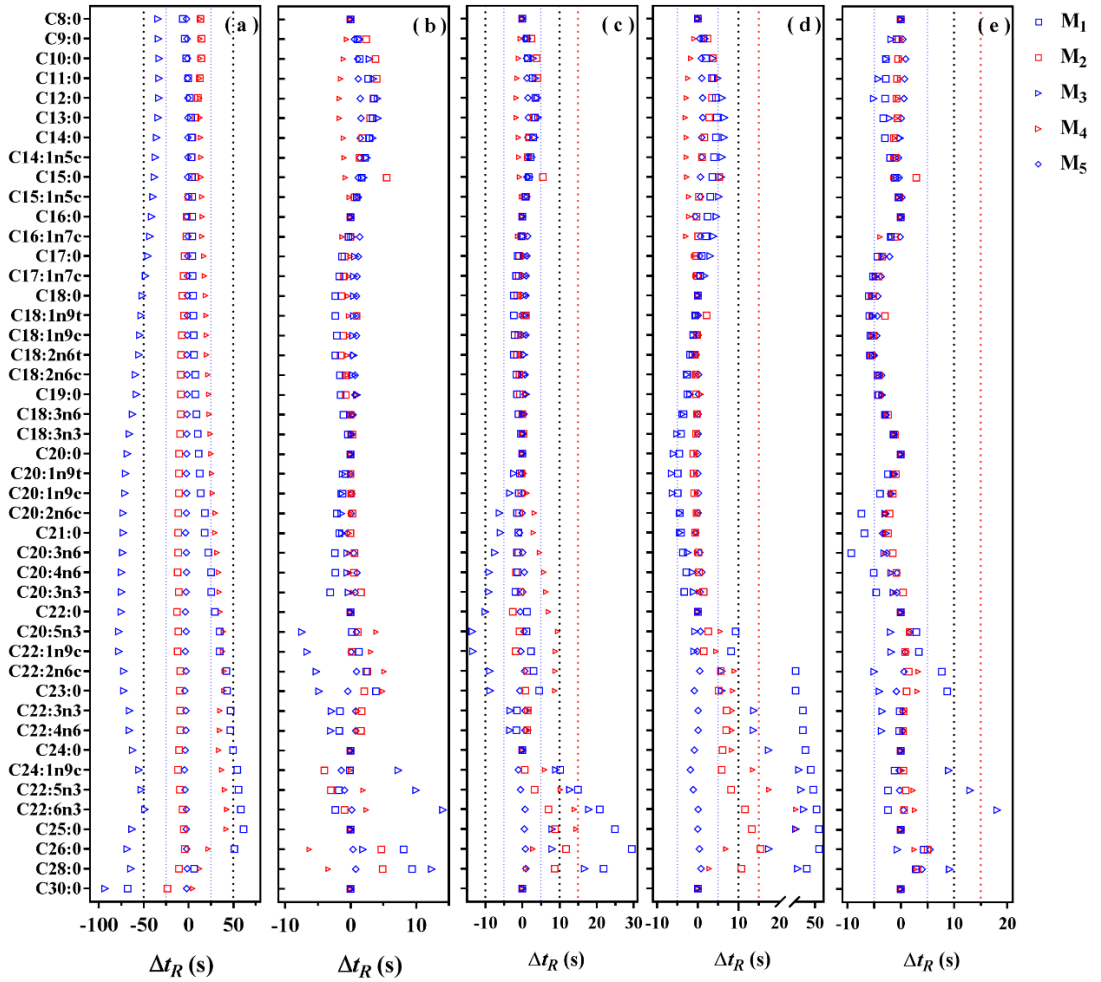

Fig. S6 Differences for t_R from different temperature-gradients, flow rates and acquisition batches compared to the standard experimental values. Data before any corrections and after corrections with the NARI derived (**a**) from 7-FAMEs (**b**), 5-FAMEs (**c**), 4-FAMEs (**d**), and the alkanes-based classical RI (**e**)

Table S1 Optimized temperature-gradient for GC-FID/MS analysis of FAMEs

	Rate (℃/min)	Temperature (℃)	Hold time (min)	Duration (min)
(Initial)		90	0.50	0.50
step1	65	130	0.10	1.22
step2	250	200	3.16	4.66
step3	80	215	0.50	5.34
step4	250	205	0.40	5.78
step5	200	250	1.40	7.41

