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Accelerating lipid profiling of human samples for biomarker discovery using UFLC-IT-TOF

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Overview

A global metabolomics approach was used to study the measurement of lipid levels in human plasma samples.

In this paper, we describe the application of a LCMS-IT-TOF to detect lipid ion signals using a LC cycle time of 10 minutes [peak widths between 5-7 seconds] with high mass accuracy.

Ion signal stability was assessed by a pooled QC sample analyzed throughout the batch . Despite the compressed nature of the LC cycle time, the peak area variance was less than 10% (n=17; batch analysis time 25 hours) for a series of phospholipids.

Endogenous lipid metabolites were identified using high accuracy MS data and verified using external search engines (http://www.lipidmaps.org; http://www.hmdb.ca; http://www.genome.jp/kegg/).

References

Koulman et al, RCM 2009; 23; 1411-1418

Introduction

Untargeted metabolite profiling has become an integral part of systems biology research and is helping to further our understanding of the physiological and patho-physiological processes related to diet, environmental factors, disease and the impact of pharmaceutical treatments. In practical terms global metabolite profiling needs to take into account not only the inherent complexity and diversity of human metabolism but also analytical technologies that deliver robust, reproducible and high quality data. To help standardize and objectively measure metabolite levels in large sample numbers a standard biological Quality Control has become a widely accepted tool in metabolic profiling to assess system performance and to filter ion signals which show idiosyncratic response throughout the batch analysis. This paper discusses the application of an ion trap-time of flight mass spectrometer to measure endogenous metabolite levels in human plasma profiling studies.

Methods

The plasma of healthy volunteers was used, either undiluted, with 20% Ringer addition, with glucose in Ringer addition, with palmitate in Ringer addition, with N-octanoulsphingosine in Ringer or with glucose, palmitate and N-octanoulsphingosine together in palmitate. This resulted in sample set of 60 different samples. By combining aliquots of all samples a pooled QC samples was created. Plasma samples were analyzed by Prominence HPLC coupled with an electrospray ion trap-time of flight mass spectrometry (LCMS-IT-TOF, Shimadzu Corporation, Kyoto, Japan) using a 0.1% formic acid : acetonitrile gradient with a sample cycle time of less than 10 minutes.

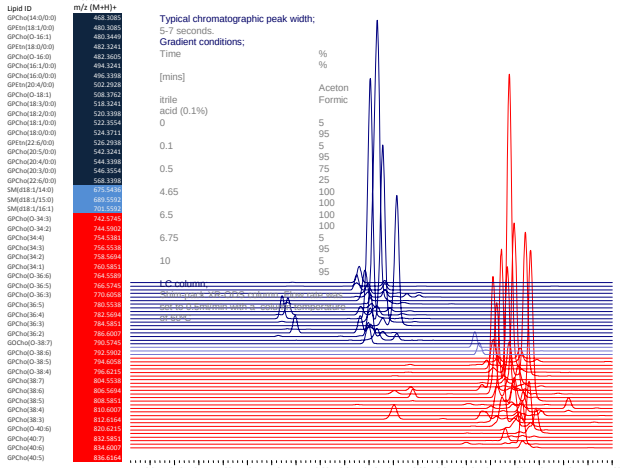

Figure 1.
Mass chromatograms for a series of phospholipids from a pooled QC human plasma sample. Typical chromatographic peak width; 5-7 seconds. MS scan speed; 100msec ; m/z 170-900; external mass calibration; ion accumulation time 20msec. In MS mode10 spectra per second were acquired. In polarity switching mode, the switching time was 100msec.

Results

In metabolite profiling studies it is important to assess the system reproducibility throughout the batch analysis. In this study the pooled QC sample was used to characterize the reproducibility of the 'system'. Whilst the FDA suggests that variability of ±15% of the nominal value represents an acceptable degree of reproducibility, in long term profiling studies the tolerance is often relaxed to between 20-40%. In this study, ion signals which resulted in a relative standard deviation (RSD) <20% and retention time RSD<1% were considered in subsequent principal component analysis.

