Metabolite Identification in Blood Plasma Using GC/MS and the Agilent Fiehn GC/MS Metabolomics RTL Library

Application Note

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Abstract

Gas chromatography / mass spectrometry (GC/MS) offers high separating power and high sensitivity for metabolomic research. The utility of metabolomic screens largely depends on the number of identified metabolites and links to their biological interpretation. Often, the challenging step is in the identification of these metabolites. The new Agilent Fiehn GC/MS Metabolomics RTL (retention time locked) Library has specifically been developed to help facilitate the identification of metabolites.

Human blood plasma was used to demonstrate metabolite identification in a complex biological matrix. The identification routines were complemented by mass spectral deconvolution and matching of sample peak spectra to Agilent Fiehn library spectra via fast, flexible, high-throughput searching. One of the most important criteria for unambiguous identification was the sample retention times, which were locked to the absolute retention time of an internal standard, d27-myristic acid. Retention time locking, a feature of the Agilent Fiehn library, results in increased identification confidence. The average retention time deviations were found to be less than 0.15 min, increasing the reliability and confidence in metabolite annotations. Implementing identification procedures such as this will become increasingly important in standardizing the reporting of metabolomics results, such as what has recently been suggested by the NIH/NIDDK, and the Metabolomics Society.

Agilent Technologies

Introduction

Comprehensive identification and quantification of small molecule metabolites (30-1,500 Da) from complex biological matrices, called "Metabolomics", is a challenging task for analytical chemistry, even with modern instrumentation. No single method can achieve this goal. For example, highly volatile compounds like terpenes cannot be assessed with liquid chromatography-based methods. Gas chromatography (GC) must be used. Similarly, liquid chromatography (LC) is clearly better suited for separation of polyphenols than capillary electrophoresis (CE), whereas bis- and trisphosphates (as in glycolysis and Calvin cycle pathways) might be most suitable for CE-separations. Nevertheless, each of these three major separation methods can detect and quantify a range of different compound classes in a multiparallel way, especially when mass spectrometry is used for unambiguous and sensitive detection. GC/MS offers high separating power and high sensitivity for metabolomic research. Often, a large overlap of identified compounds is observed when comparing results between the major separation methods, despite the large differences in separation mechanisms. The underlying reason for this high degree of overlap (even when comparing to nuclear magnetic resonance techniques) is the conserved nature of the most abundant metabolic intermediates.

Metabolites occur at very different concentrations in complex biological matrices, from which they must be extracted without compromising the structural integrity and relative abundances. Yet, a range of central metabolism pathways are highly similar in design between different species (e.g. mouse, rat and human) and even beyond (e.g. between mammals, birds and simple eukaryotes like yeast). Such conserved pathways contribute to generic metabolic needs like oxidizing carbon sources to obtain energy or, conversely, using carbohydrates, amino acids and fatty acids for cellular growth, providing the metabolic building blocks for proteins, complex lipids and storage biopolymers such as glycogen or fat. The relative abundance of these conserved intermediary metabolites can subsequently be interpreted in relation to the activity of the underlying catabolic and biosynthetic pathways. Therefore, the utility of metabolomic screens largely depends on the number of identified metabolites and links to their biological interpretation. This application note demonstrates the identification of metabolites in human blood plasma, using a single solvent mixture for protein precipitation and extraction, followed by chemical derivatization, analysis by GC/MS, data processing with mass spectral deconvolution, validation of lists of identified metabolites, and a way to use links to external chemical and biological databases.

Experimental

Blood was collected from volunteers between the hours of 12:00 and 3:00 p.m. Platelet depleted plasma (based on additional centrifugation) was isolated immediately within 15 min after plasma withdrawal. Samples were kept on ice during the procedure and plasma was immediately frozen at -80°C. Samples were thawed once, aliquoted and prepared for GC/MS analysis. 30 µL of blood plasma was extracted using 1 mL of a single phase mixture of isopropanol:acetonitrile:water (3:3:2, v/v) at 20°C for 5 min. After centrifugation, 0.5 mL of supernatant was aliquoted into a tube, and then completely dried in a SpeedVac concentrator. The tube was subsequently derivatized in two steps. First, carbonyl functional groups were protected by methoximation using 10 µL of a 40 mg/mL solution of methoxyamine hydrochloride in pyridine at 30°C for 90 min. Next, to increase the volatility of the compounds, the samples were derivatized using 90 µL of N-methyl-N-trimethylsilyltrifluoroacetamide with 1% trimethylchlorosilane (MSTFA + 1% TMCS, Pierce) at 37°C for 30 min. This procedure resulted in the detection of two peaks for derivatized aldehydes and ketones: the syn- and anti- forms such as glucose1 and glucose2.

