

Systematic evaluation of extraction methods for multi-platform based metabotyping:

Application to the *Fasciola hepatica* metabolome

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Contents of supplementary information:

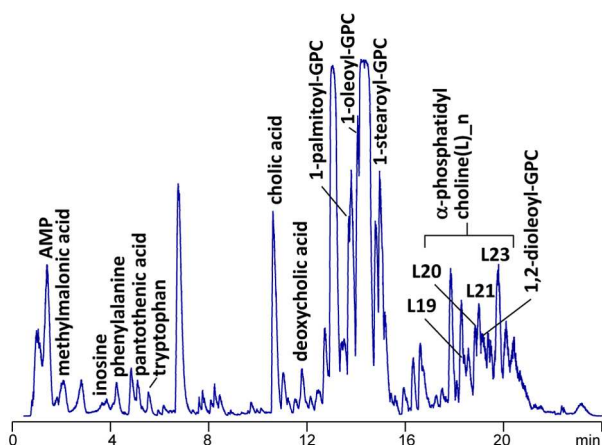
Figure S-1 presents typical base peak mass chromatograms acquired under negative ionization on the C18 column (A) and HILIC column (B), respectively, of *Fasciola hepatica* tissue samples, prepared in 80% methanol.

Figures S-2 to S-4 depict a series of mass chromatograms and/or electropherograms for the aqueous phase of mixture designed extracts of a pooled *Fasciola hepatica* tissue sample.

Table S-1 lists the standards used to confirm the identity of the chromatographic and/or electrophoretic peaks.

Table S-2 compiles all metabolites that were tentatively identified based on in house databases, comparison with authentic standards and occasionally MS/MS fragmentation.

A



B

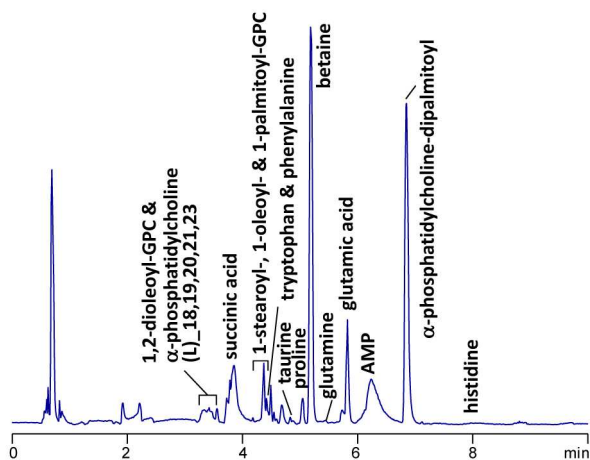


Figure S-1. Typical mass chromatograms of 80% methanol extracts of *F. hepatica* flukes acquired at ESI- mode with C18 column (A) and HILIC column (B).

Key: AMP, adenosine monophosphate; GPC, glycerophosphocholine; α-phosphatidylcholine (L)_n:* L19: 1-hexadecanoyl-2-octadecadienoyl-sn-glycero-3-phosphocholine; L20: 1-nonanoyl-2-tricosanoyl-sn-glycero-3-phosphocholine; L21: 1-hexadecanoyl-2-octadecenoyl-sn-glycero-3-phosphocholine; L23: 1-octadecanoyl-2-octadecenoyl-sn-glycero-3-phosphocholine.

*mixture of α-phosphatidylcholines (L)_n numbered 1 to 23 by order of elution in the RPLC-MS method; tentative identification based on *m/z* searches at <http://www.lipidmaps.org/data/structure/LMSDSearch.php>.

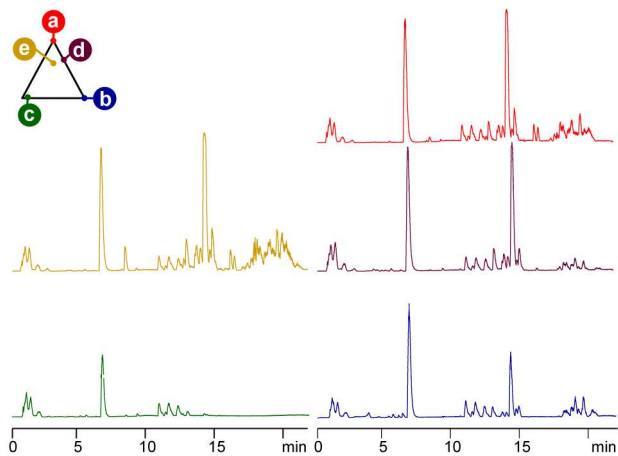


Figure S-2. Optimization of extraction of a pooled *F. hepatica* sample using a mixture design approach and RPLC-MS analysis at ESI-. Extractions performed at (a) 80% methanol, (b) 20% methanol, (c) 90:10 chloroform:20% methanol, (d) 65% methanol, and (e) 15:15:70 chloroform:20% methanol:80% methanol.