Table S2 GC-MS parameters for FAMEs

No.	Analytes	t_R (min)	Group name	Characteristic ions
1	C8:0	1.82	1	74, 87, 127.1
2	C9:0	1.93	1	74, 87, 141.1
3	C10:0	2.05	1	74, 87, 155.1
4	C11:0	2.16	1	74, 87, 157.1
5	C12:0	2.28	1	74, 87, 171.1
6	C13:0	2.39	1	74, 87, 185.2, 75.1
7	C14:0	2.51	2	74, 87, 43, 199.1
8	C14:1n5c	2.58	2	55, 74, 69.1, 84.1
9	C15:0	2.64	2	74, 87, 43, 143.1
10	C15:1n5c	2.72	2	55, 74, 69.1, 96
11	C16:0	2.80	3	43.1, 75.1, 143.1, 55.1
12	C16:1n7c	2.85	3	55.1, 74, 69.1, 83.1
13	C17:0	2.94	3	74, 87, 43.1, 75.1
14	C17:1n7c	3.03	3	55, 74, 69.1, 83.1
15	C18:0	3.14	4	75.1, 43.1, 143.1, 55
16	C18:1n9t	3.19	4	55, 74, 69.1, 83.1
17	C18:1n9c	3.23	4	55, 74, 69.1, 83.1
18	C18:2n6t	3.28	4	67, 81.1, 95, 55
19	C18:2n6c	3.39	5	79.1, 67, 81.1, 95.1
20	C19:0	3.39	5	269.2, 74.1, 75.1
21	C18:3n6	3.50	5	79.1, 67, 81.1, 93.1
22	C18:3n3	3.60	5	79.1, 67, 93.1, 81.1
23	C20:0	3.68	6	74, 87, 75, 43.1
24	C20:1n9t	3.76	6	55, 69.1, 83.1, 97.1
25	C20:1n9c	3.81	6	55, 69.1, 97.1, 74
26	C20:2n6c	4.07	7	81.1, 95.1, 82.1, 96.1
27	C21:0	4.06	7	75, 143.1, 57.1
28	C20:3n6	4.25	7	67, 79.1, 80, 81.1
29	C20:4n6	4.38	7	106.1, 105.1, 67
30	C20:3n3	4.41	7	108.1, 67, 95.1
31	C22:0	4.53	8	74, 87, 75, 43
32	C20:5n3	4.78	8	91, 105, 106
33	C22:1n9c	4.75	8	83.1, 97.1, 74, 96.1
34	C22:2n6c	5.07	9	68.1, 81.1, 95.1, 82.1
35	C23:0	5.07	9	75, 143.1, 57.1, 71.1
36	C22:3n3	5.43	10	80.9, 92.9, 40.9, 81.9
37	C22:4n6	5.43	10	80.1, 91, 93, 81
38	C24:0	5.53	10	74, 87, 75, 42.9

No.	Analytes	t_R (min)	Group name	Characteristic ions
39	C24:1n9c	5.80	11	55, 83, 97, 69
40	C22:5n3	5.91	11	79.1, 91.1, 67, 93.1
41	C22:6n3	6.03	11	91.1, 79.1, 67, 93.1
42	C25:0	6.12	11	74, 87, 43, 74.9
43	C26:0	6.39	12	74, 87, 43, 57
44	C28:0	6.78	12	74, 87, 75, 43
45	C30:0	7.17	12	74, 87, 75, 57
IS1	C17:0-d_{33}	2.88	3	77.1, 91.1, 55.2, 50.1
IS2	C19:0-d_{37}	3.28	4	77.1, 91.1, 50.1, 155.2

The first ion was employed for quantification.

Table S3 Analytical throughput, coverage and sensitivity of this method and reported methods

Data-acquisition (min)	Coverage [a]	Carbon-chain length	Platforms	LOD (fmol)	Reference
8	29	C10–C24	GC-FID/MS	NA	Masood *et al.* 2005
15	44	C8–C28	GC-FID/MS	1.9–16	Ecker *et al.* 2012
15	37	C12–C26	GC-FID/MS	4–100	An *et al.* 2013
15	36	C3–C24	LC-MS/MS	7–22287	Zhao *et al.* 2017
29	25	C10–C24	GC-FID/MS	2.3–4578.4	Han *et al.* 2011
35	33	C4–C24	GC-FID/MS	NA	Xu *et al.* 2010
35	30	C4–C24	APGC/Q-TOFMS	0.15–0.62	Xia *et al.* 2019
37	38	C2–C24	LC-MS/MS	4–40	Jiang *et al.* 2017
8	**45**	**C8–C30**	**GC-FID/MS**	**0.5–22.8**	**This method**

[a] Number of analytes; NA: not available

Table S4 Linearity and sensitivity for fatty acids as their methyl esters with this method

No.	Analytes	t_R (min)	Linearity range (μmol/L)	R^2	LLOQ (fmol)	LOD (fmol)
1	Octanoic acid (C8:0)	1.82	0.90–44.90	0.996	5.71	1.71
2	Nonanoic acid (C9:0)	1.93	0.98–48.91	0.999	8.30	2.49
3	Decanoic acid (C10:0)	2.05	0.94–47.06	0.999	11.41	3.42
4	Hendecanoic acid (C11:0)	2.16	0.53–26.71	0.999	5.91	1.77
5	Lauric acid (C12:0)	2.28	0.23–28.52	0.996	3.23	0.97
6	Tridecanoic acid (C13:0)	2.39	0.13–15.66	0.999	1.68	0.50
7	Myristic acid (C14:0)	2.51	0.27–171.64	0.999	4.66	1.40
8	Myristoleic acid (C14:1n5c)	2.58	0.31–38.87	0.999	13.19	3.96
9	Pentadecanoic acid (C15:0)	2.64	0.17–54.46	0.999	5.54	1.66
10	Pentadecenoic acid (C15:1n5c)	2.72	0.30–37.74	0.999	7.68	2.31
11	Palmitic acid (C16:0)	2.80	17.85–2230.63	0.998	16.22	4.87
12	Palmitoleic acid (C16:1n7c)	2.85	6.16–770.41	0.999	16.87	5.06
13	Heptadecanoic acid (C17:0)	2.94	0.88–110.05	0.999	7.00	2.10
14	Heptadecenoic acid (C17:1n7c)	3.03	0.62–154.15	1.000	14.27	4.28
15	Stearic acid (C18:0)	3.14	2.40–1499.16	1.000	7.44	2.23
16	Elaidic acid (C18:1n9t)	3.19	2.34–292.07	0.999	11.22	3.37
17	Oleic acid (C18:1n9c)	3.23	22.33–2790.86	1.000	19.94	5.98
18	Linoelaidic acid (C18:2n6t)	3.28	0.24–30.41	0.998	7.74	2.32
19	Linoleic acid (C18:2n6c)	3.39	0.79–2457.95	1.000	11.77	3.53
20	Nonadecanoic acid (C19:0)	3.39	0.22–69.31	0.998	5.64	1.69
21	γ-Linolenic acid (C18:3n6)	3.50	0.41–51.61	0.999	26.27	7.88
22	α-Linolenic acid (C18:3n3)	3.60	0.42–105.35	0.999	21.45	6.43
23	Arachidic acid (C20:0)	3.68	0.12–75.08	1.000	3.06	0.92
24	Trans-11-Eicosenoic acid (C20:1n9t)	3.76	0.12–30.39	1.000	5.16	1.55
25	Cis-11-Eicosenoic acid (C20:1n9c)	3.81	1.16–289.89	1.000	9.22	2.77

