# A Novel Lipid Screening Platform that Provides a Complete Solution for Lipidomics Research

### *The Lipidyzer™ Platform, powered by Metabolon®*

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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 (Figure 1). Herein, serum samples were analyzed quantitatively using a unique internal standard labeling protocol, a novel selectivity tool (differential mobility spectrometry - DMS) and novel lipid data analysis software.

## 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 (Figure 2) allowing for comprehensive coverage. Two methods were used covering thirteen 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. Positive ion mode detected the following lipid classes – SM / DAG / CE / CER / TAG. Negative ion mode detected the following lipid classes – LPE / LPC / PC / PE / FFA.

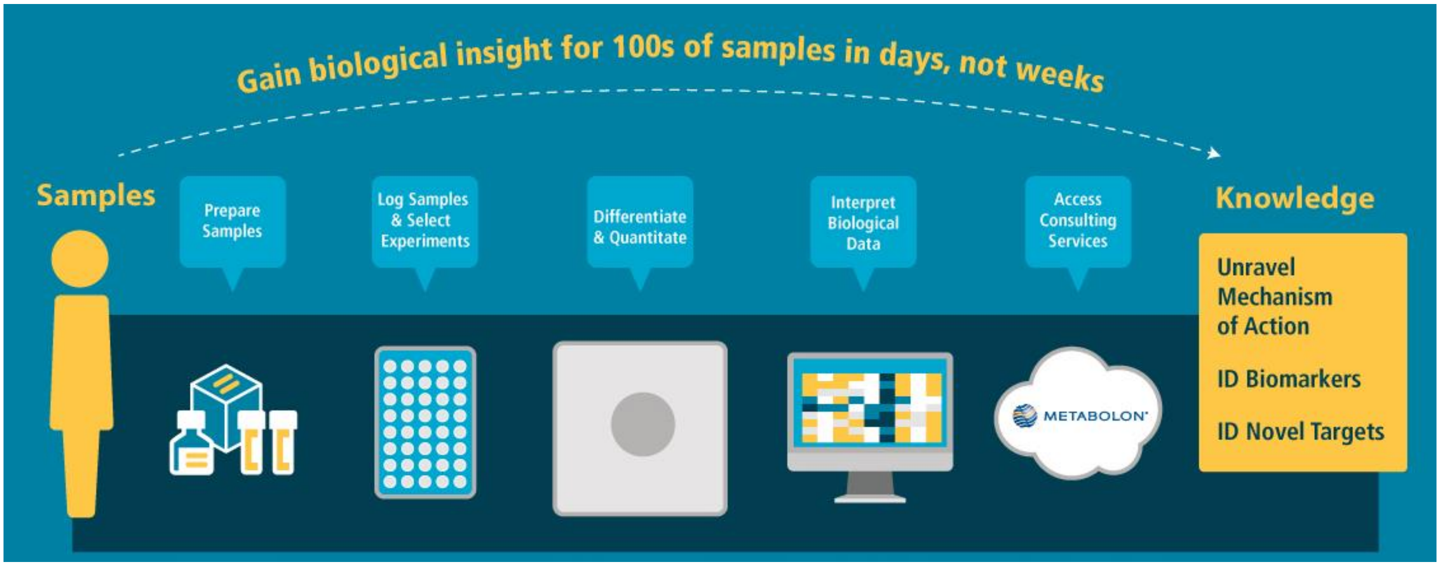

**Figure 1. One Streamlined Workflow for Peak Performance.** The Lipidyzer™ Platform makes lipid analysis easy and seamless. The simple integrated workflow allows access to comprehensive data quickly and confidently, while expert analysis services provides assurances to gain accurate biological insight.