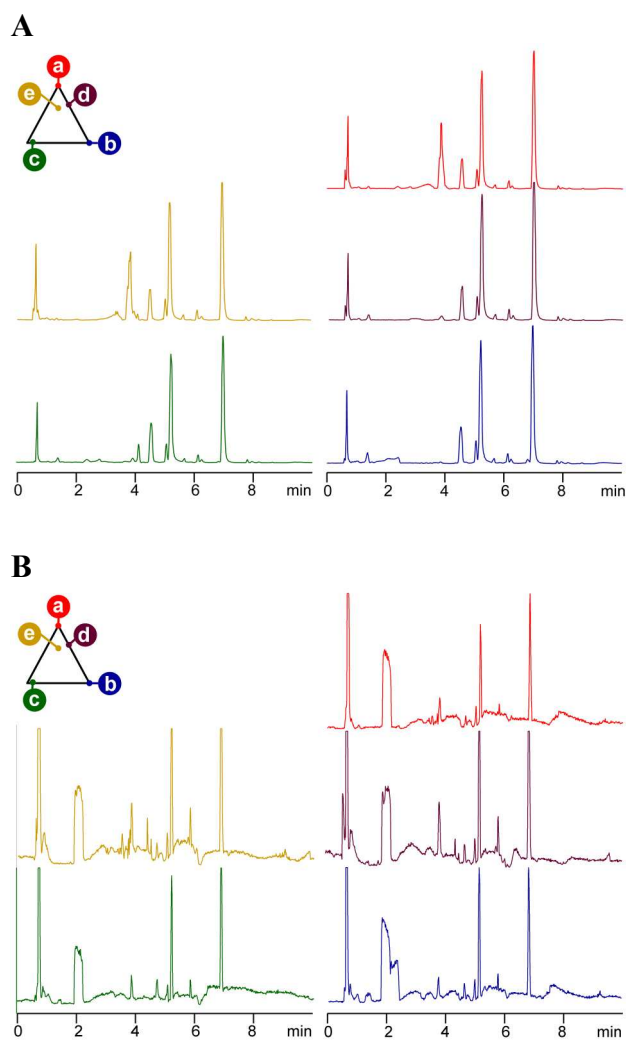


Figure S-3. Optimization of extraction of a pooled *F. hepatica* sample using a mixture design approach and HILIC-MS analysis at ESI+ mode (A) and ESI- mode (B). Extractions performed at (a) 80% methanol, (b) 20% methanol, (c) 90:10 chloroform:20% methanol, (d) 65% methanol, and (e) 15:15:70 chloroform:20% methanol:80% methanol.

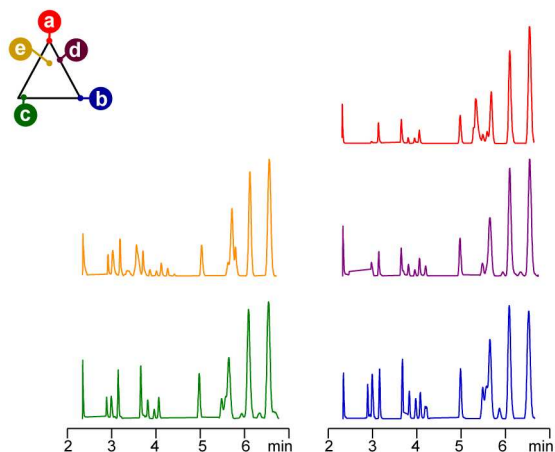


Figure S-4. Optimization of extraction of a pooled *F. hepatica* sample using a mixture design approach and CE-MS analysis at ESI+ mode. Extractions performed at (a) 80% methanol, (b) 20% methanol, (c) 90:10 chloroform:20% methanol, (d) 65% methanol, and (e) 15:15:70 chloroform:20% methanol:80% methanol.