No.	Analytes	t_R (min)	Linearity range (μmol/L)	R^2	LLOQ (fmol)	LOD (fmol)
26	Eicosadienoic acid (C20:2n6c)	4.07	0.20–49.42	1.000	5.03	7.55
27	Heneicosanoic acid (C21:0)	4.06	0.37–18.34	1.000	9.33	4.00
28	γ-Eicosatrienoic acid (C20:3n6)	4.25	1.01–125.62	0.999	25.58	10.96
29	Arachidonic acid (C20:4n6)	4.38	1.79–2239.96	0.998	45.60	22.80
30	α-Eicosatrienoic acid (C20:3n3)	4.41	0.37–18.36	0.996	7.79	2.34
31	Docosanoic acid (C22:0)	4.53	0.31–76.73	1.000	4.88	1.46
32	Eicosapentaenoic acid (C20:5n3)	4.78	1.07–133.21	0.998	33.90	10.17
33	Erucic acid (C22:1n9c)	4.75	1.32–164.74	0.999	14.58	4.37
34	Docosadienoic acid (C22:2n6c)	5.07	0.60–30.14	0.999	13.95	4.18
35	Tricosanoic acid (C23:0)	5.07	0.64–31.75	1.000	16.16	4.85
36	Docosatrienoic acid (C22:3n3)	5.43	0.59–29.29	1.000	13.55	4.07
37	Docosatetraenoic acid (C22:4n6)	5.43	0.72–36.02	0.999	18.33	18.33
38	Lignoceric acid (C24:0)	5.53	1.48–73.85	0.999	25.71	8.57
39	Nervonic acid (C24:1n9c)	5.80	1.39–173.25	0.999	24.13	14.48
40	Docosapentaenoic acid (C22:5n3)	5.91	2.20–109.81	0.999	38.23	19.12
41	Docosahexaenoic acid (C22:6n3)	6.03	1.90–237.56	1.000	33.08	16.54
42	Pentacosanoic acid (C25:0)	6.12	0.38–18.97	0.999	6.60	6.60
43	Hexacosanoic acid (C26:0)	6.39	0.75–18.97	0.998	13.09	5.61
44	Octacosanoic acid (C28:0)	6.78	1.62–40.61	0.994	28.28	10.60
45	Triacontanoic acid (C30:0)	7.17	1.66–41.44	0.997	19.24	5.77

Table S5 Accuracy and precision for this GC-FID/MS analysis of fatty acids as their methyl esters (n = 6)

No.	Analytes	L[a]	M[a]	H[a]	L[b]	M[b]	H[b]	L[c]	M[c]	H[c]
1	C8:0	80.07 ± 7.31	80.87 ± 5.89	105.12 ± 2.96	12.36	11.05	5.08	14.85	5.88	5.70
2	C9:0	86.68 ± 6.62	97.95 ± 6.43	103.81 ± 3.96	14.42	18.51	8.33	17.31	9.18	8.71
3	C10:0	83.78 ± 6.18	101.05 ± 4.74	103.82 ± 3.07	17.60	8.32	4.47	18.90	6.00	5.66
4	C11:0	87.43 ± 2.73	98.83 ± 4.38	107.14 ± 3.39	13.42	7.23	5.63	15.42	4.49	6.06
5	C12:0	80.03 ± 6.83	86.51 ± 5.45	83.95 ± 3.26	18.84	9.47	5.64	13.63	7.54	6.03
6	C13:0	109.01 ± 4.09	106.22 ± 2.16	94.64 ± 4.25	9.47	7.65	6.60	14.70	3.71	6.81
7	C14:0	82.38 ± 7.17	101.30 ± 5.58	96.70 ± 4.99	7.97	5.01	4.75	3.66	4.63	5.14
8	C14:1n5c	93.42 ± 5.60	100.55 ± 6.24	94.46 ± 5.40	8.08	6.70	5.56	8.62	4.97	6.44
9	C15:0	80.92 ± 6.15	96.38 ± 6.07	95.41 ± 4.78	7.18	4.80	5.14	3.71	3.91	6.11
10	C15:1n5c	99.86 ± 7.30	101.51 ± 3.59	97.77 ± 5.27	15.18	7.79	6.56	6.03	8.51	6.32
11	C16:0	102.62 ± 9.82	103.10 ± 7.75	95.30 ± 0.18	10.16	7.44	4.34	3.48	5.86	4.08
12	C16:1n7c	86.39 ± 9.85	110.55 ± 6.90	105.50 ± 2.13	9.29	5.12	5.99	5.94	4.41	7.07
13	C17:0	80.61 ± 9.15	108.30 ± 5.19	104.57 ± 3.92	7.57	5.47	4.35	3.57	6.15	4.27
14	C17:1n7c	86.84 ± 6.94	102.84 ± 7.32	98.05 ± 5.05	4.92	4.56	5.78	4.56	4.43	7.10
15	C18:0	109.57 ± 8.36	96.90 ± 6.94	97.56 ± 2.37	8.75	6.33	4.04	4.45	5.98	5.04
16	C18:1n9t	82.18 ± 5.37	100.36 ± 3.29	96.45 ± 4.64	5.15	4.71	5.43	2.54	4.51	6.15
17	C18:1n9c	84.89 ± 7.16	112.37 ± 5.95	96.76 ± 3.47	8.42	7.52	4.42	3.49	6.90	5.14
18	C18:2n6t	114.52 ± 8.61	100.45 ± 3.34	94.34 ± 1.70	12.76	6.09	5.78	8.94	6.92	5.52
19	C18:2n6c	112.21 ± 6.60	86.93 ± 5.52	92.55 ± 4.76	5.80	7.07	4.63	3.31	6.82	4.26
20	C19:0	94.21 ± 4.76	97.27 ± 4.10	93.16 ± 3.74	9.44	7.24	4.06	4.23	6.26	3.96
21	C18:3n6	103.85 ± 5.46	104.93 ± 2.99	97.02 ± 4.77	9.61	8.60	5.40	2.52	7.74	5.27
22	C18:3n3	97.96 ± 6.68	111.26 ± 2.53	103.05 ± 0.88	8.68	6.68	4.45	2.95	6.90	4.69
23	C20:0	80.79 ± 8.46	101.01 ± 6.30	99.13 ± 2.69	8.36	5.23	4.83	1.76	6.10	5.25
24	C20:1n9t	114.66 ± 4.41	102.96 ± 3.63	94.29 ± 3.58	8.54	4.04	5.51	11.52	4.21	5.34
25	C20:1n9c	83.02 ± 7.02	97.45 ± 3.24	96.28 ± 1.25	6.78	5.36	5.00	4.72	5.82	5.17

