Electronic Supplementary Material (ESI) for Analytical Methods.

Mixed-mode chromatography-mass spectrometry enables targeted and untargeted screening of carboxylic acids in biological samples

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Supplementary information

Table S1 Optimized MRM transitions and retention times for underivatized carboxylic acids with retention time values acquired under optimized conditions on the BEH C18 AX column.

Analyte	Precursor	Products	Collision energy [V]	Quant/qual	Retention time [min]
fumaric acid	115.0	71.1	6	quant	2.95
succinic acid	117.0	73.0	10	quant	2.21
		99.0	6	qual	
oxaloacetic acid	131.0	87.0	18	quant	3.46
malic acid	133.0	115.0	6	quant	1.32
		71.0	14	qual	
citric acid	191.0	111.1	10	quant	2.28
		87.1	18	qual	
isocitric acid	191.0	111.1	10	quant	1.57
		173.0	6	qual	
pyruvic acid	87.0	43.0	5	quant	3.02
2-oxoglutaric acid	145.0	57.0	9	quant	3.40
cis-aconitic acid	173.0	85.0	9	quant	5.90
		129.0	9	qual	
lactic acid	89.0	43.0	9	quant	1.47
		41.0	37	qual	
citric acid-D_4	195.0	113.0	5	quant	2.26
		88.6	17	qual	
malic acid-$^{13}C_4$	137.0	119.0	9	quant	1.33
		74.0	17	qual	
succinic acid-D_4	121.0	77.0	13	quant	2.22
		102.0	9	qual	
pyruvic acid-$^{13}C_3$	90.0	45.0	5	quant	3.01

2-oxoglutaric acid-$^{13}C_4$	149.0	60.0	9	quant	3.40
fumaric acid-$^{13}C_4$	119.0	74.0	5	quant	2.94
lactic acid-$^{13}C_3$	92.0	45.0 43.0	13 37	quant qual	1.49

Table S2 LOD, LOQ values, recovery, linearity and repeatability in peak areas for carboxylic acids analyzed on a BEH C18 AX column

Analyte	Internal standard	LOD [µM]	LOQ [µM]	Linear calibration range [µM]	R^2	Weight	Recovery [%]	RSD of peak areas of unlabelled metabolites [%] $n = 6$
fumaric acid	fumaric acid-$^{13}C_4$	0.1	0.5	0.5 – 200	0.9992	1/x	96	4
succinic acid	succinic acid-D_4	0.05	0.1	0.1 – 200	0.9983	1/x	99	38
malic acid	malic acid-$^{13}C_4$	0.02	0.1	0.1 – 200	0.9982	1/x	97	3
citric acid	citric acid-D_4	0.1	0.5	0.5 – 200	0.9978	1/x	98	1
pyruvic acid	pyruvic acid-$^{13}C_4$	1.0	5.0	5 – 200	0.9970	1/x	83	2
2-oxoglutaric acid	2-oxoglutaric acid-$^{13}C_4$	0.5	3.0	3 – 200	0.9975	1/x	97	1
lactic acid	lactic acid-$^{13}C_3$	0.1	1.0	1 – 200	0.9975	1/x	86	8
isocitric acid	malic acid-$^{13}C_4$	0.5	10.0	10 – 200	0.9971	1/x	no isotopically labelled standards available	2
cis-aconitic acid	fumaric acid-$^{13}C_4$	0.1	10.0	10 – 200	0.9855	1/x		4

R^2 – coefficient of determination

Table S3 Accuracy and precision for carboxylic acids evaluated on standard solutions

	Accuracy[a] [%]						Precision[b] [%]					
	intraday			interday			intraday			interday		
acid	**low**	**medium**	**high**	**low**	**medium**	**high**	**low**	**medium**	**high**	**low**	**medium**	**high**
fumaric acid	99.5	96.4	98.0	103.5	103.1	100.2	4.1	1.8	1.3	4.3	2.6	1.5
succinic acid	94.1	98.2	97.1	96.2	97.4	94.7	1.6	1.1	0.4	2.9	1.1	1.1
malic acid	98.5	97.4	96.7	95.3	92.5	90.9	1.1	0.8	0.28	1.7	2.1	3.4
citric acid	93.8	102.8	97.7	97.5	110.9	103.8	1.0	0.8	0.9	1.2	3.7	2.8
pyruvic acid	106.1	98.0	91.4	113.1	108.0	104.5	9.2	7.3	7.4	7.9	8.9	6.4
2-oxoglutaric acid	105.2	96.2	93.6	108.9	100.2	94.0	7.3	7.5	4.2	7.1	7.3	3.4
lactic acid	97.2	98.8	102.4	114.0	97.6	96.8	4.6	0.8	1.6	5.7	1.3	2.7
isocitric acid	102.9	95.6	92.9	96.0	87.8	90.4	0.1	0.3	0.6	0.6	6.0	2.0
cis-aconitic acid	139.8	89.3	110.4	131.8	84.9	116.6	0.8	1.1	0.5	1.4	1.2	5.1