Table S-1. Standards used to confirm the identity of metabolites in the *Fasciola hepatica* tissue samples.*

STANDARD SUBSTANCES		
(+)-5(S)-hydroxy-(6E,8Z,11Z,14Z)-eicosatetraenoic acid	choline chloride	N-acetylmuramic acid
(5S,6S)-dihydroxy-(7E,9E,11Z,14Z)-eicosatetraenoic acid	<i>trans</i> -cinnamic acid	N-acetylneuraminic acid
1-oleoyl-sn-glycero-3-phosphocholine	<i>cis</i> -aconitic acid	niacinamide
1-palmitoyl-sn-glycero-3-phosphocholine	citric acid, trisodium salt dihydrate	nicotinic acid
1-stearoyl-sn-glycero-3-phosphocholine	L-citrulline	O-phosphorylethanolamine
1,2-diacyl-sn-glycero-3-phosphoethanolamine	creatine	opthalmic acid
1,2-dioleoyl-sn-glycero-3-phosphocholine	creatinine	D-ornithine monohydrochloride
(14R,15S)-epoxy-(5Z,8Z,11Z)-eicosatrienoic acid	L-cysteine	orotic acid
2'-deoxyadenosine-5'-diphosphate, sodium salt	cytidine	O-succinyl-L-homoserine
2'-deoxyadenosine-5'-monophosphate	cytidine-5'-monophosphate	oxalic acid
2-deoxycytidine	deoxycholic acid	oxaloacetic acid
2-hydroxy-2-methylpropanoic acid; alpha-hydroxyisobutyric acid	DL-dihydroorotic acid	oxoproline
2-oxopentanoic acid sodium salt; 2-ketovaleric acid, sodium salt; 2-oxovaleric acid, sodium salt	5,6-dihydrothymidine	palmitic acid
3-hydroxyanthranilic acid	dihydrothymine	D-pantothenic acid hemicalcium salt
3-hydroxybutanoic acid; 3-hydroxybutyric acid	dihydrouracil	<i>p</i> -cresol
<i>trans</i> -3-hydroxycinnamic acid	dimethylamine	phenylacetyl glycine
3-(4-hydroxyphenyl)propionic acid	epinephrine	L-phenylalanine
3-methyl-2-oxobutanoic acid, sodium salt; 3-methyl-2-oxobutyric acid, sodium salt; alpha-ketoisovaleric acid, sodium salt	DL-erythro-dihydrosphingosine	L-alpha-phosphatidyl-L-serine from glycine max (soybean)

3-methyl-2-oxovaleric acid, sodium salt; 3-methyl-2-oxopentanoic acid, sodium salt	folic acid	phospho-L-arginine
3-methylglutaric acid	formic acid, sodium salt	phosphorylcholine chloride calcium salt tetrahydrate
4-methyl-2-oxovaleric acid; 2-oxoisocaproic acid; 4-methyl-2-oxopentanoic acid	D-fructose	O-phosphorylethanolamine
4-methylvaleric acid; isocaproic acid	D-fructose-6-phosphate, disodium salt hydrate	phthalic acid
alpha-aminoisobutyric acid	fumaric acid, disodium salt	pimelic acid
acetic acid, sodium salt	D-galacturonic acid, sodium salt	L-pipecolic acid
N-acetyl-L-cysteine	glycocholic acid, hydrate	porphobilinogen
adenosine	D-glucosamine-6-phosphate	L-proline
adenosine-5'-monophosphate monohydrate	D-glucose 6-phosphate, sodium salt	propionic acid
adenosine-5'-diphosphate	D-glucuronic acid	putrescine dihydrochloride
adenosine-5'-triphosphate, disodium salt hydrate	L-glutamic acid	pyridoxal-5'-phosphate hydrate
adipic acid	L-glutamine	pyridoxine
alpha-D-glucose	glutaric acid	L-pyroglutamic acid
alpha-hydroxyisobutyric acid; 2-hydroxy-2-methylpropionic acid	glutaryl coenzyme A, lithium salt	pyruvic acid
alpha-hydroxyisovaleric acid; 2-hydroxy-3-methylbutyric acid	L-gluthathione, oxidized	riboflavin
alpha-ketoglutaric acid; 2-oxoglutaric acid; 2-oxopentanedioic acid	L-gluthathione, reduced	salicylic acid
L-alanine	L-alpha-phosphatidylcholine	<i>scyllo</i> -inositol
alpha-phosphatidylcholine (L)_1**: 1-hexadecanoyl-sn-glycero-3-phosphocholine	glycerol	sebacic acid
alpha-phosphatidylcholine (L)_10**	glycine	L-serine
alpha-phosphatidylcholine (L)_11**: 1-(9Z-octadecenoyl)-2-(4Z,7Z,10Z,13Z,16Z,19Z-docosaheptaenoyl)-sn-glycero-3-phosphoethanolamine	glycogen from bovine liver	spermidine
alpha-phosphatidylcholine (L)_12**: 1-(1Z-octadecenyl)-2-(5Z,8Z,11Z,14Z,17Z-eicosapentaenoyl)-glycero-3-phosphocholine	glycocholic acid, hydrate	spermine

