Montreux 2007

Profiling and Biomarker Identification in biological fluids from differing Zucker rat strains using high mass accuracy MS^n analysis

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Overview

In this study, plasma and urine samples were taken from two Zucker rat strains and analyzed by using electrospray in positive and negative ion mode (electrospray ionization resulted in a far higher number of ion signals compared to APCI). Profiling software and Principal Component Analysis (PCA) was used not only to establish the differences between the two rat strains but also to highlight specific endogenous metabolites that could account for the PCA data.

A quadrupole ion trap mass spectrometer was applied to lipid profiling on biological samples derived from two Zucker rat strains (the Zucker (fa/fa) obese, which is a leptin deficient mutant, and the wild type, which is normal). To validate the assignments of lipid species based on both mass accuracy in MS and MSn data streams, a formula prediction software tool was used to determine the lipid profile distributions between the two rat strains.

References

1. Jones, J. J.; Stump, M. J.; Fleming, R. C.; Lay, J. O. Jr.; Wilkins,
C. L. J. Am. Soc. Mass Spectrom. 2004, 15, 1665.

Introduction

Mass spectrometry (MS) has been shown to be a powerful tool in elucidating the diversity of lipid species and their participation in regulating cellular functions particularly in biomarker discovery research. In recent years, high mass accuracy mass spectrometry has been increasingly utilized, in profiling global lipid distribution to help differentiate lipid species (to assign chemical compositions for lipid mass accuracy better than 21ppm is required, Jones et al, 2004).

To help identify individual lipid molecular species public domain databases (DBs) play an important role in component verification (for example http://lipidsearch.jp or http://metlin.scripps.edu/metabo_list.php.

By bringing together high mass accuracy MSn analysis with formula prediction software, identifying endogenous metabolites in profiling studies can be assigned with greater certainty even in the presence of a complex biological matrix.

Methods

Plasma and urine samples were taken from two Zucker rat strains (the Zucker (fa/fa) obese, which is a leptin deficient mutant, and the wild type, which is normal). Components were separated using a gradient elution into a LCMS-IT-TOF mass spectrometer (quadrupole ion trap-time of flight MS) (Shimadzu Corporation, Japan) using electrospray ionization (ESI), scanning 200-850 m/z in MS and between 50-850 in MS^n mode. The chromatographic system used a binary solvent system delivered as a gradient of Solvent A (formic acid solution 0.1%) and Solvent B (acetonitrile), and used a Shim-Pack XR ODS, 2.1x100 mm.

Profiler software (Phenomenome Informatics) was used to create a data array for all sample data; this tool was used to highlight specific components that were statistically different between the two rat strains and to export data to SIMCA-P (Umetrics) for PCA analysis.

Results

Phenomenome Profiler™ M Series (AM)+

File Edit Help

Phenomenome Profiler
Rat plasma profiling - lipid distribution
Normalized data -- null, Log(10), 0.01