The following GC/MS conditions were used. An Agilent 6890 GC oven was ramped by 10°C/min from 60°C (1 min initial time) to 325°C (10 min final time), resulting in a 37.5 min run time with cooling down to 60°C. 1 µL was injected into the Agilent split/splitless injector at 250°C by a 10µL syringe with 4 sample pumps, 1 pre-injection wash and 2 post-injection washes using both solvent A and solvent B. No viscosity delay or dwell time was applied using a fast plunger speed. Samples were introduced in both splitless and split conditions. For splitless conditions, a helium purge flow of 10.5 mL/min was applied for 1 min (8.2 psi). Gas saver was on with a saver flow rate of 20 mL/min for 3 min. A 29 m long DB5-MS column with 10 m Duragard precolumn was used with 0.25 mm diameter and 0.25 µm film thickness. A constant flow rate of 1 mL/min helium was used as carrier gas. The Mass Selective Detector (MSD) was set at a signal data rate of 20 Hz with the MSD transfer line set to 290°C. The quadrupole mass spectrometer was switched on after a 5.90 min solvent delay time, scanning from 50-600 u. The source temperature was set to 230°C and the quadrupole temperature was 150°C. Prior to acquisition, the MSD was autotuned using FC43 according to the instrument manual. When using split injections, parameters used were identical as given above but with a split ratio of 1:10 and a split flow rate of 10.3 mL/min. Absolute retention times were locked to the internal standard d27-myristic acid using the RTL system provided in Agilent's ChemStation software. Retention time locking reduces run-to-run retention time variation. The Agilent Fiehn GC/MS Metabolomics RTL Library (version June 2008) was employed for metabolite identifications. This library is the most comprehensive library of metabolite GC/MS spectra that is commercially available. It contains searchable GC/MS EI spectra and retention time indexes from approximately 700 common metabolites.

Results

A 22 year old female was tested for her blood plasma metabolome in two samples that were withdrawn four weeks apart in an ongoing pharmacogenomics research program. Plasma samples were analyzed using splitless and split injection into the GC/MS system. The major component in plasma is glucose, sometimes called 'blood sugar', as it is regulated in humans around a concentration of 5 mM to provide energy to all organs, notably to the brain. Other abundant metabolites expected are free cholesterol, saturated free fatty acids and a range of amino acids, particularly alanine, which serves as a three-carbon carrier between skeletal muscle and liver. Blood plasma withdrawals are not standardized between different clinical laboratories and can result in analytical variability. Some clinics prefer the use of EDTA to prevent clotting, whereas others use heparin or citrate for this purpose. The anti-clotting agent should be controlled for best results. Efforts to produce 'best practice' documents to standardize and harmonize protocols are under way by the National Institutes of Health; however, no documents have been released yet.

Using Agilent's ChemStation software, several abundant peaks were detected for the 1:10 split injection. Peak detection with background subtraction and subsequent matching of the resulting mass spectra to the Agilent Fiehn GC/MS Metabolomics RTL Library using either NIST MS searches or ChemStation's PBM queries confirmed that alanine, citrate, glucose and cholesterol were readily identified at retention times of 7.72, 16.59, 17.65 and 27.55 min, respectively (**Fig. 1**). Mass spectral match factors for these abundant compounds exceeded 90 in PBM search and 900 in NIST MS search even without further spectral purification. The high abundance of the citrate peak and lack of an EDTA peak confirmed that these samples had been subjected to plasma stabilization by addition of citric acid, which renders measurements for endogenous citrate impossible. Nevertheless, for detailed analysis of less abundant compounds, automatic mass spectral deconvolution (AMDIS) is required. AMDIS, an automated GC/MS identification program from the U.S. National Institute of Standards and Technology, is included with the Agilent Fiehn library. Mass spectral deconvolution automatically finds peaks, deconvolutes spectra from co-eluting compounds using model ion traces that best describe the presence of unique peaks, and subsequently match spectra against user-defined mass spectral libraries, making searches quick and simple.