alpha-phosphatidylcholine (L)_13**: 1-hexadecyl-2-(4Z,7Z,10Z,13Z,16Z,19Z-docosaheptaenoyl)-sn-glycero-3-phosphocholine	glycolic acid	suberic acid
alpha-phosphatidylcholine (L)_14**: isomers of PC(18:0/22:6)	glycolithocholic acid	succinic acid
alpha-phosphatidylcholine (L)_15**: 1-(1Z-octadecenyl)-2-(4Z,7Z,10Z,13Z,16Z,19Z-docosaheptaenoyl)-sn-glycero-3-phosphocholine	guanidine hydrochloride	taurine
alpha-phosphatidylcholine (L)_16**: 1-octadecyl-2-(4Z,7Z,10Z,13Z,16Z,19Z-docosaheptaenoyl)-sn-glycero-3-phosphocholine; 1-(1Z-eicosenyl)-2-(5Z,8Z,11Z,14Z,17Z-eicosapentaenoyl)-glycero-3-phosphocholine	guanidineacetic acid; glycocytamine	tauro-alpha-muricholic acid
alpha-phosphatidylcholine (L)_17**: 1-octadecyl-2-(4Z,7Z,10Z,13Z,16Z,19Z-docosaheptaenoyl)-sn-glycero-3-phosphocholine	hippuric acid	taurochenodeoxycholic acid, sodium salt
alpha-phosphatidylcholine (L)_18**: 1-(9Z-octadecenyl)-2-hexadecyl-sn-glycero-3-phosphocholine; 1-hexadecyl-2-(9Z-octadecenyl)-sn-glycero-3-phosphocholine	L-histidine	taurocholic acid, sodium salt hydrate
alpha-phosphatidylcholine (L)_19**: 1-hexadecanoyl-2-octadecadienoyl-sn-glycero-3-phosphocholine	D-histidinol dihydrochloride	taurodehydrocholic acid
alpha-phosphatidylcholine (L)_2**: 1-(6Z-octadecenyl)-sn-glycero-3-phosphocholine	homocarnosine	taurodeoxycholic acid, sodium salt hydrate
alpha-phosphatidylcholine (L)_20**: 1-nonanoyl-2-tricosanoyl-sn-glycero-3-phosphocholine	DL-homocysteine	taurohyocholic acid, sodium salt

alpha-phosphatidylcholine (L)_21**: 1-hexadecanoyl-2-octadecenoyl-sn-glycero-3-phosphocholine	hyocholic acid	taurohyodeoxycholic acid sodium salt hydrate
alpha-phosphatidylcholine (L)_22**	hyodeoxycholic acid	taurolithocholic acid, sodium salt
alpha-phosphatidylcholine (L)_23**: 1-octadecanoyl-2-octadecenoyl-sn-glycero-3-phosphocholine	hypoxanthine	tauro-omega-muricholic acid
alpha-phosphatidylcholine (L)_3**	indoxylsulphate, potassium salt	taurourscholic acid
alpha-phosphatidylcholine (L)_4**	inosine	tauroursodeoxycholic acid, sodium salt
alpha-phosphatidylcholine (L)_5**	inosine-5'-diphosphate, sodium salt	L-threonine
alpha-phosphatidylcholine (L)_6**: mixture of PC(8:0/18:0 to 17:0/9:0)	inosine-5'-monophosphate, disodium salt	thymidine
alpha-phosphatidylcholine (L)_7**: 1-octadecanoyl-sn-glycero-3-phosphocholine	inosine-5'-triphosphate, trisodium salt	<i>trans</i> -4-hydroxy-L-proline
alpha-phosphatidylcholine (L)_8**	L-isoleucine	alpha,beta-trehalose
alpha-phosphatidylcholine (L)_9**: mixture of PC(7:0/21:0 to 19:0/9:0)	isovaleric acid	trimethylamine N-oxide
DL-alpha-phosphatidylcholine, dipalmitoyl	L-lactic acid	L-tryptophan
alpha-muricholic acid	L-leucine	tyramine
L-arginine	leukotriene E4, methyl ester	L-tyrosine
L-ascorbic acid	alpha-lipoic acid	uridine 5'-diphosphate, disodium salt hydrate
L-asparagine	lithocholic acid	uridine 5'-diphosphoglucose, disodium salt
DL-aspartic acid	DL-lysine monohydrochloride	uridine 5'-diphosphoglucuronic acid, trisodium salt
beta-aminobutyric acid	D-malic acid	uridine 5'-monophosphate
betaine	D-mannitol	uracil
beta-muricholic acid	L-methionine	urea
beta-nicotinamide adenine dinucleotide	methylamine	uric acid
beta-glucose	2-methylbutyric acid	uridine
beta-hydroxyisovaleric acid; 3-hydroxy-3-methylbutyric acid	1-methyl-L-histidine	<i>cis</i> -urocanic acid

