

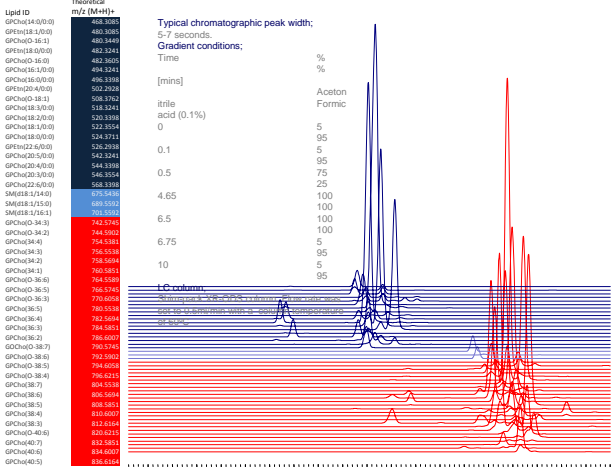
Simon Ashton<sup>1</sup>; Neil Loftus<sup>1</sup>; Chris Titman<sup>1</sup>; Albert Koulman<sup>2</sup>  
<sup>1</sup>Shimadzu, Manchester , United Kingdom ; <sup>2</sup>Lipidomics Biomarker Research Group, Elsie Widdowson laboratory, MRC, Cambridge, UK

## 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-octanoulphosphingone in Ringer or with glucose, palmitate and N-octanoulphosphingone 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; 100msc; m/z 170-900; external mass calibration; ion accumulation time 20msec. In MS mode 10 spectra per second were acquired. In polarity switching mode, the switching time was 100msec.