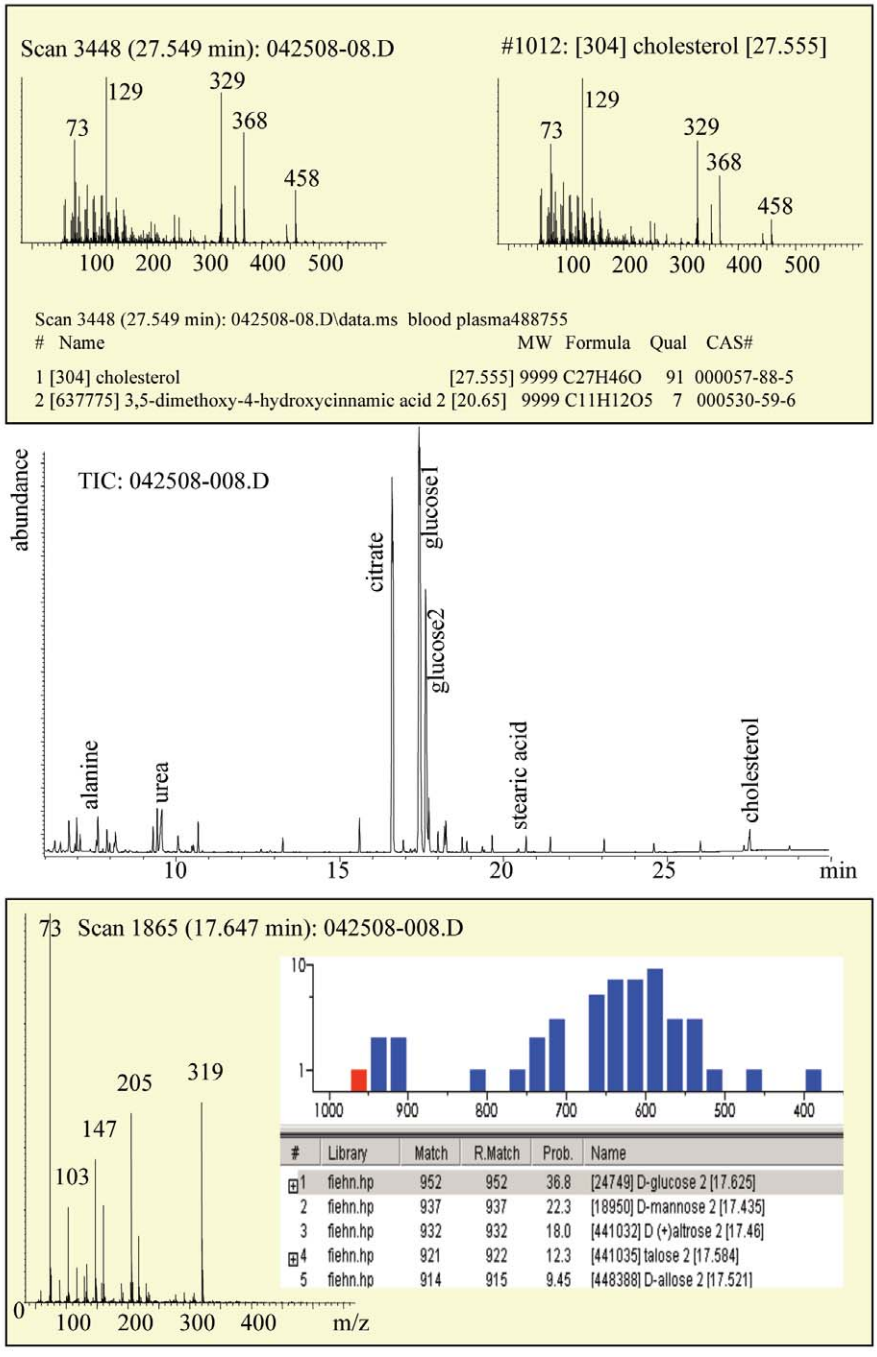

Fig. 1. Metabolite identification in human blood plasma by GC/MS after methoximation and trimethylsilylation and use of the Agilent Fiehn library.
Mid panel: Total ion chromatogram, split 1:10 injection.
Upper panel: Identification of cholesterol using the PBM Quick search in Agilent's ChemStation.
Lower panel: Identification of glucose2 using the NIST MS search and retention time information.

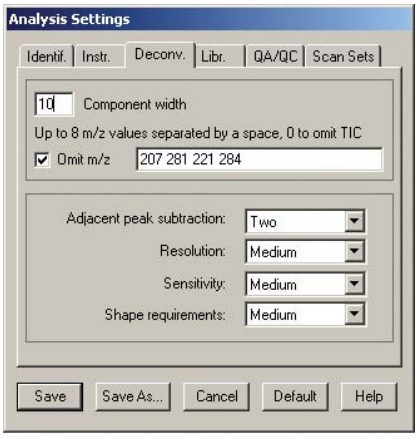

Fig. 2. Setting in AMDIS for mass spectral deconvolution.

The number of peaks and quality of deconvoluted spectra clearly depends on the complexity of the sample chromatogram and the settings that are used for AMDIS. There are no optimal AMDIS settings. Instead, users should start with 'medium' settings for resolution, sensitivity and peak shape requirements using two adjacent peak subtractions (**Fig. 2**) and then investigate results with respect to false positive and false negative peak detections.