Lipid ID	Theoretical m/z (M+H)+	Avg RT Mins	Min RT	Max RT	%RSD RT	%RSD Area	Ion signal intensity [Average response]
GPCho(14:0/0:0)	468.3085	2.395	2.392	2.403	0.13	2.76	2,173,959
GPEtn(18:1/0:0)	480.3085	2.650	2.654	2.665	0.13	4.38	685,760
GPCho(O-16:1)	480.3449	2.654	2.655	2.666	0.13	5.54	780,277
GPEtn(18:0/0:0)	482.3241	2.490	2.482	2.493	0.13	3.06	1,321,249
GPCho(O-16:0)	482.3605	2.490	2.482	2.493	0.13	3.81	649,604
GPCho(16:1/0:0)	494.3241	2.451	2.451	2.462	0.13	2.33	4,722,352
GPCho(16:0/0:0)	496.3398	2.541	2.579	2.590	0.13	0.93	50,235,899
GPEtn(20:4/0:0)	502.2928	2.520	2.518	2.529	0.12	4.61	504,206
GPCho(O-18:1)	508.3762	2.639	2.707	2.718	0.12	4.82	422,688
GPCho(18:3/0:0)	518.3241	2.405	2.407	2.417	0.13	3.62	1,241,123
GPCho(18:2/0:0)	520.3398	2.509	2.507	2.518	0.13	0.76	36,334,718
GPCho(18:1/0:0)	522.3554	2.640	2.634	2.645	0.12	1.48	25,727,526
GPCho(18:0/0:0)	524.3711	2.780	2.778	2.789	0.11	1.91	18,414,007
GPEtn(22:6/0:0)	526.2938	2.654	2.501	2.512	0.13	9.65	276,513
GPCho(20:5/0:0)	542.3241	2.507	2.507	2.519	0.14	8.75	1,206,508
GPCho(20:4/0:0)	544.3398	2.512	2.507	2.518	0.13	1.02	6,262,232
GPCho(20:3/0:0)	546.3554	2.575	2.573	2.585	0.13	2.72	1,846,463
GPCho(22:6/0:0)	568.3398	2.492	2.486	2.497	0.13	2.35	2,762,050
SM(d18:1/14:0)	675.5436	3.550	3.578	3.592	0.10	2.22	1,878,303
SM(d18:1/15:0)	689.5592	3.870	3.692	3.702	0.10	8.05	132,281
SM(d18:1/16:1)	701.5592	3.618	3.624	3.638	0.10	2.72	1,477,189
GPCho(O-34:3)	742.5745	4.030	4.043	4.057	0.09	2.23	1,307,432
GPCho(O-34:2)	744.5902	4.066	4.054	4.069	0.09	4.59	946,724
GPCho(34:4)	754.5381	3.698	3.699	3.714	0.10	4.07	588,363
GPCho(34:3)	756.5538	3.758	3.767	3.781	0.10	2.37	5,074,594
GPCho(34:2)	758.5694	3.937	3.931	3.946	0.09	0.64	43,017,246
GPCho(34:1)	760.5851	4.095	4.096	4.111	0.09	1.05	19,205,930
GPCho(O-36:6)	764.5589	3.877	3.876	3.891	0.10	2.27	473,322
GPCho(O-36:5)	766.5745	4.017	4.022	4.037	0.09	2.56	2,210,100
GPCho(O-36:3)	770.6058	3.927	4.092	4.106	0.09	5.52	572,722
GPCho(36:5)	780.5538	3.768	3.764	3.779	0.10	2.12	15,194,035
GPCho(36:4)	782.5694	3.907	3.911	3.926	0.10	1.22	17,274,519
GPCho(36:3)	784.5851	3.977	3.980	3.995	0.09	1.31	29,490,182
GPCho(36:2)	786.6007	4.145	4.149	4.164	0.09	1.71	20,403,293
GOCho(O-38:7)	790.5745	3.937	3.953	3.968	0.10	7.66	302,409
GPCho(O-38:6)	792.5902	4.533	4.539	4.555	0.09	5.89	1,332,860
GPCho(O-38:5)	794.6058	4.075	4.074	4.088	0.09	3.13	2,951,349
GPCho(O-38:4)	796.6215	4.265	4.259	4.275	0.09	7.74	636,674
GPCho(38:7)	804.5538	4.007	4.011	4.028	0.10	8.76	600,925
GPCho(38:6)	806.5694	3.847	3.850	3.865	0.10	1.72	26,501,408
GPCho(38:5)	808.5851	3.996	3.974	3.990	0.10	3.07	7,476,945
GPCho(38:4)	810.6007	4.125	4.134	4.149	0.09	2.69	6,308,132
GPCho(38:3)	812.6164	4.205	4.206	4.221	0.09	5.03	4,405,377
GPCho(O-40:6)	820.6215	4.195	4.067	4.090	0.14	8.48	210,112
GPCho(40:7)	832.5851	3.897	3.889	3.905	0.10	3.75	1,548,653
GPCho(40:6)	834.6007	4.065	4.064	4.080	0.10	2.87	5,463,972
GPCho(40:5)	836.6164	4.125	4.066	4.140	0.08	4.34	1,374,137