Normalized data -- null, Log(10), 0.01

	Ioni...	Neu...	Ret...	Anal...	P_V...	Zucker (fa/fa) obese rat										Wild type rat									
103	565....	564....	6.388	5102...	0.0003	5.87	5.92	5.52	5.75	5.78	5.84	6.02	5.36	5.79	6.02	5.39	5.53	5.51	5.4	5.37	5.4	5.59	5.48	5.58	5.45
104	564....	563....	6.3875	5102...	0.0007	6.45	6.49	6.12	6.37	6.42	6.5	6.63	5.93	6.41	6.54	6.05	6.14	6.13	6.08	6.05	6.1	6.18	6.1	6.21	6.01
105	542....	541....	6.3778	5102...	0.0004	5.58	5.59	0	5.57	5.58	5.68	5.76	0	5.55	5.71	0	0	0	0	0	0	5.27	0	0	0
106	543....	542....	6.3763	5102...	1.65...	6.82	6.85	6.48	6.78	6.8	6.87	6.99	6.31	6.78	6.9	0	0	0	0	0	0	0	0	0	0
107	542....	541....	6.373	5102...	0.0002	7.29	0	0	7.25	7.28	7.34	7.46	6.79	0	7.37	0	0	0	0	0	0	0	0	0	0
108	500....	499....	6.3019	5102...	0.0017	5.38	5.7	0	5.53	5.64	0	5.54	0	0	5.54	0	0	0	0	0	0	0	0	0	0
109	357....	356....	6.2192	5102...	1.19...	0	0	0	0	0	0	0	0	0	0	0	5.26	5.94	5.51	0	5.44	5.63	5.63	5.84	5.28
110	469....	468....	6.2017	5102...	0.0026	6.76	6.78	6.77	6.69	6.8	6.87	6.84	6.21	6.9	6.83	6.55	6.39	6.39	6.53	6.56	6.49	6.66	6.48	6.56	6.49
111	468....	467....	6.2016	5102...	0.0035	7.33	7.37	7.35	7.26	7.37	7.45	7.42	6.77	7.47	7.41	7.13	6.96	6.97	7.11	7.14	7.06	7.24	7.06	7.14	7.05
112	470....	469....	6.2004	5102...	0.0015	5.9	5.96	5.93	5.81	5.99	6.07	6.04	5.41	6.04	6.02	5.73	5.52	5.54	5.72	5.69	5.63	5.82	5.65	5.7	5.64
113	468....	467....	6.1891	5102...	0.0002	6.02	0	6.06	0	6.1	6.16	6.13	0	6.17	6.12	0	0	0	0	0	0	0	0	0	0
114	450....	449....	6.1889	5102...	0.0005	5.81	5.9	5.86	0	5.87	5.95	5.94	0	5.93	5.93	5.7	0	0	0	0	0	0	0	0	0
115	468....	467....	6.1881	5102...	0.0077	0	0	0	0	5.49	5.54	5.54	0	5.61	5.66	0	0	0	0	0	0	0	0	0	0
116	398....	397....	5.9509	5102...	5.39...	5.79	5.64	5.65	5.57	5.91	5.47	5.87	6.61	5.77	5.81	0	0	0	0	0	0	0	0	0	0
117	492....	491....	5.9391	5102...	1.29...	0	0	0	0	0	4.96	0	0	0	0	5.35	5.41	5.3	5.3	0	5.24	5.58	5.45	5.54	5.32
118	598....	597....	5.9308	5102...	0.0002	5.51	5.26	5.23	0	5.2	0	5.64	0	5.62	5.39	0	0	0	0	0	0	0	0	0	0
119	601....	600....	5.8184	5102...	0.0018	5.56	5.14	0	0	5.47	0	5.73	0	5.74	5.41	0	0	0	0	0	0	0	0	0	0
120	600....	599....	5.8138	5102...	8.99...	6.06	5.88	5.74	5.65	6.02	5.38	6.26	0	6.24	5.98	0	0	0	0	5.12	0	0	0	0	0
121	256....	255....	5.7541	5102...	0.0018	5.9	0	0	7.27	6.41	0	7.28	6.19	6.46	0	0	0	0	0	0	0	0	0	0	0
122	257....	256....	5.7523	5102...	0.0081	0	0	0	6.56	5.65	0	6.58	5.33	5.68	0	0	0	0	0	0	0	0	0	0	0
123	598....	597....	5.7095	5102...	0.0002	5.51	5.25	5.3	0	5.41	0	5.73	0	5.77	5.48	0	0	0	0	0	0	0	0	0	0
124	558....	557....	5.7078	5102...	0.0002	5.83	5.65	5.6	0	5.73	0	6.04	0	6	5.79	0	0	0	0	0	0	0	0	0	0
125	585....	584....	5.6286	5102...	4.83...	5.58	5.68	5.21	5.33	5.84	5.3	5.59	0	5.64	5.68	0	0	0	0	0	0	0	0	0	0
126	601....	600....	5.614	5102...	0.0018	5.46	5.23	0	0	5.41	0	5.71	0	5.68	5.35	0	0	0	0	0	0	0	0	0	0
127	562....	561....	5.5128	5102...	0.0017	5.51	5.65	0	5.38	5.45	0	0	0	5.47	5.66	0	0	0	0	0	0	0	0	0	0
128	558....	557....	5.4958	5102...	0.0026	5.87	5.69	5.7	5.37	5.82	0	6.06	0	6.05	5.76	0	0	0	5.17	5.1	0	0	0	0	0
129	598....	597....	5.493	5102...	0.0002	5.5	5.32	5.24	0	5.44	0	5.75	0	5.74	5.33	0	0	0	0	0	0	0	0	0	0
130	600.33	599....	5.3337	5102...	0.0028	5.93	0	5.65	5.62	5.96	5.31	6.13	0	0	5.84	0	0	0	0	5.11	0	0	0	0	0
131	585....	584....	5.2743	5102...	0.0077	0	5.64	0	5.4	0	5.3	5.61	0	5.56	0	0	0	0	0	0	0	0	0	0	0
132	558....	557....	5.2691	5102...	0.0026	5.78	5.63	5.57	5.53	5.61	0	5.93	0	5.82	5.69	0	0	0	4.95	5.15	0	0	0	0	0

