Poster Reprint

ASMS 2022
Poster number WP365

Comprehensive Accurate Mass Metabolomics EI Library and Validation of the Data Processing Workflows Using Human Plasma Samples

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Introduction

EI GC/MS mass spectral libraries have been proven to be an essential tool that enables a quick compound identification. Both retention time/index as well as accurate mass information provide additional evidence that helps to confirm or reject compound identity. Accurate mass GC/MS libraries are particularly useful for applications that involve difficult matrices and/or where the compounds of interest have a complex elemental formula, and metabolomics is one of these applications. To help improve reliability and increase throughput in the metabolomics applications, we created an accurate mass spectral Retention Time Locked EI library of compounds generally found in biological matrices containing over 800 entries. We have also validated the library using both target and non-target screening workflows with human plasma samples.

Experimental

Metabolite standards and human plasma extracts were derivatized by methoximation followed by silylation with MSTFA + 1 % TMCS. EI spectra for individual standards or mixtures of metabolites, known to well separate chromatographically have been acquired using an accurate mass high resolution 7250 GC/Q-TOF system (Figure 1). Accurate mass EI fragments were converted to the theoretical m/z using MassHunter Qualitative Analysis software version 10, prior to importing the spectra into the accurate mass metabolomics Personal Compound Database and Library (PCDL). The metabolites were extracted from human plasma using acetonitrile : isopropanol : water (3:3:2). The extracts were dried, derivatized as described above and were further used to evaluate accurate mass library screening workflows.

Figure 1. Agilent 7250 GC/Q-TOF

GC and MS Conditions:	Q-TOF (7250)
GC	7890
Column	DB-5MS UI, 30 m, 0.25 mm, 0.25 µm, DuraGuard, 10m
Inlet	SSL, 4-mm UI liner single taper
Injection volume	1 µL
Injection mode	Splitless
Inlet temperature	280°C
Oven temperature program	50°C for 0.5 min; 10°C/min to 325°C, 10 min hold
Carrier gas	Helium
Column flow	1 mL/min
Transfer line temperature	280°C
Quadrupole temperature	150°C
Source temperature	200°C
Electron energy	70 eV
Emission current	5 µA
Spectral acquisition rate	5 Hz
Mass range	50 to 1200 m/z

Table 1. GC/Q-TOF Acquisition Parameters

Building the Accurate Mass Metabolomics Library

The metabolites for the accurate mass Metabolomics PCDL were selected to make sure that majority of the GC/MS-amenable primary metabolites are included. To convert each fragment ion m/z into the theoretical m/z, an automated fragment formula annotation for each metabolite spectrum was performed and reviewed and corrected, when necessary, prior to exporting the spectrum into the Metabolomics PCDL (Figure 2). The current Metabolomics PCDL also includes RTs as well as both alkanes and fatty acid methyl esters (FAMEs)-based retention indices (RIs). The current version of the accurate mass Metabolomics library contains over 800 accurate mass EI spectra and over 500 unique compounds.

