Understanding Complex Lipid Metabolism through Quantitative Lipidomics

Baljit K Ubhi[1], Alex Conner[2], Eva Duchoslav[3], Annie Evans[2], Richard Robinson[2], Paul RS Baker[1] and Steve Watkins[2]. [1]SCIEX, USA, [2]Metabolon, USA and [3]SCIEX, Canada

ABSTRACT

Lipids play a role in a variety of metabolic disorders, inflammatory processes and signaling pathways that they are deemed important to human health. High throughput screening assays which can be set-up with minimal effort is the goal of the non-lipid expert. A major challenge in lipid analysis is the many isobaric interferences present in highly complex samples that confound identification and accurate quantitation. Herein we present a novel lipidomics platform that enables facile, quantitative lipid analysis and allows targeted profiling of over 1100 lipid molecular species from 13 different lipid classes across complex lipid metabolism.

INTRODUCTION

A major challenge in lipid analysis is the many isobaric interferences present in highly complex samples that confound identification and accurate quantitation. This problem, coupled with complicated sample preparation techniques and data analysis, highlights the need for a complete solution that addresses these difficulties and provides a simplified method for analysis. A novel lipidomics platform was developed that includes simplified sample preparation, automated methods, and streamlined data processing techniques that enable facile, quantitative lipid analysis. Herein, serum samples were analyzed quantitatively using a unique internal standard labeling protocol, a novel selectivity tool (differential mobility spectrometry; DMS, Figure 3) and novel lipid data analysis software.

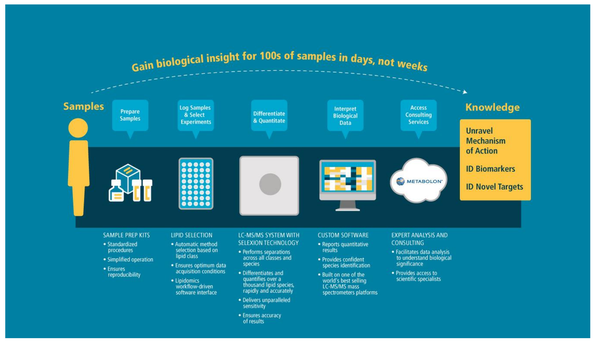

Figure 1. One Streamlined Workflow for Peak Performance. Lipidyzer™ Platform makes lipid analysis easy and seamless. The simple integrated workflow allows you access to comprehensive data quickly and confidently, while expert analysis services provide you with the assurance to gain accurate biological insight.

MATERIALS AND METHODS

Applying the kit for simplified sample extraction and preparation, a serum matrix was used following the protocols provided. A QTRAP® System with SelexION® DMS Technology (SCIEX) was used for targeted profiling of over a thousand lipid species from 13 different lipid classes (Table 1) allowing for comprehensive coverage. Two methods covering ten lipid classes using a flow injection analysis (FIA); one injection with SelexION® Technology ON and another with the SelexION® Technology turned OFF. The lipid molecular species were measured using MRM and positive/negative switching.

Negative ion mode detected the following lipid classes – FFA/LPE/LPC/PC/PE. Positive ion mode detected the following lipid classes – SM/DAG/CE/CER/TAG. Samples were quantitated using the Lipidomics Workflow Manager (LWM) software accompanying the full solution, which incorporates the novel labeled internal standards available as a kit (over 50 internal standards across 13 classes), developed for this platform (Avanti Lipids). The kits include not only the labeled internal standards but unlabeled internal standards for the COV tuning of the SelexION® device as well as lyophilized plasma to use as a QC sample throughout the analysis. System suitability test mixtures for system performance measurements are supplied to run either as a more regular test (daily) or a more comprehensive (monthly).