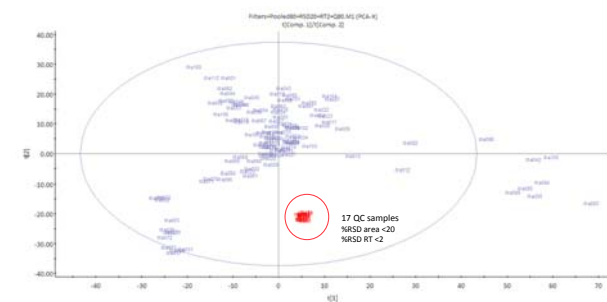
## Results

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  $\pm 15\%$  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) <sup>+</sup>	Avg RT	Min RT	Max RT	%RSD RT	%RSD Area	Ion signal intensity [Average response]
GPCol(14:0/0)	468.3085	2.395	2.392	2.403	0.13	2.26	2,173,959
GPCol(18:1/0:0)	480.3085	2.650	2.654	2.665	0.13	4.38	685,760
GPCol(0:16:1)	480.3449	2.654	2.655	2.666	0.13	5.54	780,777
GPCol(18:0/0:0)	482.3241	2.490	2.482	2.493	0.13	3.08	1,323,240
GPCol(0:16:0)	482.3605	2.490	2.482	2.493	0.13	3.81	649,604
GPCol(16:1/0:0)	494.3241	2.451	2.451	2.462	0.13	2.33	4,722,352
GPCol(16:0/0:0)	496.3398	2.541	2.579	2.590	0.13	0.93	50,235,899
GPCol(20:0/0)	507.2928	2.520	2.518	2.529	0.12	4.01	504,206
GPCol(0:18:1)	508.3762	2.639	2.707	2.718	0.12	4.83	423,688
GPCol(18:3/0:0)	518.3241	2.405	2.407	2.417	0.13	3.02	1,241,123
GPCol(18:2/0:0)	520.3398	2.509	2.507	2.518	0.13	0.76	36,334,718
GPCol(18:1/0:0)	522.3554	2.640	2.634	2.645	0.12	1.48	25,727,526
GPCol(18:0/0:0)	524.3711	2.780	2.778	2.789	0.11	1.91	18,414,007
GPCol(22:0/0)	536.2938	2.654	2.501	2.512	0.13	9.65	272,533
GPCol(20:5/0:0)	542.3241	2.507	2.507	2.519	0.14	8.75	1,208,538
GPCol(20:4/0:0)	544.3398	2.512	2.507	2.518	0.13	1.02	6,262,322
GPCol(20:3/0:0)	546.3554	2.575	2.573	2.585	0.13	2.72	1,846,463
GPCol(22:5/0:0)	568.3398	2.492	2.486	2.497	0.13	2.35	2,762,070
SM(18:1/14:0)	692.5436	5.550	5.578	5.592	0.13	2.23	1,273,303
SM(18:1/15:0)	689.5592	3.870	3.692	3.702	0.10	8.05	132,281
SM(18:1/16:1)	701.5592	3.618	3.624	3.638	0.10	2.71	1,477,189
GPCol(0:34:3)	742.5745	4.030	4.043	4.057	0.09	2.23	1,307,432
GPCol(0:34:2)	744.5902	4.066	4.054	4.069	0.09	4.59	946,724
GPCol(34:1)	754.5745	3.698	3.699	3.714	0.10	4.07	589,607
GPCol(34:3)	758.5902	3.758	3.767	3.781	0.10	2.37	5,074,504
GPCol(34:2)	758.5904	3.937	3.931	3.946	0.09	0.64	43,017,246
GPCol(34:1)	760.5851	4.095	4.096	4.111	0.09	1.05	19,205,930
GPCol(36:6)	764.5589	3.877	3.876	3.891	0.10	3.27	473,322
GPCol(36:5)	765.5745	4.017	4.022	4.037	0.09	2.58	629,327
GPCol(36:3)	770.6003	3.827	4.092	4.106	0.09	5.52	572,722
GPCol(36:5)	780.5538	3.768	3.764	3.779	0.10	2.12	15,194,035
GPCol(36:4)	782.5694	3.907	3.911	3.926	0.10	1.22	17,274,518
GPCol(36:3)	784.5851	3.977	3.980	3.995	0.09	1.31	29,490,182
GPCol(36:2)	786.6007	4.145	4.149	4.164	0.09	1.71	20,403,263
GPCol(0:38:7)	790.5745	3.937	3.950	3.968	0.10	7.66	302,489
GPCol(0:38:6)	792.5902	4.533	4.539	4.555	0.09	5.89	1,332,860
GPCol(0:38:5)	794.6058	4.075	4.074	4.088	0.09	3.13	2,951,349
GPCol(0:38:4)	796.6215	4.265	4.259	4.275	0.09	7.74	636,674
GPCol(38:7)	804.5538	4.007	4.011	4.028	0.10	8.76	629,327
GPCol(38:6)	804.5694	3.847	3.850	3.865	0.10	1.72	26,501,488
GPCol(38:5)	808.5851	3.996	3.974	3.990	0.10	3.07	7,476,945
GPCol(38:4)	810.6007	4.125	4.134	4.149	0.09	2.69	6,308,132
GPCol(38:3)	812.6164	4.205	4.206	4.221	0.09	5.03	4,405,377
GPCol(0:40:6)	820.5745	4.195	4.067	4.087	0.10	8.48	4,090,967
GPCol(40:7)	832.5851	3.897	3.889	3.905	0.10	3.75	1,548,653
GPCol(40:6)	834.6007	4.065	4.064	4.080	0.10	2.87	5,463,972
GPCol(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; 1 $\mu$ L 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 1 $\mu$ L injection volume data only].

Measured	Theoretical	Mass accuracy	RT Range	Ion RT	#	q01	q04	q05	q06	q07			
100	m/z	ppm											
520	520.3398	1.73	002.44-002.53	2.507	17	112	28433396	27191868	25643330	28505517	263242919	26001039	
408	3857-408.3834	5.63	001.18-003.25	2.507	17	112	24519187	24294177	24475130	24529175	23541062	23716137	24353038
758	5709-758.5694	1.98	003.90-003.96	3.933	17	112	22881968	22515941	23008426	22344578	22928781	22380036	22600799
522	524.3711	-1.15	002.56-002.65	2.634	17	112	20680254	19967149	19829518	20307489	19321215	199912096	197801254
534	536.592	-2.48	002.77-002.79	2.777	17	112	15444494	16208490	15845183	16223438	15951256	16068710	163002410
835	836.6164	0.37	003.71-003.87	3.853	17	112	35447823	35082863	34747676	35038993	35073231	34498811	34470462
786	6005-786.6007	-2.25	004.09-004.17	4.151	17	112	12928697	13171423	12790519	13326273	12802929	13232591	12646797
782	5695-782.5694	0.13	003.78-003.83	3.914	17	112	12207656	11945942	12216128	11906276	11927999	11796744	12129750
784	5853-784.5851	0.25	003.95-004.01	3.982	17	112	9879755	9910688	10410538	10107200	9995321	10042110	9924339
780	5538-780.5538	1.28	003.68-003.79	3.767	17	112	9796279	9787057	10190088	9957328	9580785	9660253	9622806

**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 [fs] 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 (Metrics). The PCA plot shows a cluster of QC signals indicating robust detection of the ion signals detected.

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