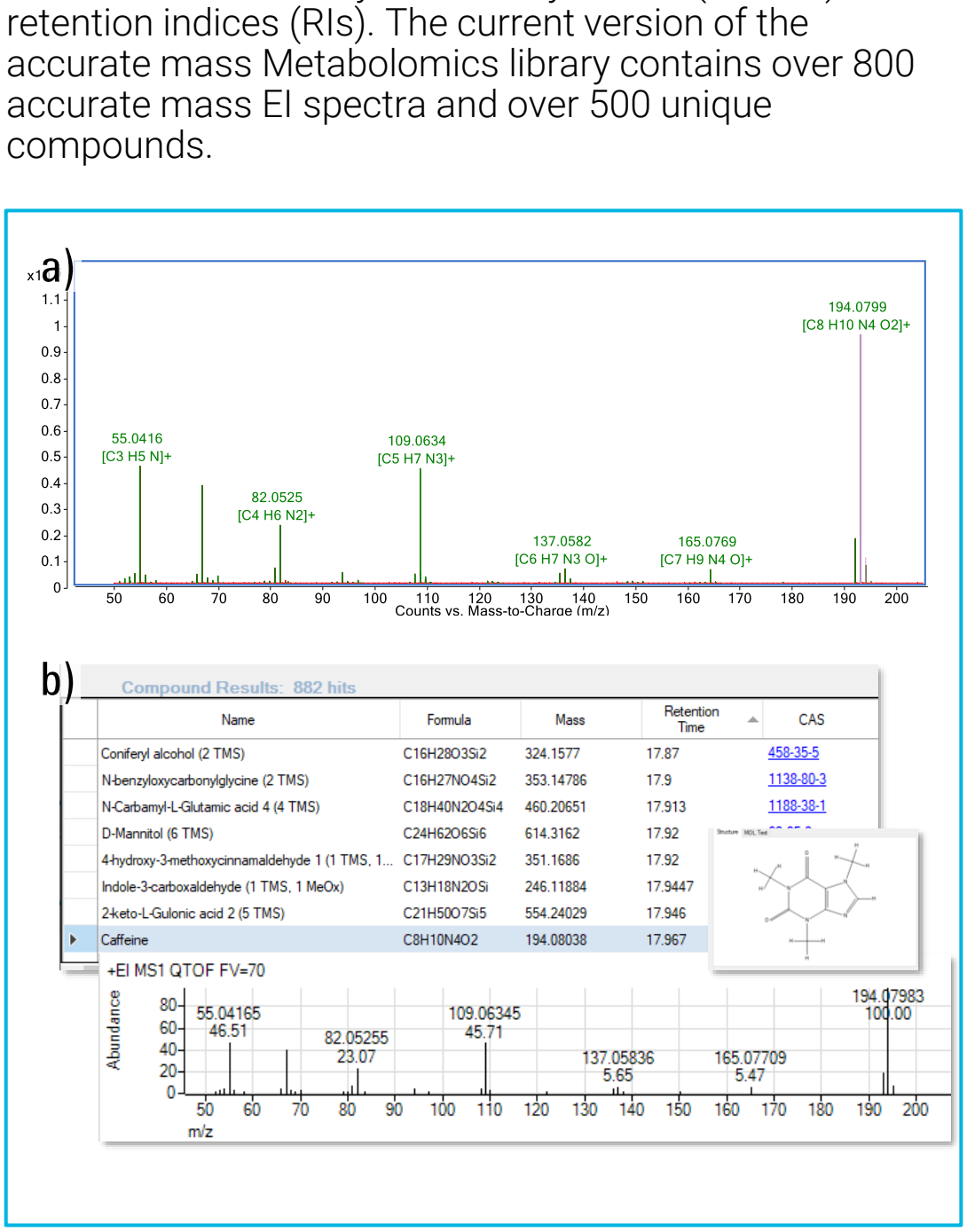

Figure 2. Creation of the PCDL. A) Fragment Formula Annotation for subsequent conversion of the accurate m/z of the entire spectrum to the theoretical m/z. B) The Metabolomics accurate mass library can be viewed in the PCDL Manager

The Accurate mass Metabolomics PCDL included a comprehensive variety of GC-amenable compound classes (Figure 3).