Fraction	Lipid Classes	Number of Species*
Neutral Lipids	Triacylglycerols (TAG)	502
	Diacylglycerols (DAG)	67
	Free Fatty Acids (FFA)	28
	Cholesterol Esters (CE)	34
Polar Lipids	Phosphatidylcholines (PC)	161
	Phosphatidylethanolamines (PE)	233
	Lysophosphatidylcholines (LPC)	28
	Lysophosphatidylethanolamines (LPE)	28
	Sphingomyelins (SM)	16
	Ceramides (CER)	56

Table 1. Full Coverage of Complex Lipid Metabolism. The Lipidyzer™ Platform fully elucidates the class and fatty acid composition of each lipid molecular species. Thirteen classes covering over 1000 species means the Lipidyzer™ Platform offers comprehensive coverage of complex lipid metabolism. *total no of MRMs measured = 1153 (which includes the internal standards). The Ceramides listed above includes the further three classes, DCER, HCER and LCER.

RESULTS

This system allows for (1) Quantitative results for each lipid class (listed in Table 1) as a sum of individual species (nmol/g). (2) Mole percent composition determined computationally from lipid molecular species data (%) and (3) Accurate lipid molecular species concentrations as compared with historical data generated by alternative methods. The Lipidyzer™ platform enables ease of use, specificity and quantitative rigor. The ease of use is offered by the Lipidomics Workflow Manager which guides you through your workflow. Specificity is offered by SelexION® Technology (Figure 3). Finally quantitative rigor is insured by a stable spray through the use of PEEKsil tubing and a micro flow electrode (65µm) which minimizes carryover (with the addition of a post-injection wash) and finally the internal standards which neutralize quantitative bias.

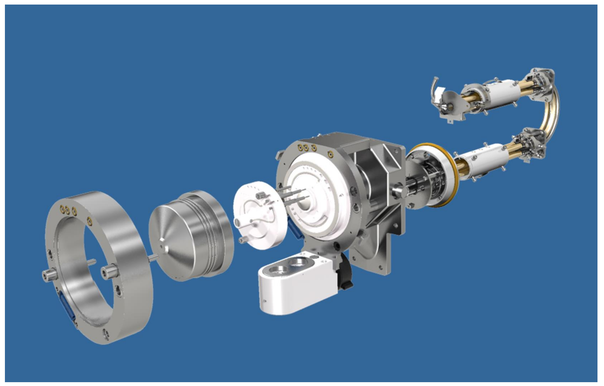

Figure 2. Differential Mobility Spectrometry (DMS). A hardware schematic for the 5500 QTRAP® system with the SelexION® device, attached to the instrument interface. To the right of the interface is the high vacuum region containing the QJet® ion guide, Q0, and the analytical quadrupoles, and to the left of the interface, at atmospheric pressure, is the DMS unit. Ions are generated by the instrument source, and are directed towards the DMS cell where they are differentially selected based on their dipole moment. The SelexION® unit is located at the atmospheric pressure side of the interface and can be easily removed without breaking vacuum. Additionally, the SelexION® device can use chemical modifiers to enhance separation of structurally similar molecules, a function not possible in high vacuum. A consequence to having the instrument configured with the DMS unit at the interface is that the isobaric overlap resulting from the relatively wide isolation window of Q1 during MS/MS analysis can be minimized by selecting molecules prior to entering the mass spectrometer for analysis.

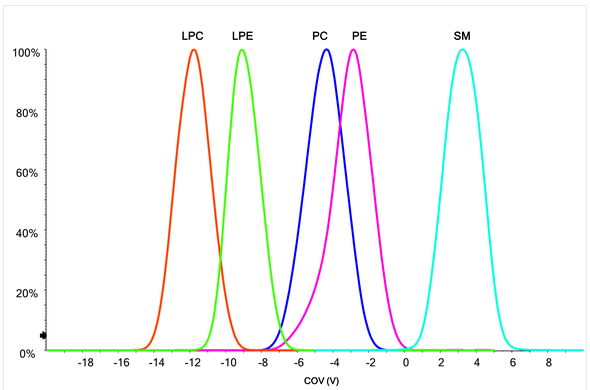

Figure 3. Differential Mobility Spectrometry (DMS). A compensation voltage (COV) trace. COV ramped from -25V to 10V separates lipid classes. This figure highlights the specificity of the SelexION® device to separate lipid classes from complex mixtures. The COV per class is added to the MRM tables in the method and at any one voltage across the DMS, only that specific lipid class is selected and allowed and passed through to Q1 for subsequent MRM analysis. In this figure the COV tuning mixture is infused at 7ul/min and the software automatically tunes the COV value per class by collecting LPC/LPE/PC/PE MRMs in the negative ion mode and SM MRMs in the positive ion mode. The COVs are automatically updated into the method files required to run the Lipidyzer™ platform.