bilirubin	methylmalonic acid	ursodeoxycholic acid
carnitine hydrochloride	<i>myo</i> -inositol	uridine 5'-triphosphate, trisodium salt dihydrate
L-carnosine	N-acetyl- α -D-glucosamine-1-phosphate, disodium salt	L-valine
chenodeoxycholic acid	N-acetyl-DL-glutamic acid	L-xylose
cholic acid	N-acetyl-L-cysteine	

*cholic acid derivatives are from Steraloids Inc; phospholipids are from Avanti Polar Lipids Inc.; other standards were acquired from Sigma-Aldrich.

** mixture of α -phosphatidylcholines (L)_n numbered 1 to 23 by order of elution in the RPLC-MS method; tentative identification based on *m/z* searches at <http://www.lipidmaps.org/data/structure/LMSDSearch.php>.

Table S-2. Tentatively assigned metabolites according to the analytical platform and ionization mode.

ID #	METABOLITES	RPLC- MS <i>ESI</i> +	RPLC- MS <i>ESI</i> -	HILIC- MS <i>ESI</i> +	HILIC- MS <i>ESI</i> -	CE-MS <i>ESI</i> +
1	(5S,6S)-dihydroxy-(7E,9E,11Z,14Z)-eicosatetraenoic acid	X	X	X		
2	(14R,15S)-epoxy-(5Z,8Z,11Z)-eicosatrienoic acid	X				
3	1-oleoyl-sn-glycero-3-phosphocholine	X	X	X	X	
4	1-palmitoyl-sn-glycero-3-phosphocholine	X	X	X	X	
5	1-stearoyl-sn-glycero-3-phosphocholine	X	X	X	X	
6	1,2-dioleoyl-sn-glycero-3-phosphocholine	X	X	X	X	
7	2'-deoxyadenosine-5'-diphosphate		X*		X	
8	2'-deoxyadenosine-5'-monophosphate	X	X			
9	2-deoxycytidine	X	X	X	X	
10	3-hydroxyanthranilic acid			X	X	X
11	3-hydroxycinnamic acid		X			
12	3-hydroxyphenylpropionic acid		X		X	
13	3-methylglutaric acid	X			X	
14	α -ketoglutaric acid		X*		X	
15	α -muricholic acid	X			X	
16	α -phosphatidylcholine (L)_1	X		X		
17	α -phosphatidylcholine (L)_10			X		
18	α -phosphatidylcholine (L)_12			X		
19	α -phosphatidylcholine (L)_13			X		
20	α -phosphatidylcholine (L)_14				X	
21	α -phosphatidylcholine (L)_18	X			X	
22	α -phosphatidylcholine (L)_19	X	X	X	X	
23	α -phosphatidylcholine (L)_2	X		X	X	
24	α -phosphatidylcholine (L)_20	X	X	X	X	
25	α -phosphatidylcholine (L)_21	X	X	X	X	
26	α -phosphatidylcholine (L)_22				X	
27	α -phosphatidylcholine (L)_23	X	X		X	
28	α -phosphatidylcholine (L)_3				X	
29	α -phosphatidylcholine (L)_4			X	X	
30	α -phosphatidylcholine (L)_6	X		X		
31	α -phosphatidylcholine (L)_7	X		X	X	
32	α -phosphatidylcholine (L)_9	X				
33	α -phosphatidylcholine-dipalmitoyl			X	X	
34	acetate				X	
35	adenosine	X		X		X
36	adenosine monophosphate	X	X	X	X	
37	adenosine-5'-diphosphate	X*	X*	X		