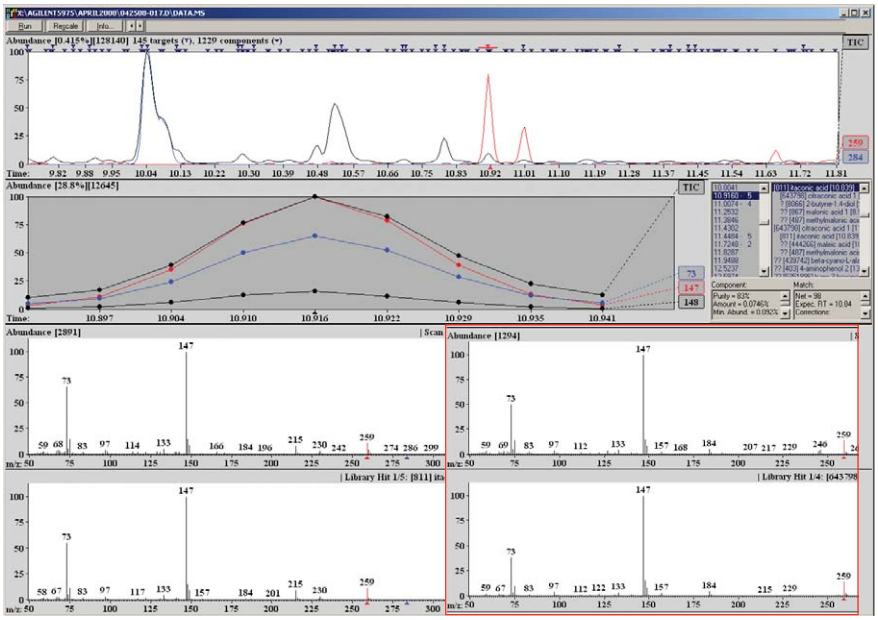

Fig. 3. Identification of itaconic acid and citraconic acid (peaks labeled red, chromatogram upper panel) in human blood plasmas despite high spectral similarity (lower panels) and close retention times.

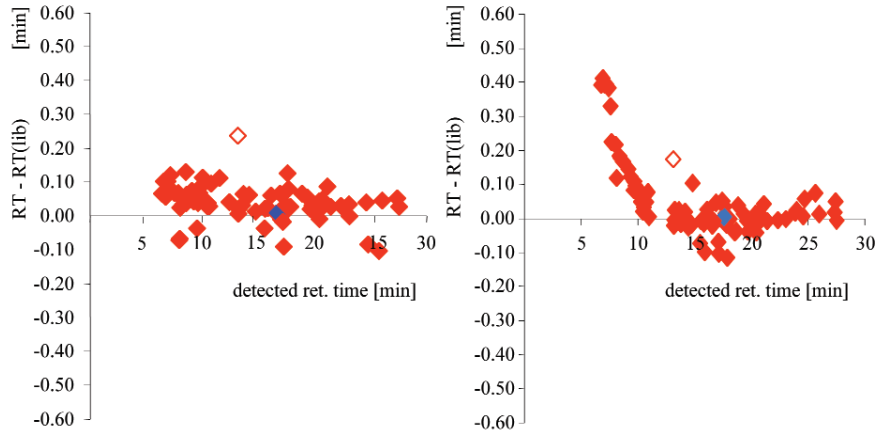

Fig. 4. Deviation of detected retention times from the Agilent Fiehn library retention times. Left panel: Split 1:10 injection (method used for generating library spectra). Right panel: Splitless injection. Blue rhomb: retention time locking compound d27-myristic acid. Empty rhomb: threitol, likely misidentified as evidenced by a retention time shift of > 0.15 min. The drift of retention deviation for metabolites with retention times of < 9 min (right panel) is caused by the altered gas flows in splitless vs. split injections.

We have performed this strategy for samples that were run in split and splitless injection and altered settings from medium to high and very high settings, which we found to influence the number of detected peaks but not largely the number of metabolites identified in the samples. When combining these approaches, 102 peaks were identified for splitless injected plasma samples by matching deconvoluted mass spectra to the Agilent Fiehn GC/MS Metabolomics RTL Library, constraining the hits by retention time difference between detected and Agilent Fiehn library retention time, and manually validating the spectral matches (**Table 1**). In about 90% of the cases, the correct match was found as the top hit in the AMDIS peak lists. In **Fig. 3**, such automatic matches are exemplified for two organic acids (itaconic and citraconic acid, detected at signal/noise ratios of 319 and 172, respectively). These acids shared high spectral similarity when comparing ion abundances of *m/z* 73, 147, 215 and both eluted in close proximity of less than 0.1 min (~ 5 s). Despite these similarities, spectral differences in lower abundant ions and the retention time difference unambiguously identified such less common acids. In other cases (roughly 10% of the identified peaks), however, identification had to rely on small differences in retention times when the spectra of the correct metabolite had slightly worse spectral matches than one of its isomer structures. This occurred especially for carbohydrates and sugar alcohols, e.g. for glucose, fructose, ribose and ribitol. Nevertheless, in most instances erroneous identifications could easily be sorted out using the differences of Agilent Fiehn library retention times to detected retention times (**Fig. 4**). However, a caveat must be considered for splitless injections: due to the altered gas flow conditions, metabolites that elute with a retention time earlier than 9 min tend to elute up to 0.4 min later than the predicted retention times, which were generated under split 1:10 conditions (**Fig. 4**). In almost all cases, the retention time difference was smaller than 0.15 min for identified peaks. When larger differences were observed as in threitol, it is likely that the peak was misidentified despite the high spectral similarity. The threitol isomer, erythritol, was not included in the 2008 version of the Agilent Fiehn GC/MS Metabolomics RTL Library.