[a] accuracy calculated as a closeness of back-calculated concentration to the nominal concentration, $n = 3$

[b] precision calculated as RSD of repeated measurements of the same solution, $n = 6$

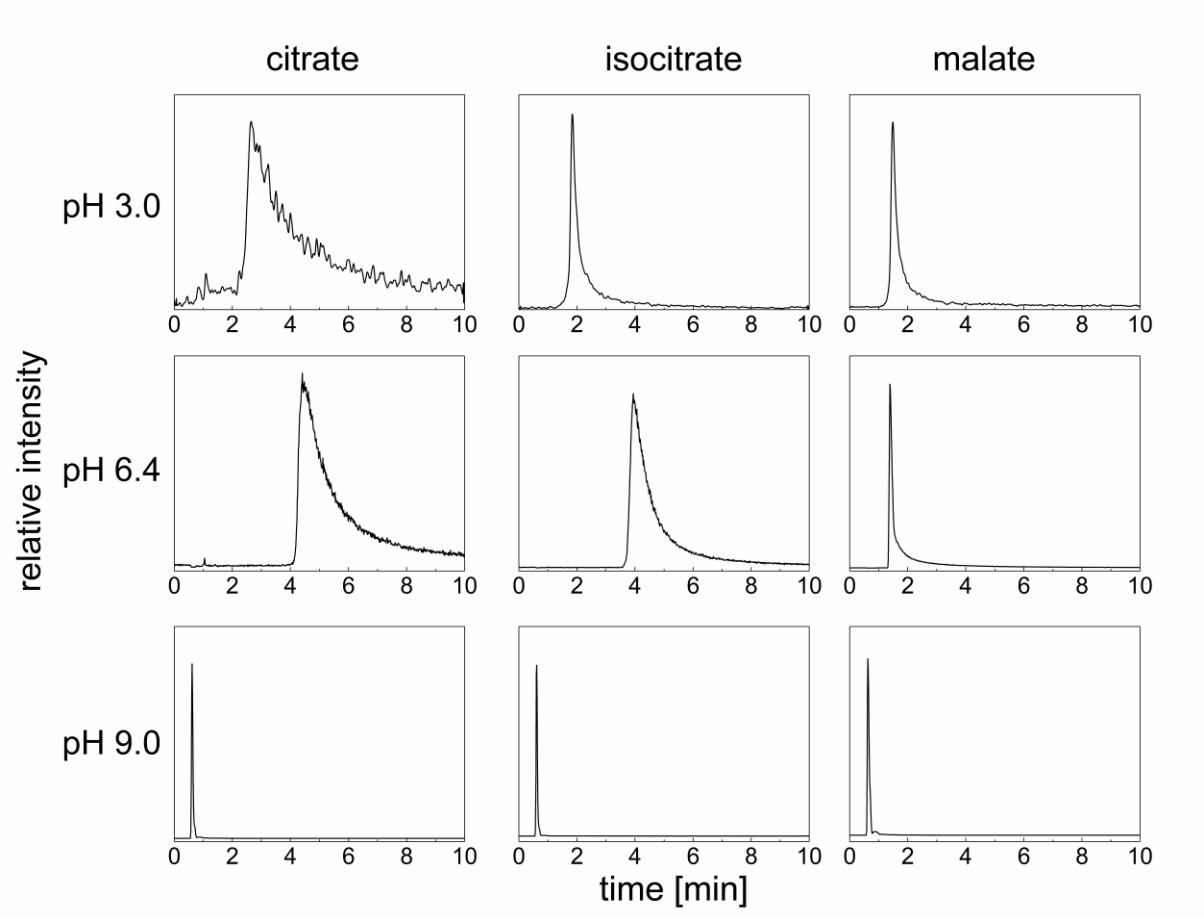

Fig. S1 Comparison of retention of 50 µM citrate, isocitrate and malate at different pH of 10 mM ammonium formate in water/acetonitrile 80/20 (v/v), isocratic elution. Measured by LC-MS/MS in MRM mode. MRM transitions: citrate – 191.0 > 111.1, isocitrate – 191.0 > 173.0, malate – 133.0 >115.0