Figure 1.

Profiler software was used to transform LC/MS raw data into a data array of retention time and mass pairs. The data array was then normalized to highlight differences in component identities between the Zucker (fa/fa) obese, which is a leptin deficient mutant, and the wild type, which is normal.

Results

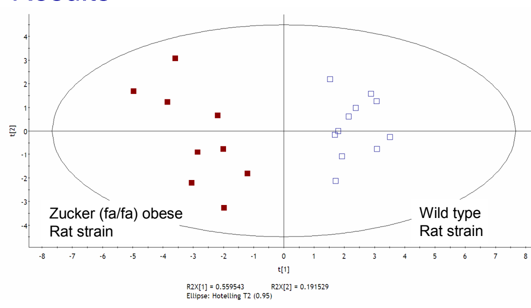


Figure 2. PCA discriminates between the two rat strains (the Zucker (fa/fa) obese, which is a leptin deficient mutant, and the wild type, which is normal). The difference between the two clusters reflects a different distribution of plasma phospholipid concentrations.

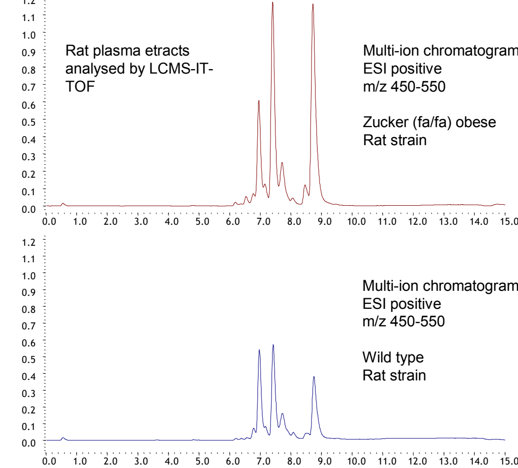
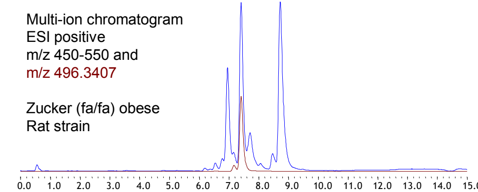
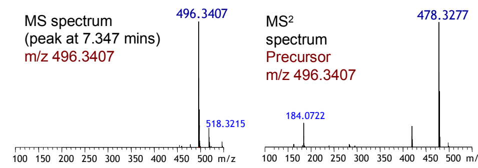


Figure 3. Mass chromatogram range between m/z 450-550 for the Zucker (fa/fa) obese (upper chromatogram) and wild type (lower chromatogram) normalised to the same intensity. The distribution of phospholipids is the same between the two strains, the difference in PCA is with the ion signal intensity between the groups.