Name	CAS	PubChem	MSnet	RT	RT (lib)	RT-RT(lib)	Purity	*m/z*	S/N *m/z*
4-hydroxyproline 2	51-35-4	5810	68	13.29	13.27	0.02	21%	230	37
alanine 1	56-41-7	5950	95	7.72	7.50	0.22	33%	116	125
beta-cyano-L-alanine	45159-34-0	439742	83	11.25	11.29	0.05	29%	158	73
creatinine	60-27-5	588	95	13.65	13.63	0.02	64%	329	44
cystine 3	56-89-3	67678	90	21.15	21.10	0.04	31%	266	19
epsilon-caprolactam	105-60-2	7768	97	6.78	6.39	0.39	67%	170	252
glutamic acid 1	56-86-0	33032	53	13.33	13.34	-0.01	12%	174	17
glutamic acid 2	56-86-0	33032	76	14.41	14.40	0.01	20%	246	35
glutamic acid 3 (oxoproline)	56-86-0	33032	98	13.21	13.23	-0.02	73%	156	152
glutamine 3	56-85-9	738	96	16.12	16.09	0.03	96%	156	185
glycine	56-40-6	750	97	10.48	10.46	0.02	91%	174	245
isoleucine 2	443-79-8	791	84	10.28	10.23	0.05	10%	158	73
leucine 1	61-90-5	6106	91	8.48	8.30	0.18	62%	86	123
leucine 2	61-90-5	6106	66	9.99	9.95	0.05	39%	158	102
L-methionine 1	63-68-3	6137	84	11.83	11.84	-0.04	12%	396	20
lysine 2	56-87-1	5962	88	17.68	17.64	0.03	57%	317	142
ornithine	70-26-8	6262	60	14.35	14.35	0.00	21%	142	22
phenylalanine 1	63-91-2	994	79	13.55	13.55	0.00	23%	120	61
proline 1	147-85-3	145742	85	8.73	8.57	0.17	53%	70	177
proline 2	147-85-3	145742	74	10.35	10.32	0.03	66%	142	102
serine 1	56-45-1	5951	96	9.80	9.71	0.10	76%	132	98
serine 2	56-45-1	5951	87	11.18	11.17	0.01	31%	204	62
threonine 1	72-19-5	6288	95	10.29	10.22	0.07	81%	147	71
tryptophan 2	73-22-3	6305	89	20.44	20.47	-0.02	40%	202	207
tyrosine 2	60-18-4	6057	97	17.84	17.86	-0.02	93%	218	242
urea	57-13-6	1176	99	9.68	9.60	0.08	93%	147	479
valine 1	72-18-4	6287	91	7.63	7.30	0.33	31%	72	168
valine 2	72-18-4	6287	88	9.26	9.15	0.11	85%	144	159
2-hydroxypyridine	142-08-5	8871	92	6.93	6.52	0.41	23%	152	83
3-indoleacetic acid	87-51-4	802	80	18.09	18.09	0.00	5%	202	36
3-indolelactic acid 2	1821-52-9	92904	65	20.06	20.08	-0.02	5%	202	24
4-hydroxybenzoic acid	114-63-6	135	62	14.48	14.51	-0.03	47%	267	29
6-hydroxynicotinic acid	5006-66-6	72924	90	13.82	13.83	0.04	8%	266	19
benzene-1,2,4-triol	533-73-3	10787	94	14.18	14.16	0.02	29%	342	47
benzoic acid	65-85-0	243	97	9.70	9.59	0.11	18%	179	61
caffeic acid	331-39-5	1549111	84	19.72	19.75	-0.04	13%	396	20
nicotinic acid	59-67-6	938	71	10.32	10.27	0.05	8%	180	25
p-cresol	95-48-7	2879	90	8.39	8.21	0.18	4%	165	30
picolinic acid	98-98-6	1018	78	10.68	10.60	0.00	6%	202	36
pyrogallol	87-66-1	1057	89	13.48	13.46	-0.01	35%	312	61
uric acid 1	66-22-8	1175	98	19.34	19.33	0.01	89%	441	184
arabitol	488-82-4	94154	77	15.53	15.60	-0.07	37%	217	30
fructose 1	57-48-7	5984	92	17.11	17.18	-0.07	85%	307	138
fructose 2	24259-59-4	5984	75	17.18	15.11	-0.11	75%	217	77
fucose 1	2438-80-4	17106	91	15.61	15.61	0.09	5%	147	184
glucose 1	59-23-4	24749	95	17.48	17.43	0.05	100%	319	420
glucose 2	87-78-5	18950	94	17.62	17.62	0.00	100%	205	497
glycerol	56-81-5	753	91	10.03	9.94	0.09	27%	147	184
glycerol 1-phosphate	34363-28-5	754	93	15.96	16.06	-0.10	44%	357	45
glycolic acid	79-14-1	757	87	7.43	7.05	0.38	21%	147	72
isomaltose 1	499-40-1	439193	93	25.70	25.63	0.07	44%	361	54
lyxose 1	1114-34-7	65550	65	14.85	14.74	0.10	21%	217	19
maltose 1	69-79-4	6255	88	24.76	24.70	0.06	67%	361	69