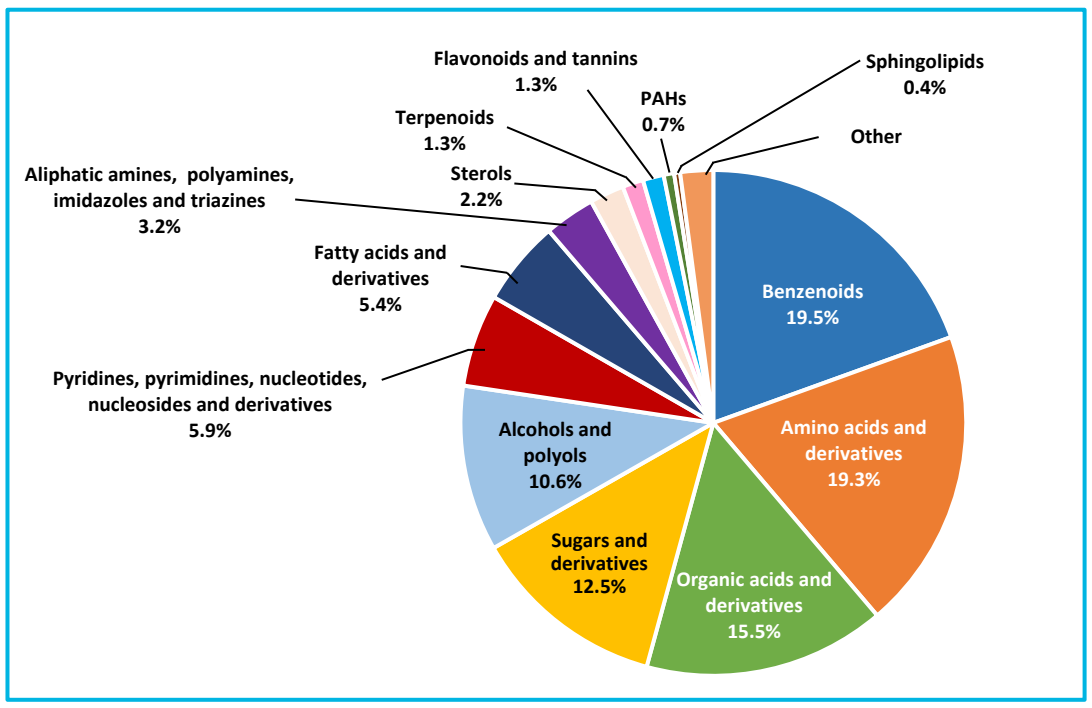

Figure 3. Compound classes included into the Metabolomics PCDL and their distribution

Validation of the Metabolomics PCDL Using Human Plasma

The Metabolomics PCDL has been validated using the human plasma extracts in a non-targeted (Figure 4) as well as targeted (Figure 5) screening workflows incorporated into the Unknowns Analysis (UA) as well as Quantitative Analysis of MassHunter, respectively.