Component identification

Predicting a candidate list based on MS and MSn data takes into account mass accuracy and mass resolution of the experimentally derived pseudo-molecular peak and related fragment ion data generated using MSn spectra (or complement ion data) together with conventional chemical rules (such as nitrogen, electron configuration, DBE range, H/C ratio).





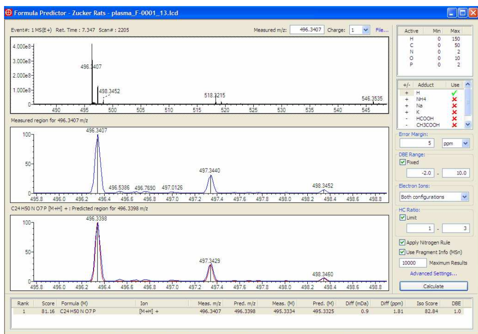
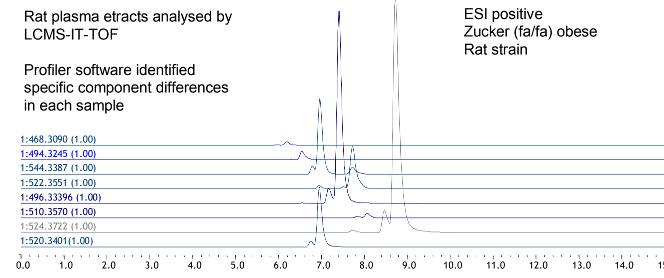


Figure 4. Formula prediction software used to help identify the component at 7.347 minutes. The most probable empirical formula for the component was $C_{24}H_{50}NO_7P$. Using public databases this formula corresponded to 1-Palmitoyllysophosphatidylcholine. The same approach was used to identify all other lipids components in the plasma samples in both populations.

Positive identification using high mass accuracy MSn

Using the formula prediction software a number of phospholipid components were positively identified (the components showed a strong agreement with published high mass accuracy data for MS and MS/MS and also showed the same fragment ions as the public databases)



Rt (mins)	Measured (M+H)+	Theoretical (M+H)+	accuracy ppm	Mass Formula	ID
6.200	468.3090	468.3085	1.07	C22 H46 N O7 P	Myristoyl L-a-lysophosphatidylcholine
6.560	494.3245	494.3241	0.81	C24 H48 N O7 P	1-(9E-hexadecenoyl)-sn-glycero-3-phosphocholine
6.940	520.3401	520.3398	0.58	C26 H50 N O7 P	LPC(1-acyl 18:2)
6.944	544.3387	544.3398	-2.02	C28 H50 N O7 P	Arachidonyl lysolecithin
7.393	496.3396	496.3398	0.00	C24 H50 N O7 P	1-Palmitoyllysophosphatidylcholine
7.677	522.3551	522.3554	-0.57	C26 H52 N O7 P	1-Oleoylglycerophosphocholine
8.099	510.3570	510.3554	-1.57	C32 H47 N O4	1-octadecyl-sn-glycero-3-phosphocholine
8.750	524.3722	524.3711	2.10	C26 H54 N O7 P	1-Stearoylglycerophosphocholine

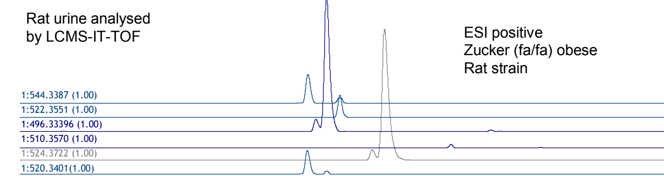


Figure 5. A series of lipids were identified using the formula prediciton software. In all instances the predicted formula was the first hit; in most instances only one candidate was reported illustrating the usefulness of the MSn as a candidate filter in plasma and urine samples.

Conclusions

Although profiling studies are useful tools in biomarker research it is also important to provide positive identification for specific components. Using high mass accuracy MSn data with a software tool that calculates empirical formula assignments helps to increase the probability of positive identification using LC/MS.