38	adipate	X			X	
39	alanine	X*		X		X
40	arginine	X*		X	X	X
41	ascorbic acid		X*	X		
42	asparagine		X*		X	X
43	aspartic acid		X*	X	X	X
44	betaine	X*		X	X	X
45	β-muricholic acid	X			X	
46	carnitine			X		
47	carnosine	X*		X	X	X
48	chenodeoxycholic acid	X			X*	
49	cholic acid	X	X		X	
50	choline	X*		X		X
51	citrate		X			
52	citrulline	X*		X	X	
53	creatine	X*		X	X	X
54	creatinine		X*	X		
55	cysteine dimer		X*			
56	cytidine	X*		X		
57	cytidine-5'-monophosphate	X*	X*	X	X	
58	deoxycholic acid	X	X		X	
59	dihydroorotic acid				X	
60	epinephrine					X
61	fructose		X*	X	X	
62	fructose-6-phosphate		X*	X	X	
63	fumarate		X*		X*	
64	galacturonic acid		X*			
65	glucosamine-6-phosphate	X*	X*		X	
66	glucose-6-phosphate	X*	X*	X	X	
67	glucuronic acid	X*				
68	glutamic acid	X		X	X	X
69	glutamine		X*	X	X	X
70	glutaric acid			X	X	
71	glutathione	X	X	X	X	X
72	glutathione disulfide					X
73	glycerophosphocholine	X*	X*	X	X	
74	glycocholic acid	X		X	X	
75	glycolic acid		X*			
76	guanidoacetic acid	X*	X*	X	X	
77	histidine	X*	X*	X	X	X
78	homocysteine			X	X	X
79	hyocholic acid	X		X	X	
80	hyodeoxycholic acid	X			X*	

81	hypoxanthine	X	X	X	X	X
82	indoxylsulphate		X		X*	
83	inosine	X	X	X	X	X
84	isoleucine	X	X	X	X	X
85	leucine	X	X	X	X	X
86	lipoic acid				X	
87	lysine	X*		X		X
88	malate		X*		X	
89	mannitol	X		X	X	
90	methionine	X	X	X	X	X
91	methylhistidine	X		X	X	
92	methylmalonic acid		X		X	
93	<i>myo</i> -inositol		X*			
94	N-acetyl- α -glucosamine-1-phosphate	X*	X*	X	X	
95	N-acetylcysteine			X		
96	N-acetylmuramic acid	X*				
97	N-acetylneuraminic acid			X		
98	niacinamide					X
99	nicotinic acid	X*	X*	X	X	X
100	<i>o</i> -phosphorylethanolamine	X*	X*	X	X	
101	ornithine	X*		X	X	X
102	orotic acid			X	X	
103	pantothenic acid	X	X	X	X	
104	phenylalanine	X	X	X	X	X
105	phospho-L-arginine	X*		X		X
106	phosphorylcholine	X*		X	X	
107	pimelic acid	X		X	X	
108	pipecolic acid	X*		X	X	X
109	porphobilinogen		X	X	X	
110	proline	X*	X*	X	X	X
111	pyridoxal-5-phosphate	X*				
112	pyridoxine	X*		X	X	
113	pyroglutamate		X*	X	X	
114	salicylic acid				X	
115	sebacic acid		X		X	
116	serine		X*		X	
117	spermidine			X		
118	spermine			X		X
119	suberic acid		X	X	X	
120	succinic acid		X		X	
121	taurine		X*	X	X	
122	taurochenodeoxycholic acid	X		X		
123	taurodeoxycholic acid	X		X		

124	taurohyodeoxycholic acid			X		
125	tauroolithocholic acid	X		X	X	
126	tauroursodeoxycholic acid			X		
127	threonine			X	X	X
128	thymidine		X	X		
129	<i>trans</i> -4-hydroxyproline	X*		X	X	X
130	tryptophan	X	X	X	X	X
131	tyramine	X		X		X
132	tyrosine	X	X	X	X	X
133	uracil				X	
134	uric acid	X	X		X	
135	uridine	X*	X*		X	
136	uridine diphosphate glucose		X*		X	
137	uridine diphosphate glucuronic acid		X*		X	
138	uridine monophosphate	X*	X*		X	
139	urocanic acid				X	
140	ursodeoxycholic acid	X				
141	valine	X*	X*	X	X	X
142	xylose		X*	X		

*k<1.0