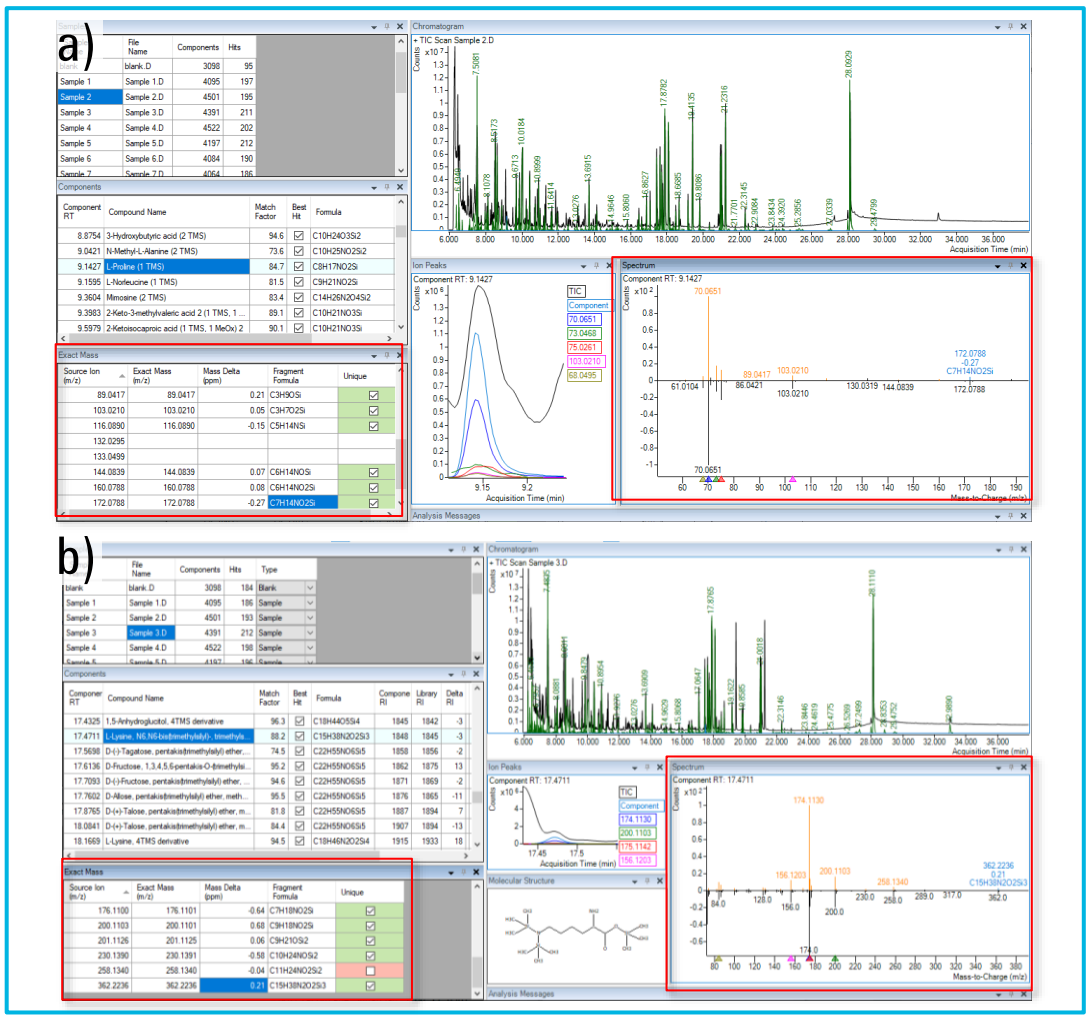

Figure 4. Non-targeted analysis using a) accurate mass Metabolomics PCDL and b) unit mass NIST17.L ExactMass tool (see the tables and mirror plots outlined in red rectangles) helped to get rid of false positives. Compound ions are highlighted when m/z corresponds to the library hit formula

Results and Discussion

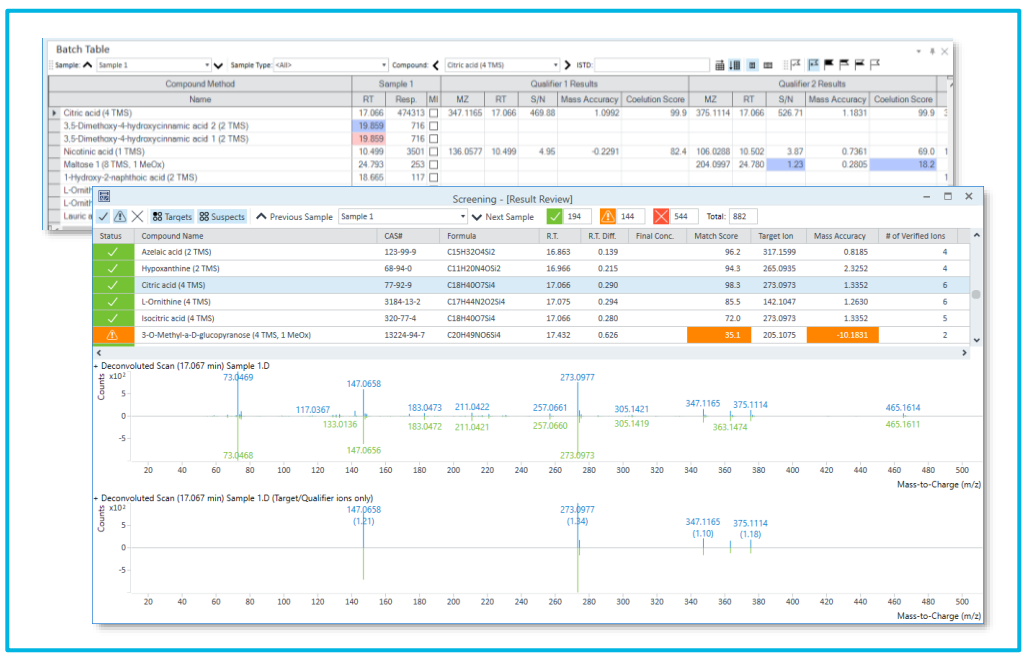

Figure 5. Target screening results and visualization. The Screener summary window allows to quickly review the results for further method optimization in order to minimize false positives and false negatives

Screening Method Parameters and Results Filtering

Method parameters for both target and non-target screening approaches were optimized to reduce false positives and false negatives. In particular, this applied to Library Match score and RT/RI window limits as well as forward-reverse search weight factor.

The results obtained using NIST library were filtered out based on the accurate mass information using the ExactMass tool. Examples of false negatives are shown in Figure 6. 36 to 48 compounds per sample were found to be false positives only based on the accurate mass information. This represented approximately 30% of total hits. Using the accurate mass Metabolomics PCDL (in target and non-target approaches) allowed to automatically exclude false positives due to accurate mass discrepancies since it is built into the screener/library search tool.