Table 1. Identification of 102 compounds in blood plasma using splitless injection quadrupole GC/MS with retention time locking and combined AMDIS-Agilent Fiehnlib queries. Compounds that are incompletely derivatized produce more than one type of derivatized species. These are denoted by 1, 2, 3 etc. at the end of the compound names. Such tables are created using the 'generate report' option in AMDIS.

Name	CAS	PubChem	MSnet	RT	RT (lib)	RT-RT(lib)	Purity	*m/z*	S/N *m/z*
maltose 2	69-79-4	6255	98	24.96	24.92	-0.03	12%	218	244
myo-inositol	87-89-8	892	94	19.39	19.35	0.03	75%	318	78
saccharic acid	87-73-0	5460673	64	18.57	18.61	-0.04	14%	333	22
sucrose	111-11-5	5988	96	24.01	23.99	0.02	46%	361	55
threitol	6968-16-7	169019	93	13.13	12.95	0.17	43%	217	31
methyl caprate	110-42-9	C10	93	10.70	10.65	0.05	71%	74	110
methyl docosanoate	929-77-1	C22	96	23.08	23.08	0.00	66%	74	70
methyl eicosanoate	1120-28-1	C20	88	21.43	21.44	-0.01	43%	74	75
methyl hexacosanoate	5802-82-4	C26	94	26.03	26.02	0.01	40%	74	58
methyl laurate	111-82-0	C12	84	13.24	13.25	-0.01	54%	74	80
methyl linocerate	2442-49-1	C24	96	24.61	24.60	0.01	49%	74	55
methyl myristate	124-10-7	C14	90	15.59	15.60	-0.01	70%	74	119
methyl octacosanoate	55682-92-3	C28	91	27.37	27.35	0.02	15%	74	38
methyl palmitate	112-39-0	C16	93	17.74	17.72	0.01	60%	74	129
methyl pelargonate	1731-84-6	C9	95	9.37	9.25	0.12	48%	74	107
methyl stearate	112-61-8	C18	93	19.65	19.66	-0.01	65%	74	81
myristic acid d27	60658-41-5	C14 RTL	80	16.72	16.73	-0.01	21%	312	72
arachidic acid	506-30-9	10467	95	22.36	22.37	-0.01	51%	369	38
behenic acid	112-85-6	8215	89	23.91	23.90	0.02	23%	397	25
capric acid	334-48-5	2969	85	12.41	12.40	0.15	8%	200	39
caprylic acid	124-07-2	379	87	9.89	9.81	0.09	13%	201	37
heptadecanoic acid	506-12-7	10465	92	19.80	19.80	0.00	38%	327	36
lauric acid	143-07-7	3893	69	14.77	14.79	-0.02	23%	257	32
linoleic acid	60-33-3	5280450	94	20.41	20.40	0.01	81%	337	85
myristic acid	544-63-8	11005	70	16.93	16.89	0.05	2%	285	58
oleic acid	112-80-1	445639	90	20.46	20.50	-0.04	64%	339	106
palmitic acid	64519-82-0	985	98	18.88	18.85	0.04	93%	313	293
palmitoleic acid	373-49-9	445638	62	18.69	18.73	-0.04	2%	311	29
pimelic acid	111-16-0	385	60	14.19	14.19	0.00	23%	155	20
stearic acid	57-11-4	5281	98	20.69	20.68	0.02	95%	341	330
10-hydroxydecanoic acid	362-06-1	74300	92	16.36	16.51	-0.02	76%	202	207
1-hexadecanol	36653-82-4	2682	75	17.93	18.05	-0.12	3%	299	30
2-furoic acid	88-14-2	6919	73	8.19	8.07	0.12	2%	125	18
2-hydroxybutyric acid	565-70-8	11266	75	8.07	7.85	0.22	33%	147	41
2-ketoisocaproic acid 2	4502-00-5	70	85	9.19	9.04	0.15	65%	200	39
adipic acid	124-04-9	196	86	13.02	13.00	0.00	27%	120	60
alpha ketoglutaric acid	328-50-7	51	74	13.84	13.86	-0.02	35%	147	21
citraconic acid 1	498-23-7	643798	91	11.01	11.00	0.00	70%	147	107
citric acid	5949-29-1	311	96	16.59	16.61	-0.02	100%	347	290
glyceric acid	473-81-4	439194	87	10.78	10.73	0.05	30%	189	38
itaconic acid	97-65-4	811	95	10.92	10.84	0.08	87%	147	180
lactic acid	79-33-4	107689	95	7.24	6.85	0.39	60%	147	293
phosphoric acid	7664-38-2	1004	97	10.04	9.97	0.08	88%	299	633
pyruvic acid	127-17-3	1060	76	7.11	6.71	0.39	24%	174	64
succinic acid	29915-38-6	1110	82	10.54	10.51	0.03	22%	148	88
trans-aconitic acid	585-84-2	444212	95	15.83	15.84	-0.02	91%	229	146
oxalic acid	144-62-7	971	63	7.78	7.88	-0.11	25%	147	367
alpha tocophereol	10191-41-0	2116	84	27.43	27.38	0.05	15%	502	67
cholesterol	57-88-5	304	97	27.55	27.56	-0.01	89%	368	198