CONCLUSIONS

The Lipidyzer™ Platform offers the following benefits:

Benefit 1 – Comprehensive coverage across ten lipid classes employing over 50 internal standards, a complete and novel approach to lipid quantitation.

Benefit 2 – Specificity harnessed by the power of SelexION® DMS Technology allows for the specificity and eliminates isobaric interferences from overlapping lipid species within the same m/z range.

Benefit 3 – Quantitation that is accurate and precise data for class/species concentration and composition determination allowing highly reproducible data (Figures 4, 5 and 7).

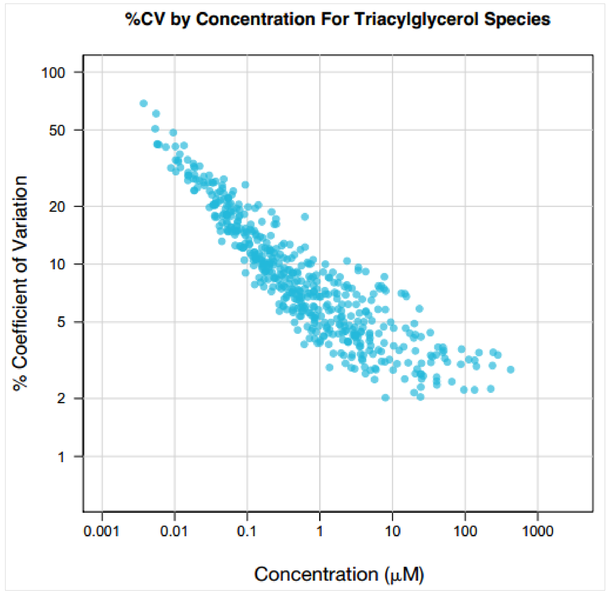

Figure 4. Reproducibility Data of TAG Species Measurements. Blue dots represent the individual TAG species measurements plotted by average concentration in human serum (x-axis) and the precision of the measurement (y-axis). The majority of TAG species measurements had CVs less than 20% (88.3% of measurements), and the accuracy of the platform related strongly to the original concentration of the TAG profile in the sample. In fact, 99.6% of the molar mass of TAG was comprised by TAG chains that were measured with better than 10% CVs.

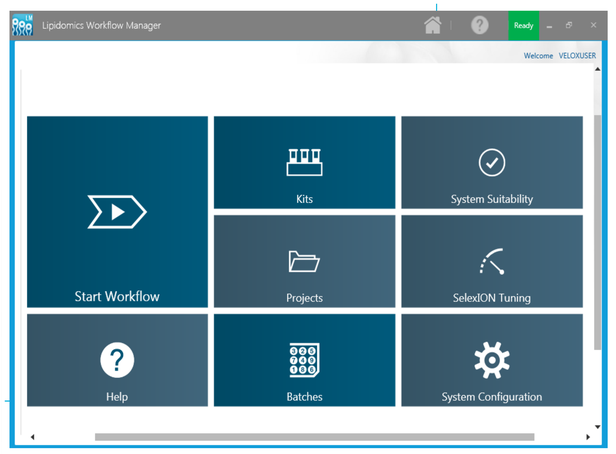

Figure 6. Lipidyzer™ Workflow Manager. The software controlling the Lipidyzer™ Platform is the Lipidomics Workflow Manager (LWM) This software system provides LIMS capabilities for sample-tracking and workflow management, complete control of the overall system as well as the workflow. This includes automated data-processing for signal detection and result calculations, and finally reporting and visualization functionalities. All of this is accessible in the cloud environment which allows the user to secure and sharable data.