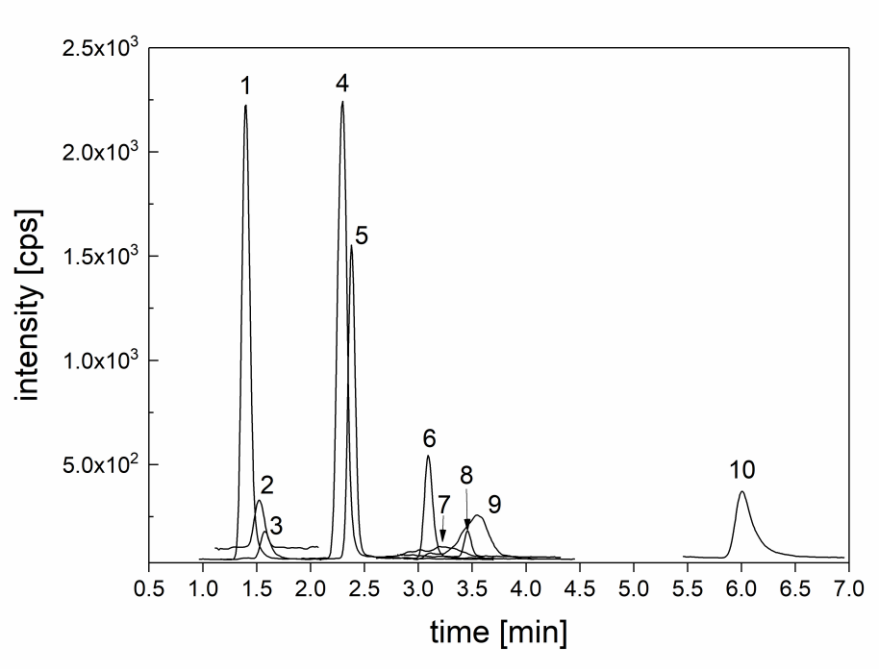

Fig. S2 Dynamic MRM chromatogram of a standard mixture of underivatized carboxylic acids analyzed on a BEH C18 AX column. Mixture of 5 µM acids, except for fumaric acid (20 µM), 2-oxoglutaric acid (80 µM) and pyruvic acid (80 µM). Peak assignment: 1 – malic acid, 2 – lactic acid, 3 – isocitric acid, 4 – succinic acid, 5 – citric acid, 6 – fumaric acid, 7 – pyruvic acid, 8 – oxaloacetic acid, 9 – 2-oxoglutaric acid, 10 – cis-aconitic acid. Instrumental conditions described in footnote

5 µL of a sample injected to an Atlantis Premier BEH C_{18} AX column (100×2.1 mm, 1.7 µm, Waters, MA, USA). The mobile phase composed of (A) 10 mM ammonium formate in water of final pH 2.6 and (B) 10 mM ammonium formate in 50/50 water/methanol (*v*/*v*) of pH 9.4 (pH of aqueous buffer before mixing with methanol). The mobile phase flow rate of 0.35 mL/min with the following gradient: 0.0 min (0% B), 0.5 min (0% B), 3.0 min (70% B), 6.0 min (70% B), 7.0 min (0% B), 10.0 min (0% B). Column was thermostated at 30 °C. Analytes ionized in ESI operated in the negative mode. The source and gas parameters as follows: ion spray voltage -3.5 kV, gas temperature 150 °C, drying gas flow 11 L/min, nebulizer pressure 30 psi, sheath gas temperature 400 °C, sheath gas flow 12 L/min, fragmentor 380 V.