Table 1.
The table above shows the reproducibility of the system to a number of phospholipids with different signal intensities for a pooled QC sample (n=17; 1uL injection volume; interspersed between every 12 samples; total run sequence time was approximately 25 hours ; batch analysis size was 129 files which included 17 QC samples). In this analysis the ion signal reproducibility was less than 10% for all phospholipids measured and the retention time variation was equal to or less than 0.1%. Peak areas calculated using LabSolutions software.

Results

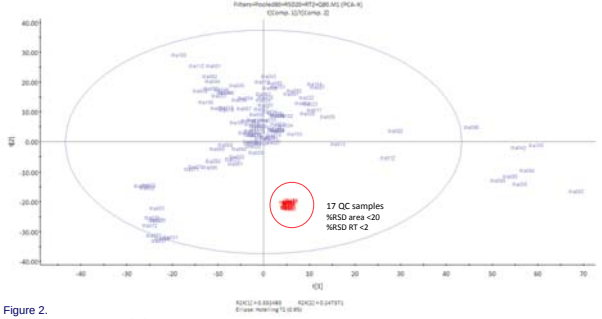

Figure 2.
Principal Component Analysis.
Pooled QC data are closely clustered together and show no 'run order' change in signal response over the analytical run. [The data presented above consider the 1uL injection volume data only].

Measured Ion m/z	Theoretical m/z	Mass accuracy ppm	RT Range	Ion RT	#q	#s	Aligned data array - filtered - sorted according to ion intensity q01	q02	q03	q04	q05	q06	q07
520.3407	520.3398	1.73	002.44 - 002.53	2.507	17	112	25833996	27191868	25683360	26590517	25369279	26324919	26001039
408.3857	408.3834	5.63	003.18 - 003.25	3.231	17	46	24519187	24294177	24475130	24529375	23541062	23716372	24353098
758.5709	758.5694	1.98	003.90 - 003.96	3.933	17	112	22881968	22515941	23098426	22344759	22928781	22380036	22800799
522.3548	522.3554	-1.15	002.56 - 002.65	2.634	17	112	20685254	19967149	19825518	20397499	19382163	19912099	19780154
524.3698	524.3711	-2.48	002.77 - 002.79	2.777	17	112	15444494	16208490	15845183	16223438	15953266	16068710	16300210
806.5697	806.5694	0.37	003.71 - 003.87	3.853	17	112	15447521	15082963	14747676	15284933	15073221	14458811	14870462
786.6005	786.6007	-0.25	004.09 - 004.17	4.151	17	112	12928697	13171423	12790519	13326723	12902929	13523591	12646797
782.5695	782.5694	0.13	003.78 - 003.83	3.914	17	112	12207563	11945942	12216128	11906276	11927999	11796744	12129750
784.5853	784.5851	0.25	003.95 - 004.01	3.982	17	112	9879755	9910688	10410558	10107200	9995221	10042110	9924339
780.5538	780.5528	1.28	003.68 - 003.79	3.767	17	112	9796279	9787057	10139088	9957328	9580785	9960253	9828606

Table 2.
Following the alignment of the raw data files the data array was filtered to include ions above a set tolerance (%RSD area <20%; %RSD RT <2%; minimum number of pooled QC ions was >80%). Plasma samples prepared by protein precipitation with cold acetonitrile typically results in a data array dominated by phospholipid ion signals. The table shows the average mass accuracy measured throughout the batch analysis (the mass accuracy was calculated for each sample [#s] using the m/z value for the most intense ion in the spectrum bin). For example, the average mass accuracy throughout the run of 112 samples and 17 QC controls for m/z 520.3398 was 1.73ppm (n=129).

Conclusions

- This study reports the application of a LCMS-IT-TOF to human plasma metabolite profiling. Using a scan speed of 10 scans per second the peak area variance for a number of phospholipid ion signals was less than 10% with a retention time variance less than or equal to 0.10% for peak widths typically between 5-7 seconds.
- Following the alignment of ion signals and applying filtering parameters (ion signals present in the pooled QC must vary less than 20% in peak area throughout the run and must be present in 80% of the QC samples) 916 ions were submitted for statistical analysis using Simca-P (Umetrics) . The PCA plot shows a cluster of QC signals indicating robust detection of the ion signals detected.