REFERENCES

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2. Baker PR et. al. Three-dimensional enhanced lipidomics analysis combining UPLC, differential ion mobility spectrometry, and mass spectrometric separation strategies. Journal Lipid

TRADEMARKS/LICENSING

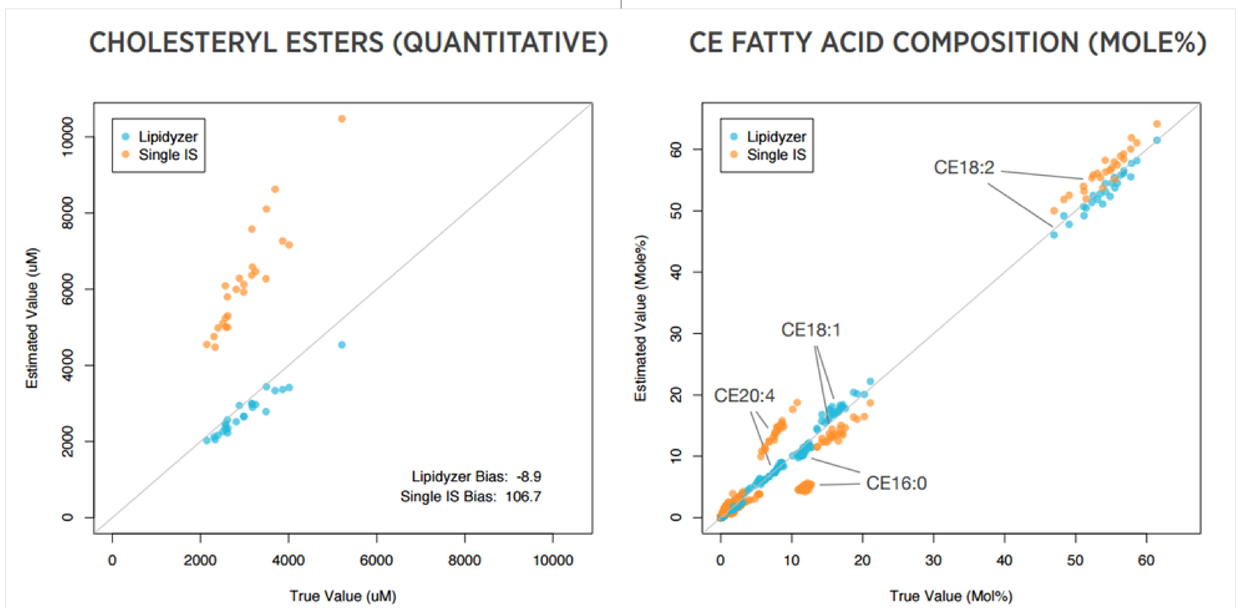

Figure 5. Concentration and compositional accuracy for Cholesteryl Esters (CE). The Lipidyzer™ internal standards were compared to the use of a single internal standard (dCE(16:0)) for their ability to accurately calibrate the concentration of total cholesteryl esters (left) and the fatty acid composition of cholesteryl esters expressed as a mole% fatty acid composition (right) in human serum. Twenty-five human serum samples with known total CE and CE fatty acid compositions were profiled using the Lipidyzer™ Platform. The Lipidyzer™ Platform quantified total CE with less than 10% bias, compared to a 100% bias in the estimate made using a single internal standard (A). The results indicated that using a single internal standard greatly overestimated the concentration of CE, likely by overestimating the contribution of the major unsaturated fatty acids. Plot B shows the individual fatty acid profiles of CE (expressed as a mole % of total CE) when quantified using the Lipidyzer™ Platform and the single internal standard. The composition is clearly warped by the bias caused by using one internal standard, whereas using a mixture of internal standards provided an accurate fatty acid composition of CE. Estimated value = measurement from the LipidyzerTM Platform. The True Value is the measured value of this data previously using Metabolon's gold standard GC-FID platform. Here 8 different internal standards for CE and we are assigning multiple target analytes to a single IS using the following hierarchy of rules: (1) degree of unsaturation > (2) chain length.