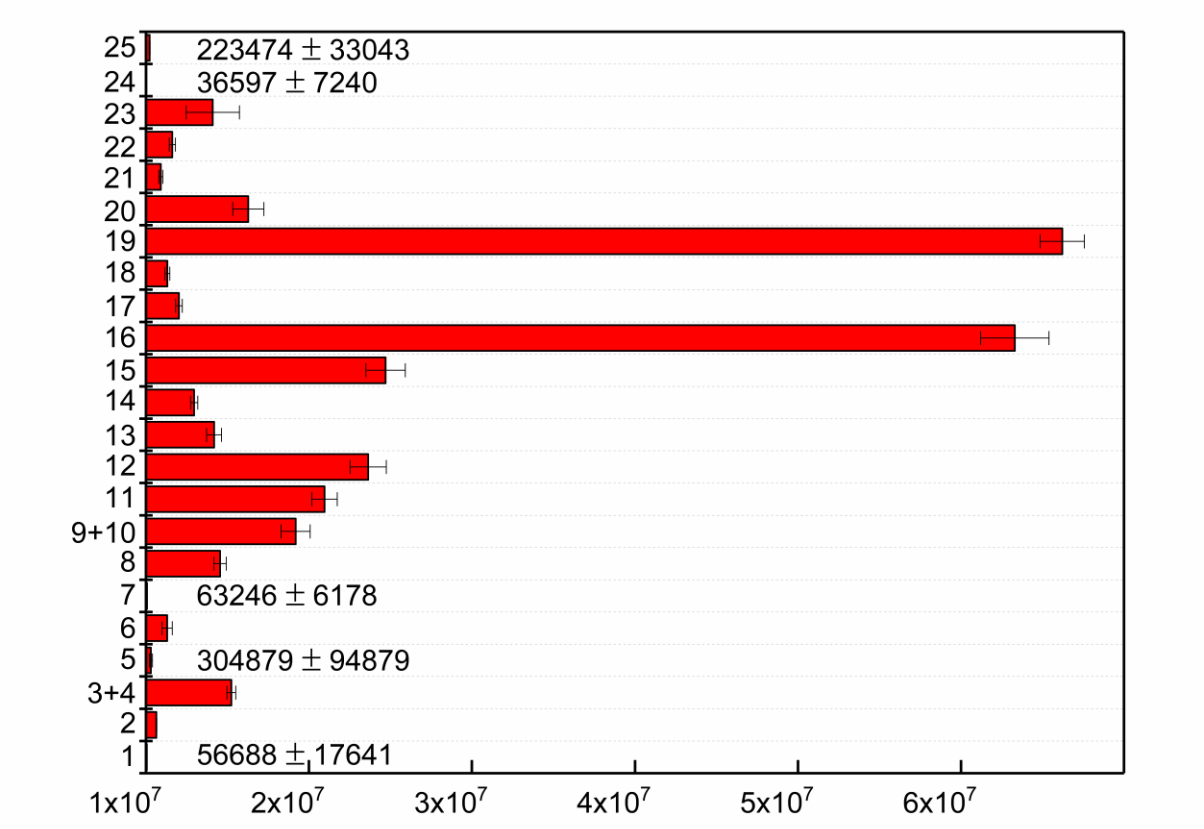

Fig. S3 Peak areas ± SD of fatty acids identified with HR-MS in human plasma samples (*n* = 10). The number of each bar corresponds with the number of the identified fatty acid in Table S4 and Fig. 3.

Table S4 A complete list of all fatty acid standards tested on the BEH C18 AX column. The fatty acids identified in human plasma, cells, and plants are highlighted in bold

# of identified acid	Carboxylic acid	$[M-H]^-$ m/z theoretical	$[M-H]^-$ m/z experimental	t_R[min]	Peak area (RSD, %)		
					plasma	cells	plant
	2-Hydroxyoctanoic acid	159.10267	159.09480	1.09			
	Prostaglandin E2	351.21770	333.20658	1.10			
	Prostaglandin D2	351.21770	351.21816	1.10			
	13,14-Dihydro-15-keto prostaglandin E2	351.21770	333.20816	1.13			
	13,14-Dihydro-15-keto prostaglandin D2	351.21770	333.20816	1.14			
	Leukotriene B4	335.22278	335.22335	1.24			
	Traumatic acid	227.12888	227.12939	1.30			
	2-hydroxydecanoic acid	187.13397	187.13429	1.34			
1	**Octanoic acid**	**143.10775**	**143.10810**	**1.35**	88649 (31)	2992 (10.1)	
	9,10-Dihydroxy-octadecenoic acid 12,13-Dihydroxy-octadecenoic acid	313.23843	313.23909	1.36			
	9-Hydroxy-octadecatrienoic acid	293.21221	293.21285	1.44			
2	**12-Hydroxy-eicosapentaenoic acid**	**317.21222**	**317.21307**	**1.45**	635262 (4.2)		
	13-Hydroxy-octadecatrienoic acid	293.21221	293.21285	1.46			
	13-Hydroxy-octadecatrienoic acid (gamma)	293.21221	293.21285	1.46			
	16-Hydroxy-docosahexaenoic acid	343.22787	343.22880	1.55			
3+4	**9-Hydroxyoctadecadienoic acid**	**295.22787**	**295.22851**	**1.56**	5239113 (5.1)		5101 (15.8)
	13-Hydroxyoctadecadienoic acid	**295.22787**	**295.22851**	**1.57**			
	17-Hydroxy-docosahexaenoic acid	343.22787	343.22880	1.56			
	14-Hydroxy-docosahexaenoic acid	343.22787	343.22880	1.56			