Table 1 continued

A further investigation of the identified peaks revealed that both signal/noise ratios and peak purity determined the final net match for mass spectral similarities of blood plasma metabolites (**Fig. 5**). Above a signal/noise ratio of 100, all peaks reliably yielded Agilent Fiehn library net matches with a score of better than 80. For peak purity, a general trend was that a better spectral net match was associated with higher peak purity. However, this trend for peak purity did not have a clear and sharp relationship and cut-off range as observed for signal/noise ratio. The 102 identified metabolites were subsequently classified by major chemical groups into 'sterols and aromatics', 'amino compounds', 'hydroxyl acids', 'carbohydrates' and 'fatty acids and fatty alcohols'. Each of the classes was represented by 15 or more identified peaks (**Fig. 6**) with a slightly higher representation for amino acids and other compounds bearing amino groups (such as urea). This figure demonstrates that all major compound classes of primary metabolism can be targeted and identified in blood plasma by GC/MS.

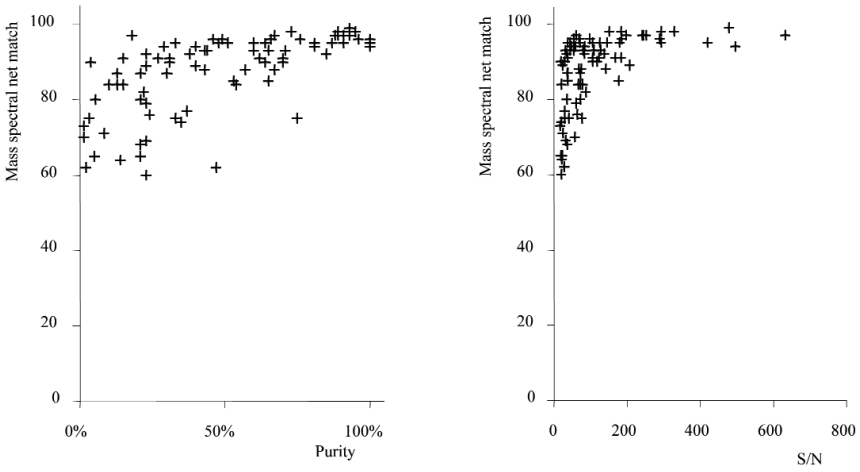

Fig. 5. Dependence of mass spectral net match for identified metabolites. Left panel: mass spectral purity after AMDIS deconvolution. Right panel: Signal/noise ratio of quantification ion.

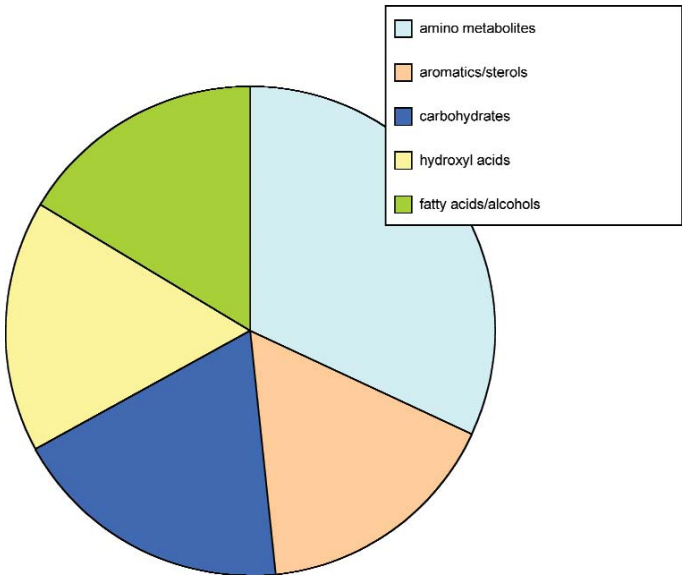

Fig. 6. Fraction of compound classes in identified blood plasma metabolites.