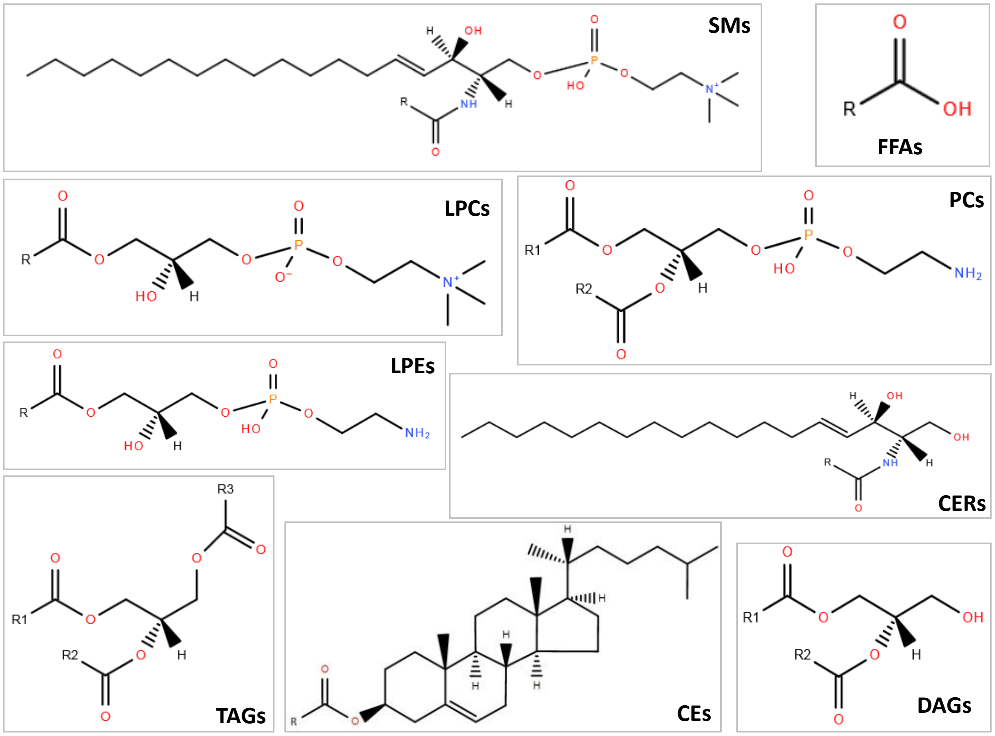

**Figure 2. Comprehensive Coverage of Complex Lipid Metabolism.** The Lipidyzer™ Platform allows comprehensive coverage of complex lipid metabolism by allowing the measurement across thirteen lipid classes and over 1100 lipid molecular species. DCER, HCER and LCER not shown.

Samples were quantitated using the Lipidomics Workflow Manager (LWM) software accompanying the hardware, 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 compensation voltage (COV) tuning of the SelexION® device as well as control lyophilized plasma used as a QC sample. A system suitability test for system performance measurements is supplied to run either as a more regular test (if system is used routinely) or a more comprehensive test, run once every month (or for infrequent use).

## 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 the user through the workflow. Specificity is provided by SelexION® Technology (Figure 3). Finally quantitative rigor is ensured by a stable spray through the use of PEEKsil tubing and a microflow hybrid electrode (65µm), an additional post-injection wash for low carryover, and finally the internal standards which neutralize quantitative bias[3].

**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 1100 species means comprehensive coverage of complex lipid metabolism. The Ceramides include a further three classes, DCER, HCER and LCER.*# of MRMs measured = 1153 (including internal standards).

| Fraction | Lipid Classes | # 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 (PE)* | *28* |
| | *Sphingomyelins* | *16* |
| | *Ceramides* | *56* |

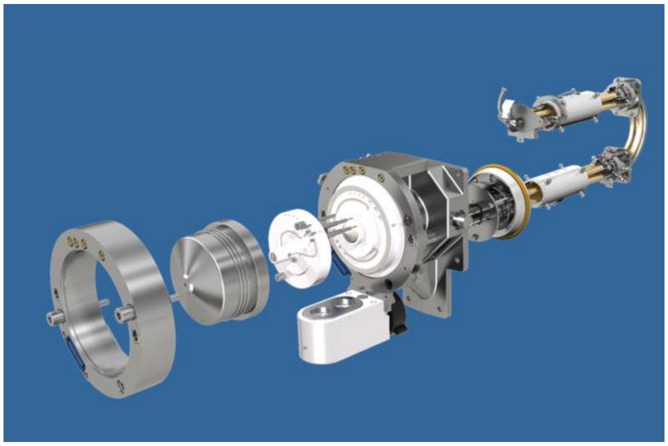

**Figure 3. Differential Mobility Spectrometry (DMS).** A hardware schematic for the QTRAP® 5500 system with the SelexION® device, attached to the instrument interface. To the right of the interface is the high vacuum region containing the QJet® ion guide, Q0, and the analytical quadrupoles, and to the left of the interface, at atmospheric pressure, is the DMS unit. Ions are generated by the source, and are directed towards the DMS cell where they are differentially selected based on their dipole moment. The SelexION device 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 or with non-planar DMS systems. Using DMS separations, 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.