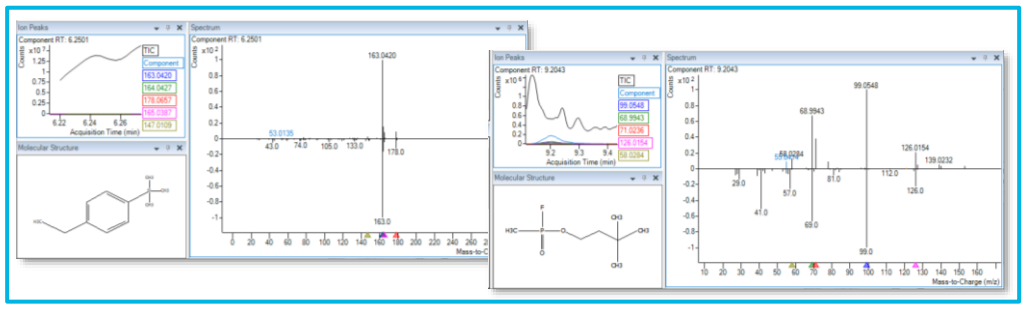

Figure 6. Examples of the false negatives that can be easily recognized with a help of the ExactMass tool

Comparison between Different Screening Approaches Using the Metabolomics PCDL and NIST

Number of compounds identified in human plasma samples in the target screening approach with PCDL and non-target analysis using both NIST and PCDL were compared (Table 2).

Sample name	Number of identified compounds		
	Screener	UA PCDL	UA NIST
Sample A	153	151	140
Sample B	149	146	144
Sample C	151	146	138
Sample D	152	146	143
Sample E	145	138	145
Sample F	141	141	139
Sample G	139	148	146
Sample H	164	144	147
Sample I	146	139	131
Sample J	152	148	136
Sample K	151	146	132
Sample M	168	151	140
Sample N	167	152	157

Table 2. Number of the identified compounds when using different screening approaches and libraries

Typically, a higher number of compounds was identified when using the Metabolomics PCDL as compared to NIST. However, interestingly target and non-target approached were complimentary with regards to the specific compounds identified by each method.