No.	Analytes	L[a]	M[a]	H[a]	L[b]	M[b]	H[b]	L[c]	M[c]	H[c]
26	C20:2n6c	82.47 ± 7.64	99.35 ± 7.76	100.50 ± 4.64	7.98	6.37	5.49	2.30	6.11	4.20
27	C21:0	84.48 ± 7.26	94.40 ± 4.08	96.50 ± 3.60	7.75	4.40	5.17	4.67	4.75	5.20
28	C20:3n6	85.29 ± 7.11	106.35 ± 5.23	95.35 ± 4.44	9.10	7.75	5.08	4.68	6.30	4.78
29	C20:4n6	107.49 ± 5.91	108.67 ± 6.10	102.52 ± 5.72	9.16	8.72	4.50	3.28	6.91	5.93
30	C20:3n3	116.23 ± 8.60	107.94 ± 7.22	94.25 ± 5.29	13.50	11.35	5.22	6.24	11.20	6.00
31	C22:0	107.43 ± 9.25	101.12 ± 7.94	97.50 ± 2.59	9.02	6.53	4.65	4.34	6.33	5.48
32	C20:5n3	115.35 ± 8.68	85.80 ± 9.45	88.42 ± 4.53	15.31	13.82	15.36	6.94	3.96	12.77
33	C22:1n9c	91.26 ± 9.00	116.51 ± 3.65	108.00 ± 3.10	10.57	8.94	4.26	9.93	7.34	4.81
34	C22:2n6c	84.61 ± 8.94	95.80 ± 2.91	90.85 ± 2.68	8.43	5.54	5.87	8.60	6.67	6.44
35	C23:0	85.92 ± 9.59	115.32 ± 5.56	102.82 ± 3.59	8.87	6.78	4.36	6.16	6.16	5.22
36	C22:3n3	81.52 ± 8.20	83.15 ± 4.09	91.15 ± 4.62	9.13	6.15	4.65	3.68	6.31	4.59
37	C22:4n6	101.61 ± 9.87	96.62 ± 3.70	96.44 ± 1.36	9.40	6.94	4.44	4.46	6.53	3.44
38	C24:0	90.68 ± 6.36	96.59 ± 3.10	92.41 ± 3.90	10.10	7.94	5.77	8.75	6.45	4.77
39	C24:1n9c	80.32 ± 7.29	86.56 ± 5.83	89.69 ± 4.09	6.89	8.31	4.59	6.92	6.93	4.57
40	C22:5n3	86.27 ± 8.23	94.47 ± 7.64	89.02 ± 4.98	7.56	6.71	4.69	7.37	4.95	4.95
41	C22:6n3	100.75 ± 6.24	93.09 ± 3.69	98.52 ± 2.45	10.32	9.59	5.15	4.25	8.50	4.62
42	C25:0	91.73 ± 6.01	112.68 ± 5.32	100.70 ± 3.87	18.34	18.29	6.57	17.52	6.50	8.00
43	C26:0	120.01 ± 6.42	99.75 ± 5.98	89.15 ± 3.84	11.93	6.97	5.05	15.71	7.55	4.75
44	C28:0	119.75 ± 6.26	108.31 ± 4.87	88.78 ± 2.14	9.42	7.11	5.81	8.86	7.35	6.66
45	C30:0	91.48 ± 3.77	94.16 ± 5.23	86.82 ± 2.61	16.95	10.62	5.38	9.20	5.55	5.36

L, M, H: low, medium, high concentration of QC working solutions; [a]: Recovery (%); [b] Inter-day and [c] Intra-day CV (%)

Table S6 Stability (CV, %) of methylated fatty-acids during GC-FID/MS analysis (n = 6)