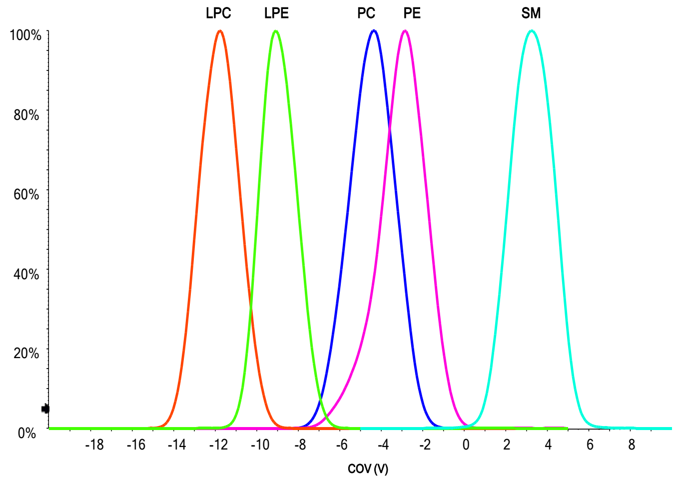

**Figure 4. Differential Mobility Spectrometry (DMS).** Ramping the COV from -25V to 10V generates a COV trace and highlights the separation of lipid classes and the addition specificity that can be achieved. In this workflow, the COV tuning mixture is infused at 7µL/min and the software automatically 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 MRM method files required to run the Lipidyzer™ platform. During operation, the COV is set only that specific lipid class is selected and allowed and pass through to Q1 for subsequent MRM analysis.

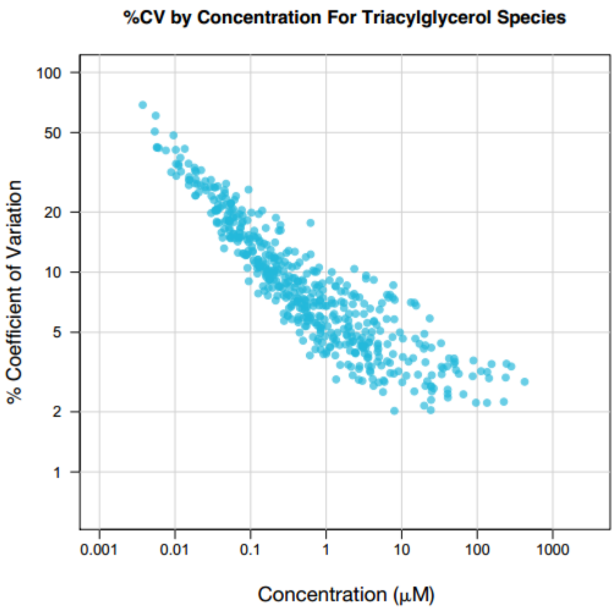

**Figure 5. Reproducibility Data of TAG Species Measurements.** Blue dots represent the individual TAG species measurements plotted by average concentration in human serum (x-axis) and the precision of the measurement (y-axis). The majority of TAG species measurements had CVs less than 20% (88.3% of 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. Lipidomics 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 SelexION® tuning and system suitability tests than can be run as a daily or monthly check to monitor the performance of the platform. Automated data-processing for signal detection and result calculations, and reporting and visualization functionalities are all part of the software.