RT	Compound Name	Derivatized Formula	UA Library Match Score	Screener Library Score	RT	Compound Name	Derivatized Formula	UA Library Match Score	Screener Library Score
6.48	Boric acid	C9H27BO3Si3	93.2	82.1	17.06	Citric acid	C18H40O7Si4	99.3	98.2
7.33	L-Lactic acid	C9H22O3Si2	95.4	83.8	17.07	L-Ornithine	C17H44N2O2Si4	95.7	87.1
8.39	2-hydroxybutanoic acid	C10H24O3Si2	96.7	99.6	17.51	Caffeine	C8H10N4O2	88.2	99.6
8.78	p-Cresol	C10H16OSi	85.3	99.6	17.57	D-tagatose 2	C22H55NO6Si5	94.4	90.4
8.85	L-Leucine	C9H21NO2Si	81.3	98.5	17.76	Mannose 1	C22H55NO6Si5	98.8	99.8
8.85	3-Hydroxybutyric acid	C10H24O3Si2	95.6	81.9	17.83	L-Tyrosine 1	C15H27NO3Si2	84.7	84.8
8.90	2-Hydroxy-3-methylbutyric acid	C11H26O3Si2	92.2	92.6	17.87	D-Glucose 1	C22H55NO6Si5	83.6	99.8
9.58	2-Ketoisocaproic acid 2	C10H21NO3Si	94.2	97.4	18.23	D-Mannitol	C24H62O6Si6	98.6	80.8
9.85	Ethanolamine	C11H31NOSi3	97.6	98.9	18.26	Glucuronic acid 1	C21H50O7Si5	92.7	82.1
10.14	Benzoic acid	C10H14O2Si	96.8	99.6	18.34	L-Tyrosine 2	C18H35NO3Si3	95.0	99.5
10.21	L-Serine	C9H23NO3Si2	98.0	98.8	18.48	Sinigrin	C22H44NO9S2Si4	87.2	85.1
10.40	Phosphoric acid	C9H27O4PSi3	89.3	98	18.48	Myo-Inositol	C24H60O6Si6	88.0	82.5
10.78	L-Proline	C11H25NO2Si2	93.8	99.7	18.59	Indole-3-acetic acid	C16H25NO2Si2	88.5	82.9
10.89	Glycine	C11H29NO2Si3	97.8	99.6	18.93	Gluconic acid	C24H60O7Si6	90.9	97.6
11.59	L-Serine	C12H31NO3Si3	96.0	99.2	19.21	Palmitoleic acid	C19H38O2Si	96.0	99.7
11.64	Nonanoic acid	C12H26O2Si	98.2	99.5	19.41	Palmitic acid	C19H40O2Si	97.2	99.9
12.50	ß-Alanine	C12H31NO2Si3	94.3	99.3	19.41	Cyclohexanecarboxylic acid	C10H20O2Si	98.1	85.8
12.90	Capric acid	C13H28O2Si	93.9	99.4	19.76	Indole-3-propionic acid	C17H27NO2Si2	83.4	84.6
13.55	O-Acetylsalicylic acid	C13H22O3Si2	87.9	99.5	19.80	Conduritol-ß-expoxide 2	C18H38O5Si4	93.7	86.8
13.69	L-Glutamic acid (dehydrated)	C11H23NO3Si2	99.1	98.9	19.85	Uric acid	C17H36N4O3Si4	96.4	99.7
13.81	L-Glutamate	C11H25NO4Si2	87.3	99.1	20.58	Indole-3-lactate	C20H35NO3Si3	89.0	93.5
14.05	L-Phenylalanine	C12H19NO2Si	95.6	98.3	20.89	L-Tryptophan	C19H30N2O3Si2	80.3	83.9
14.10	Creatinine	C13H31N3OSi3	86.3	99.3	20.96	N-Acetyl-L-Tryptophan	C20H36N2O2Si3	83.4	81.9
14.32	a-Hydroxyglutaric acid	C14H32O5Si3	94.9	80	21.00	Oleic acid	C21H42O2Si	97.9	99.9
14.64	Pyrogallol	C15H30O3Si3	83.3	82.7	21.22	Stearic acid	C21H44O2Si	97.2	99.2
14.86	L-Glutamate	C14H33NO4Si3	90.2	99.7	22.31	Adrenic acid	C25H44O2Si	85.3	92
14.96	L-Phenylalanine	C15H27NO2Si2	98.1	99.1	24.03	1-Monopalmitin	C25H54O4Si2	83.7	94.5
14.98	4-Hydroxybenzoic acid	C13H22O3Si2	82.9	95.2	24.39	Sucrose	C36H86O11Si8	94.5	96
15.27	Lauric acid	C15H32O2Si	96.3	99.5	24.46	Behenic acid	C25H52O2Si	82.4	95.7
15.49	Arabinose	C18H45NO5Si4	96.8	85	25.15	Maltose 1	C37H89NO11Si8	91.4	98.7
15.90	3-Indoxylsulfate	C14H23NO4SSi2	91.5	86	25.31	1-Monoolein	C27H56O4Si2	87.3	96
16.13	3-(3-hydroxyphenyl)propionic acid	C15H26O3Si2	80.9	99.6	25.47	Glyceryl monostearate	C27H58O4Si2	84.9	97.4
16.38	L-Ornithine	C14H36N2O2Si3	96.0	98.1	25.79	Squalene	C30H50	90.1	98.9
16.48	Ribonic acid	C20H50O6Si5	93.5	93.1	27.03	ß-Tocopherol	C31H56O2Si	90.6	99.7
16.51	4-Hydroxy-3-methoxybenzoic acid	C14H24O4Si2	92.7	99.8	27.98	a-Tocopherol	C32H58O2Si	95.2	94.7
16.86	Azelaic acid	C15H32O4Si2	97.8	94.5	28.09	Cholesterol	C30H54OSi	88.0	99.8
16.96	Hypoxanthine	C11H20N4OSi2	91.1	93.8	29.48	ß-Sitosterol	C32H58OSi	92.2	98.6

Table 3. Compounds identified in human plasma using target (Screener) and non-target (UA) approaches with the Metabolomics PCDL. Only shown compounds identified with the library match score > 80 in both cases

Conclusions

- A comprehensive accurate mass EI Metabolomics library has been created validated using human plasma samples
- Accurate mass library ensured more specific and sensitive detection of the metabolites as compared to a unit mass library such as NIST

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RA44677.5561111111

Published in USA, May 20, 2022

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