No.	Analytes	L[a]	M[a]	H[a]	L[b]	M[b]	H[b]	L[c]	M[c]	H[c]
1	C8:0	14.00	11.90	8.29	18.58	6.35	2.75	9.23	6.73	3.83
2	C9:0	9.68	4.50	10.06	15.00	7.05	3.14	12.17	5.76	4.33
3	C10:0	14.21	5.92	6.20	16.50	4.60	2.25	13.36	4.07	3.26
4	C11:0	13.80	4.77	5.29	19.40	3.42	1.87	11.77	4.11	3.17
5	C12:0	15.89	7.56	6.78	4.97	2.00	1.62	8.78	2.64	2.68
6	C13:0	7.07	3.98	7.51	7.04	3.22	2.46	10.03	2.72	2.96
7	C14:0	8.22	4.62	6.10	1.65	1.37	1.48	2.43	1.83	2.53
8	C14:1n5c	4.29	5.23	6.15	8.91	2.02	1.85	10.50	1.57	2.41
9	C15:0	8.05	4.24	6.28	1.94	2.83	1.55	3.82	3.08	2.84
10	C15:1n5c	16.59	11.29	6.55	18.07	4.62	2.16	6.38	6.16	2.72
11	C16:0	8.05	4.82	4.80	1.38	1.16	1.23	1.61	1.27	1.34
12	C16:1n7c	8.86	3.80	5.00	3.86	7.84	3.62	3.51	4.15	4.21
13	C17:0	7.64	4.71	5.21	6.07	2.95	2.11	7.17	3.24	3.09
14	C17:1n7c	4.52	4.40	5.41	9.79	3.40	1.60	3.69	3.26	2.13
15	C18:0	8.93	5.01	4.50	0.89	1.05	0.68	2.97	0.82	0.70
16	C18:1n9t	7.32	4.59	4.88	5.36	2.81	0.93	4.34	2.57	1.62
17	C18:1n9c	9.37	5.69	4.47	0.63	1.14	1.00	1.62	2.02	0.93
18	C18:2n6t	13.32	5.37	7.36	17.64	5.96	2.52	15.27	3.05	2.65
19	C18:2n6c	10.69	5.85	4.70	0.60	0.41	0.59	1.74	0.58	1.12
20	C19:0	7.42	4.90	5.44	2.36	2.00	2.19	2.15	2.79	3.17
21	C18:3n6	12.93	6.76	5.35	5.31	3.21	3.87	3.34	1.17	1.57
22	C18:3n3	8.22	5.79	4.87	3.71	3.25	1.56	3.13	2.32	2.32
23	C20:0	9.39	5.22	4.50	9.09	7.55	4.36	7.40	2.91	3.24
24	C20:1n9t	8.81	4.54	5.50	6.51	6.21	3.32	13.54	2.44	1.68
25	C20:1n9c	6.02	4.82	6.03	7.65	2.54	1.56	6.33	2.77	2.69

No.	Analytes	L[a]	M[a]	H[a]	L[b]	M[b]	H[b]	L[c]	M[c]	H[c]
26	C20:2n6c	7.51	5.35	5.00	3.66	3.81	2.82	3.05	2.79	1.91
27	C21:0	8.85	4.33	5.67	3.94	3.90	2.74	17.91	2.89	1.43
28	C20:3n6	8.91	5.86	4.60	2.21	2.45	2.78	2.18	1.64	2.07
29	C20:4n6	7.27	6.02	5.17	3.00	2.34	1.35	4.28	2.59	1.35
30	C20:3n3	11.45	9.37	5.94	5.71	2.33	1.85	5.72	2.90	3.14
31	C22:0	11.18	5.64	5.57	3.65	3.66	2.87	4.38	2.25	4.79
32	C20:5n3	15.24	18.26	10.90	8.76	12.19	7.40	7.88	9.41	7.37
33	C22:1n9c	10.16	6.63	3.36	6.96	3.89	2.80	4.88	4.04	4.19
34	C22:2n6c	8.52	10.09	5.61	19.62	3.75	2.82	6.59	8.88	4.09
35	C23:0	10.59	5.41	6.14	9.22	6.46	3.51	7.86	3.03	2.24
36	C22:3n3	9.34	5.06	4.27	11.14	7.52	2.07	11.21	10.17	2.92
37	C22:4n6	11.15	8.84	5.07	14.52	4.31	2.21	9.85	4.95	7.27
38	C24:0	10.66	5.96	4.94	2.21	3.63	3.15	3.91	2.96	1.65
39	C24:1n9c	10.65	6.84	4.92	3.39	1.57	1.31	4.09	3.62	3.07
40	C22:5n3	10.29	7.05	6.28	4.86	2.93	2.79	6.22	2.58	2.82
41	C22:6n3	9.87	7.64	5.48	1.84	2.29	0.94	4.40	2.56	1.61
42	C25:0	19.51	11.26	8.48	13.96	8.97	7.95	16.97	14.30	3.03
43	C26:0	15.00	6.88	9.50	3.83	8.72	3.43	11.14	11.19	2.95
44	C28:0	11.82	9.78	6.71	11.26	8.29	2.05	12.01	6.28	2.52
45	C30:0	14.75	11.47	7.59	3.94	11.61	2.27	17.75	7.51	1.93

L, M, H: low, medium, high concentration of QC working solutions; [a] 20 ℃; [b] 4 ℃; [c] –80 ℃

Table S7 Fatty acid composition of some typical biological matrices (Mean ± SD, n = 5)