	11-Hydroxy-eicosatetraenoic acid	319.22787	319.22861	1.57			
	15-Oxo-eicosatetraenoic acid	317.21222	317.21323	1.58			
	15-Hydroxy-eicosatetraenoic acid	319.22787	319.22861	1.59			
	8-Hydroxy-eicosatetraenoic acid	319.22787	319.22861	1.59			
	9-Hydroxy-eicosatetraenoic acid	319.22787	319.22861	1.60			
5	**Decanoic acid**	**171.13905**	**171.13939**	**1.63**	304880 (31.1)		
	12-Oxo-eicosatetraenoic acid	317.21222	317.21323	1.64			
	2-hydroxylauric acid	215.16527	215.16575	1.70			
	3-hydroxytridecanoic acid	229.18092	229.18143	1.79			
	12(13)epoxy-octadecenoic acid	295.22787	295.22880	1.82			
	9(10)epoxy-octadecenoic acid	295.22787	295.22880	1.83			
	Lithocholic Acid	375.29047	375.29128	1.98			
6	**Lauric acid**	**199.17035**	**199.17072**	**2.04**	1306001 (23.8)		94661 (7.2)
7	**3-Hydroxymyristic acid**	**243.19657**	**243.19704**	**2.05**	63246 (9.8)		
8	**Eicosapentaenoic acid**	**301.21730**	**301.21797**	**2.15**	4551723 (8.2)		
9+10	**α-Linolenic acid (18:3n−3)** **γ-Linolenic acid (18:3n−6)**	**277.21730**	**277.21797**	**2.23**	9187632 (9.7)		9936 (12.7)
	2-Hydroxymyristic acid	243.19657	243.19706	2.28			
11	**Docosahexaenoic acid (22:6n−3)**	**327.23295**	**327.23368**	**2.31**	10954545 (7.1)	119407 (28.8)	
12	**Arachidonic acid**	**303.23295**	**303.23373**	**2.35**	13632487 (8.1)	153483 (37.4)	
13	**Myristic acid**	**227.20165**	**227.20213**	**2.38**	4182248 (10.9)		
14	**Docosapentaenoic acid**	**329.24860**	**329.24963**	**2.38**	2960287 (7.4)		
15	**Palmitoleic acid**	**253.21730**	**253.21783**	**2.40**	14701224 (8.3)	213190 (11.8)	
16	**Linoleic acid (18:2n−6)**	**279.23295**	**279.23370**	**2.46**	53297022 (3.9)	157840 (9.4)	157840 (9.4)

17	**Dihomo-γ-linolenic acid (20:3n-6)**	**305.24860**	**305.24926**	**2.53**	2028418 (10.1)	
18	**Heptadec-10-enoic acid**	**267.23295**	**267.23351**	**2.57**	1314551 (11.5)	
	Adrenic acid (22:4n−6)	331.26425	331.26643	2.59		
19	**Oleic acid**	**281.24860**	**281.24939**	**2.72**	56215540 (2.4)	1197760 (4.5)
20	**Palmitic acid**	**255.23295**	**255.23351**	**2.73**	6281818 (15.3)	
21	**Eicosadienoic acid**	**307.26425**	**307.26500**	**2.81**	912988 (13.3)	26929 (27.2)
	Heptadecanoic acid	269.24860	269.24911	2.92		
	Arachidic acid	311.29555	311.29621	2.99		
22	**Eicosenoic acid**	**309.27990**	**309.28056**	**3.06**	1621579 (11.6)	54810 (6.4)
23	**Stearic acid**	**283.26425**	**283.26486**	**3.07**	4102902 (39.7)	
24	**Docosadienoic acid**	**335.29556**	**335.29674**	**3.09**	36598 (19.8)	
	Nonadecanoic acid	297.27990	297.28061	3.23		
25	**Erucic acid**	**337.31120**	**337.31179**	**3.34**	223474 (14.8)	
	Behenic acid	339.32685	339.32720	3.64		