Results from the Agilent Fiehn GC/MS Metabolomics RTL Library searches not only identify metabolites but also provide unique compound identifiers, the PubChem numbers, that allow retrieving physiological and biochemical information. This is particularly helpful for less well-known compounds such as 'itaconic acid'. When querying the PubChem number (which is found in brackets preceding each compound name in the Agilent Fiehn library), the PubChem website provides links to the biochemical pathway database KEGG (**Fig. 7**). Following the link, KEGG displays information on enzymes and pathway overviews in which these compounds are involved. Exemplified is itaconate which leads to 'C5-branched dibasic acid metabolism', which comprises both citraconic acid and itaconic acid (identified in **Fig. 3**) and derives the hypothesis that their abundance is regulated over citramalate. When using such KEGG pathways, it is important to understand that human blood plasma metabolite queries must not be constrained to 'human' pathways because of the diversity of gut microbes that excrete metabolites into the vascular system and because of the exogenous compounds that are ingested by humans through food (e.g. of plant origin). Hence, a high diversity of compounds and a resulting high complexity of the plasma metabolome can be expected. This is the primary reason why many detected peaks in plasma profiles yet remain to be identified.

Conclusions

The new Agilent Fiehn GC/MS Metabolomics RTL Library was applied to metabolic analysis of human blood plasma. It was shown that the identification routines comprised in the ChemStation software can be complemented by AMDIS mass spectral deconvolution and matching peak spectra to the Agilent Fiehn library spectra, which enable fast, flexible, high-throughput searching. One of the most important criteria for unambiguous identification was the retention times, which were locked to the absolute retention time of the internal standard, d27-myristic acid. Retention time locking, a feature of the Agilent Fiehn library, increases identification confidence. Retention time deviations were found to be less than 0.15 min, increasing the reliability and confidence in metabolite annotations. Such procedures will be important in recent efforts to standardize reporting of metabolomic results, as suggested by the NIH/NIDDK and the Metabolomics Society.

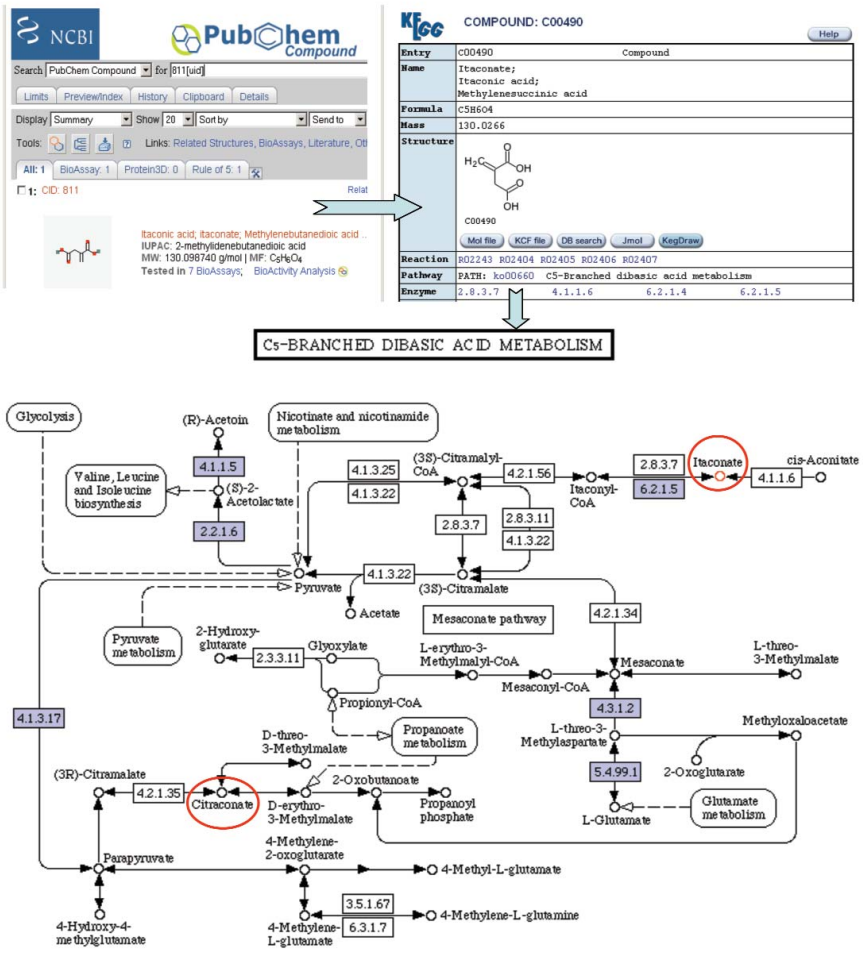

Fig. 7. Retrieving information about biological relevance for identified metabolites using the PubChem number.

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