Analytes	Human urine (μmol/L)	Human plasma (μmol/L)	Mouse cecum content (μg/g)	H1299 cells (μg/g)	Rabbit liver tissue (μg/g)
C10:0	4.56 ± 0.37	5.02 ± 0.54[*]	2.57 ± 0.19	1.64 ± 0.15	1.82 ± 0.14
C11:0	ND	2.52 ± 0.32[*, #]	1.75 ± 0.14	0.64 ± 0.05	0.95 ± 0.08
C12:0	1.32 ± 0.15	3.78 ± 0.38[*]	9.54 ± 0.63	7.02 ± 0.44	4.32 ± 0.62
C14:0	9.40 ± 0.09	34.72 ± 3.21	79.80 ± 8.71	257.55 ± 8.96	59.13 ± 11.44
C14:1n5c	ND	7.47 ± 0.10[#]	ND	21.38 ± 0.83	4.22 ± 0.64
C15:0	ND	16.08 ± 1.15[*]	187.69 ± 19.38	16.42 ± 0.61	98.98 ± 10.68
C16:0	13.74 ± 1.08	1968.39 ± 100.91	1444.76 ± 145.24	1951.09 ± 62.18	5693.39 ± 523.05
C16:1n7c	ND	70.27 ± 4.24	67.17 ± 5.03	1482.05 ± 55.62	91.57 ± 11.17
C16:1n9c	ND	85.76 ± 6.91	ND	62.32 ± 18.89	30.41 ± 9.88
C17:0	ND	25.86 ± 0.73[*]	71.97 ± 7.02	9.60 ± 0.29	256.27 ± 23.91
C17:1n7c	ND	25.38 ± 0.18[*, #]	11.25 ± 0.56	40.10 ± 1.40	53.05 ± 4.49
C18:0	58.49 ± 0.96	463.62 ± 40.88	958.14 ± 91.32	366.83 ± 15.08	7315.10 ± 657.80
C18:1n7c	ND	230.73 ± 24.54	ND	3159.07 ± 751.15	ND
C18:1n9c	90.34 ± 1.77	1152.98 ± 126.56	933.82 ± 75.00	2539.72 ± 111.47	2501.75 ± 283.58
C18:2n6c	168.28 ± 2.20	4131.60 ± 297.00	884.17 ± 121.82	145.30 ± 4.28	11274.30 ± 1079.85
C19:0	ND	ND	10.06 ± 0.58	ND	33.13 ± 2.54
C18:3n6	ND	39.75 ± 1.27[#]	12.59 ± 0.56	10.33 ± 0.84	32.89 ± 3.81
C18:3n3	ND	59.37 ± 3.32[*]	28.35 ± 1.19	10.28 ± 1.20	267.77 ± 37.41
C20:0	ND	27.10 ± 1.42	106.45 ± 8.79	14.45 ± 0.50	87.14 ± 7.31
C20:1n9c	ND	17.05 ± 0.75	67.67 ± 5.62	235.46 ± 8.77	49.42 ± 5.66
C20:1n11c	ND	ND	168.44 ± 43.1	47.69 ± 2.13	ND
C20:2n6c	ND	43.34 ± 1.21	26.58 ± 2.05	17.75 ± 1.00	739.18 ± 107.90
C20:3n6	ND	130.45 ± 7.07	198.95 ± 22.40	46.72 ± 1.91	404.10 ± 38.66
C20:4n6	ND	879.95 ± 31.47	236.82 ± 34.76	216.87 ± 8.87	3361.34 ± 346.47
C20:3n3	ND	ND	9.87 ± 0.79	10.79 ± 0.84	44.69 ± 7.07

Analytes	Human urine (μmol/L)	Human plasma (μmol/L)	Mouse cecum content (μg/g)	H1299 cells (μg/g)	Rabbit liver tissue (μg/g)
C22:0	ND	127.54 ± 4.62	184.66 ± 20.95	21.09 ± 2.74	327.01 ± 33.90
C22:1n9c	15.80 ± 0.36	19.18 ± 2.83	18.17 ± 1.62	24.85 ± 1.55	22.71 ± 1.48
C22:1n11c	ND	ND	ND	5.91 ± 1.17	ND
C20:5n3	ND	76.04 ± 3.04	36.45 ± 3.23	31.53 ± 1.04	59.92 ± 8.07
C22:2n9c	ND	ND	ND	3.66 ± 0.47	ND
C23:0	ND	15.95 ± 0.68*, #	14.16 ± 1.20	4.83 ± 0.28	114.14 ± 13.31
C22:3n3	ND	12.29 ± 1.15*, #	8.09 ± 0.91	18.97 ± 0.90	384.56 ± 55.96
C22:4n6	ND	12.97 ± 0.54#	11.16 ± 1.18	23.40 ± 1.10	523.82 ± 77.68
C24:0	ND	77.19 ± 3.76	97.14 ± 9.66	62.64 ± 3.07	126.68 ± 11.54
C24:1n9c	ND	93.85 ± 3.46	22.84 ± 1.82	180.22 ± 5.10	147.24 ± 10.75
C22:5n3	ND	56.18 ± 2.33	32.81 ± 2.90	33.06 ± 1.29	202.43 ± 22.57
C22:5n6	ND	ND	ND	ND	173.05 ± 18.98
C22:6n3	ND	284.57 ± 14.16	144.48 ± 13.04	140.92 ± 8.76	206.03 ± 22.92
C25:0	ND	ND	8.14 ± 1.36	2.34 ± 0.60	7.55 ± 1.13
C26:0	ND	ND	34.07 ± 4.12	20.14 ± 0.72	ND
SFA	94.73 ± 5.66	2770.02 ± 148.01	3020.36 ± 76.35	2736.63 ± 90.25	14128.98 ± 1266.55
MUFA	106.14 ± 2.02	1702.67 ± 166.77	1242.7 ± 41.87	7798.76 ± 829.38	2900.37 ± 318.47
PUFA	168.28 ± 2.20	5726.51 ± 354.92	1533.18 ± 39.30	705.93 ± 22.53	17674.08 ± 1793.37
n3PUFA	ND	488.45 ± 20.85	249.58 ± 18.68	245.55 ± 9.58	1165.41 ± 150.58
n6PUFA	168.28 ± 2.20	5238.06 ± 334.13	1283.59 ± 43.80	460.38 ± 13.30	16508.67 ± 1644.65
n3/n6	ND	0.09 ± 0.00**	0.19 ± 0.02	0.53 ± 0.01	0.07 ± 0.00**
ToFA	369.14 ± 4.14	10199.2 ± 657.05	5796.24 ± 147.47	11244.98 ± 845.13	34703.43 ± 3331.68
C16:1n7c/C16:0 (SCD1)	ND	0.04 ± 0.00**	0.05 ± 0.00	0.76 ± 0.01	0.02 ± 0.00
C18:1n9c/C18:0 (SCD1)	1.55 ± 0.05	2.48 ± 0.07	0.98 ± 0.02	6.93 ± 0.34	0.34 ± 0.02
C18:3n6/C18:2n6 (FADS2)	ND	0.01 ± 0.00	0.01 ± 0.00	0.07 ± 0.00	ND
C20:4n6/C20:3n6 (FADS1)	ND	6.75 ± 0.28	1.19 ± 0.16	4.64 ± 0.06	8.31 ± 0.11