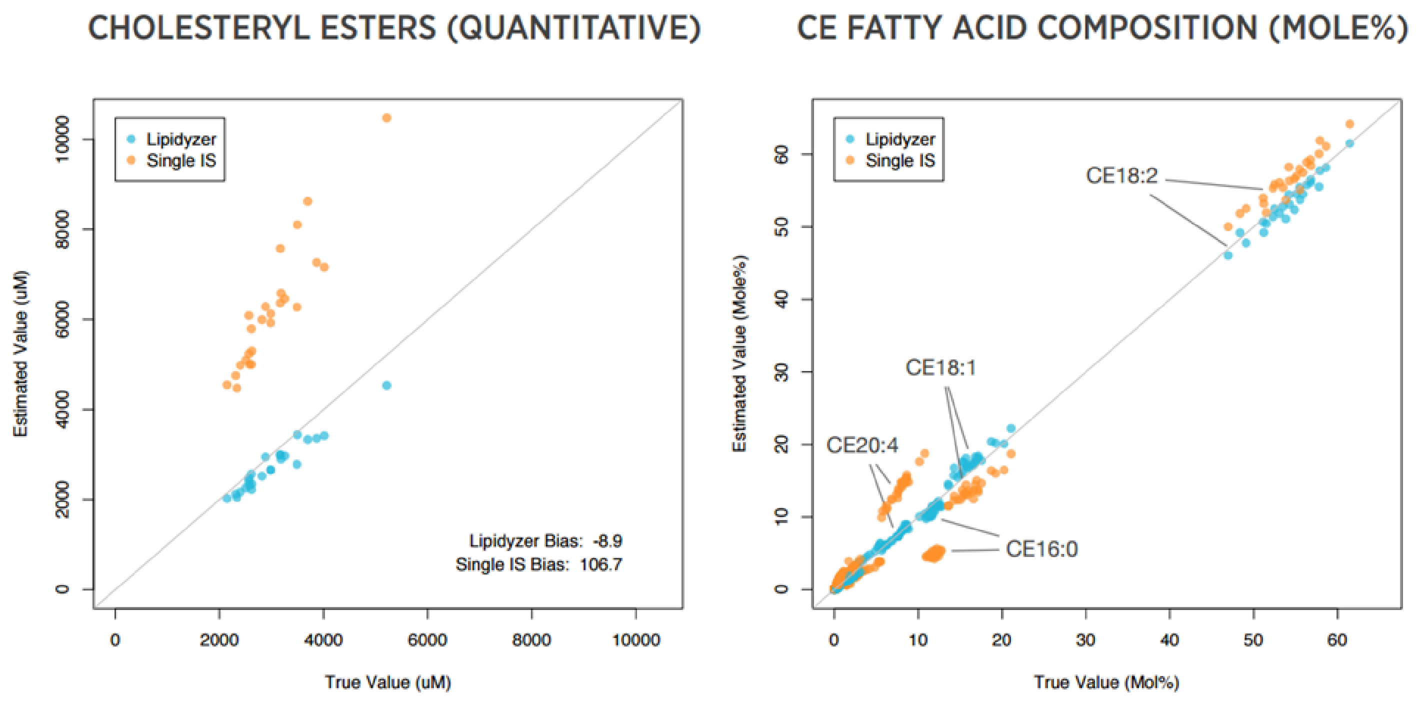

**Figure 7. Concentration and Compositional Accuracy for Cholesteryl Esters (CE).** The Lipidyzer internal standards were compared to the use of a single internal standard (dCE(16:0)) for their ability to accurately calibrate the concentration of total cholesteryl esters (left) and the fatty acid composition 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 total CE was quantified 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 a 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 Lipidyzer 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 are used and multiple target analytes are assigned to a single IS using the following hierarchy of rules: (1) degree of unsaturation > (2) chain length.

## Conclusions

The Lipidyzer™ Platform offers the following benefits:

**Benefit 1** – Comprehensive coverage across thirteen 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).

## References

1. Lintonen *et al.* (2014). Differential mobility spectrometry-driven shotgun lipidomics. *Analytical Chemistry* **86(19)**: 9662-9.
2. Baker PR *et. al*. Three-dimensional enhanced lipidomics analysis combining UPLC, differential ion mobility spectrometry, and mass spectrometric separation strategies. *Journal Lipid Research* (2014) **55(11)**: 2432-42.
3. Novel Chemical Standards Kits Enable Facile Lipid Quantitation, SCIEX Technical Note RUO-MKT-02-3879-A.

Document number: RUO-MKT-02-2871-A

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