Analytes	Human urine (μmol/L)	Human plasma (μmol/L)	Mouse cecum content (μg/g)	H1299 cells (μg/g)	Rabbit liver tissue (μg/g)
C20:3n6/C18:3n6 (ELOVL5)	ND	3.28 ± 0.16	15.8 ± 1.52	4.55 ± 0.47	12.32 ± 0.65
C22:5n3/C20:5n3 (ELOVL2)	ND	0.74 ± 0.03	0.90 ± 0.08	1.05 ± 0.03	3.39 ± 0.18
C22:4n6/C20:4n6 (ELOVL5)	ND	0.01 ± 0.00	0.05 ± 0.00	0.11 ± 0.00	0.16 ± 0.01
C18:0/C16:0 (ELOVL6)	4.28 ± 0.31	0.24 ± 0.01	0.66 ± 0.02	0.19 ± 0.00	1.29 ± 0.04
C22:4n6/ C18:3n6 (Elongases)	ND	0.33 ± 0.01	0.89 ± 0.07	2.27 ± 0.17	15.91 ± 1.4

ND: not detected; [*]not detected in Tremblay-Franco *et al.* (2015) and [#] not detected in Han *et al.* (2011). [**] Ratio value without unit. SCD1: stearoyl-CoA desaturase 1. FADS: Fatty acid desaturase. ELOVL: elongation of very long-chain fatty acids protein 6 (*i.e.*, elongation of very long-chain fatty acid-like fatty acid elongase)

Table S8 Retention time from QSRR calculation (t_R^C), experimental measurements in biological samples (t_R^E) and analyte standards (t_R^{std}) for six tested fatty-acids in the form of methyl esters, which were not included in model building

Analytes	t_R^C (min)	t_R^E (min)	Δt_R ($\lvert t_R^E - t_R^C \rvert$)	t_R^{std} (min)	Δt_R ($\lvert t_R^E - t_R^{std} \rvert$)	Characteristic Ions
C16:1n9c	2.72	2.82	0.10	2.80	0.02	74, 55, 69.1, 83.1
C18:1n7c	3.02	3.18	0.16	3.19	0.01	55, 69.1, 74, 83.1
C20:1n11c	3.40	3.71	0.31	3.72	0.01	55, 69.1, 74, 40.9
C22:1n11c	4.20	4.58	0.38	4.58	0.00	55, 74, 83.1, 97.1
C22:2n9c *	4.48	4.85	0.37	/	/	96.1, 81.1, 82.1, 95.1
C22:5n6	5.14	5.42	0.28	5.42	0.00	79, 91, 80.1, 93

* Commercial standard is unavailable for the time being and identified based on NIST database

Table S9 Retention time variations (Δt_R, min) for 45 FAMEs (C8–C30) against the first injection of mixed FAME standards (Stds-Level 1) for human urine, plasma, H1299C cells, mouse feces and rabbit liver tissue analyzed in the same batch (2–31 injections); Δt_R was below 0.04 min for all (in an Excel file)

Table S10 Standards for fatty acids and sources

No.	Analytes	CAS	Supplier	Stock solution (mmol/L)
1	Octanoic acid (C8:0)	124-07-2	Shanghai Aladdin Bio-Chem Technology Co., LTD	0.40
2	Nonanoic acid (C9:0)	112-05-0	Shanghai Aladdin Bio-Chem Technology Co., LTD	0.44
3	Decanoic acid (C10:0)	334-48-5	Shanghai Aladdin Bio-Chem Technology Co., LTD	0.43
4	Hendecanoic acid (C11:0)	112-37-8	Shanghai Aladdin Bio-Chem Technology Co., LTD	0.24
5	Lauric acid (C12:0)	143-07-7	Shanghai Aladdin Bio-Chem Technology Co., LTD	0.52
6	Tridecanoic acid (C13:0)	638-53-9	Shanghai Aladdin Bio-Chem Technology Co., LTD	0.29
7	Myristic acid (C14:0)	544-63-8	Shanghai Aladdin Bio-Chem Technology Co., LTD	3.15
8	Myristoleic acid (C14:1n5c)	544-64-9	Sigma-aldrich (Shanghai) Trading Co., LTD	0.71
9	Pentadecanoic acid (C15:0)	1002-84-2	Shanghai Aladdin Bio-Chem Technology Co., LTD	0.99
10	Pentadecenoic acid (C15:1n5c)	84743-29-3	Anpel Laboratory Techno logies (Shanghai) Inc.	0.35
11	Palmitic acid (C16:0)	57-10-3	Shanghai Aladdin Bio-Chem Technology Co., LTD	20.28
12	Palmitoleic acid (C16:1n7c)	373-49-9	Shanghai Aladdin Bio-Chem Technology Co., LTD	7.08
13	Heptadecanoic acid (C17:0)	506-12-7	Shanghai Aladdin Bio-Chem Technology Co., LTD	1.01
14	Heptadecenoic acid (C17:1n7c)	29743-97-3	Shanghai Aladdin Bio-Chem Technology Co., LTD	1.42
15	Stearic acid (C18:0)	57-11-4	Shanghai Aladdin Bio-Chem Technology Co., LTD	13.77
16	Elaidic acid (C18:1n9t)	112-79-8	Shanghai Aladdin Bio-Chem Technology Co., LTD	2.66

No.	Analytes	CAS	Supplier	Stock solution (mmol/L)
17	Oleic acid (C18:1n9c)	112-80-1	Shanghai Aladdin Bio-Chem Technology Co., LTD	25.37
18	Linoelaidic acid (C18:2n6t)	506-21-8	Shanghai yuanye Bio-Technology Co., Ltd	0.28
19	Linoleic acid (C18:2n6c)	60-33-3	Shanghai Aladdin Bio-Chem Technology Co., LTD	22.35
20	Nonadecanoic acid (C19:0)	646-30-0	Shanghai Aladdin Bio-Chem Technology Co., LTD	1.27
21	γ-Linolenic acid (C18:3n6)	506-26-3	Shanghai Aladdin Bio-Chem Technology Co., LTD	0.48
22	α-Linolenic acid (C18:3n3)	463-40-1	Shanghai Aladdin Bio-Chem Technology Co., LTD	0.96
23	Arachidic acid (C20:0)	506-30-9	Sigma-aldrich (Shanghai) Trading Co., LTD	0.68
24	Trans-11-Eicosenoic acid (C20:1n9t)	62322-84-3	Sigma-aldrich (Shanghai) Trading Co., LTD	0.28
25	Cis-11-Eicosenoic acid (C20:1n9c)	5561-99-9	Shanghai Aladdin Bio-Chem Technology Co., LTD	2.66
26	Eicosadienoic acid (C20:2n6c)	2091-39-6	Sigma-aldrich (Shanghai) Trading Co., LTD	0.45
27	Heneicosanoic acid (C21:0)	2363-71-5	Shanghai Aladdin Bio-Chem Technology Co., LTD	0.17
28	γ-Eicosatrienoic acid (C20:3n6)	1783-84-2	Shanghai Aladdin Bio-Chem Technology Co., LTD	1.14
29	Arachidonic acid (C20:4n6)	506-32-1	Shanghai Aladdin Bio-Chem Technology Co., LTD	20.36
30	α-Eicosatrienoic acid (C20:3n3)	2091-27-2	Anpel Laboratory Techno logies (Shanghai) Inc.	0.17
31	Docosanoic acid (C22:0)	112-85-6	Shanghai Aladdin Bio-Chem Technology Co., LTD	0.70
32	Eicosapentaenoic acid (C20:5n3)	10417-94-4	Shanghai Aladdin Bio-Chem Technology Co., LTD	1.22
33	Erucic acid (C22:1n9c)	112-86-7	Shanghai Aladdin Bio-Chem Technology Co., LTD	1.50
34	Docosadienoic acid (C22:2n6c)	7370-49-2	Anpel Laboratory Techno logies (Shanghai) Inc.	0.27
35	Tricosanoic acid (C23:0)	2433-96-7	TCI(Shanghai)Development Co., Ltd.	0.30
36	Docosatrienoic acid (C22:3n3)	28845-86-5	Shanghai yuanye Bio-Technology Co., Ltd	0.27

No.	Analytes	CAS	Supplier	Stock solution (mmol/L)
37	Docosatetraenoic acid (C22:4n6)	28874-58-0	Nu-Chek Prep.INC.	0.33
38	Lignoceric acid (C24:0)	557-59-5	Shanghai Aladdin Bio-Chem Technology Co., LTD	0.68
39	Nervonic acid (C24:1n9c)	506-37-6	Shanghai Aladdin Bio-Chem Technology Co., LTD	1.59
40	Docosapentaenoic acid (C22:5n3)	24880-45-3	Shanghai yuanye Bio-Technology Co., Ltd	1.01
41	Docosahexaenoic acid (C22:6n3)	6217-54-5	Shanghai Aladdin Bio-Chem Technology Co., LTD	2.18
42	Pentacosanoic acid (C25:0)	506-38-7	Shanghai Aladdin Bio-Chem Technology Co., LTD	0.17
43	Hexacosanoic acid (C26:0)	506-46-7	Shanghai Aladdin Bio-Chem Technology Co., LTD	0.16
44	Octacosanoic acid (C28:0)	506-48-9	Sigma-aldrich (Shanghai) Trading Co., LTD	0.17
45	Triacontanoic acid (C30:0)	506-50-3	Sigma-aldrich (Shanghai) Trading Co., LTD	0.37
46	Heptadecanoic-d_{33} acid (C17:0-d_{33})	352431-41-5	Beijing Zhen Xiang Technology Co., LTD	1.55
47	Nonadecanoic-d_{37} acid (C19:0-d_{37})	1219798-49-8	Beijing Zhen Xiang Technology Co., LTD	0.98

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