Novel Chemical Standards Kits Enable Facile Lipid Quantitation

The Lipidyzer™ Platform Provides more Accurate Lipid Analysis with less Quantitative Bias

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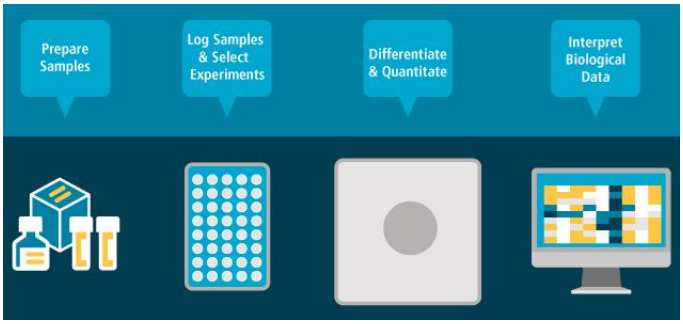
A novel lipidomics platform was developed that includes simplified sample preparation, automated methods, and streamlined data processing techniques, for facile, quantitative lipid analysis[1]. Unique internal standards, tuning kits and system suitability kits were designed to overcome a gap in the lipidomics field. Traditionally a single internal standard per class had been used as a strategy in quantitating lipid molecular species, however the diversity of fatty acid chain lengths and degrees of unsaturation for molecular species that provide differential fragmentation efficiency were unaccounted for, which fundamentally impacts quantitation. The Lipidyzer™ platform internal standards, which contain over 50 labeled molecular species across thirteen classes, neutralize this quantitative bias and allow for more accurate measurement.

In addition to the labelled internal standards, the platform is equipped with additional kits which allow a user to assess the reproducibility and sensitivity of their system before running samples. The System Suitability kit enables the user to assess the sensitivity of the assay and the reproducibility (robustness) of the platform. The SelexION® Technology Tuning kit allows the automated optimization of the differential mobility spectrometry (DMS) cell which aids in definitive lipid identification[2]. Finally, the QC Spike Standards kit, which contains unlabeled molecular lipid species, can be added to the QC control plasma at a known concentration and monitored throughout the analysis as a QC sample.



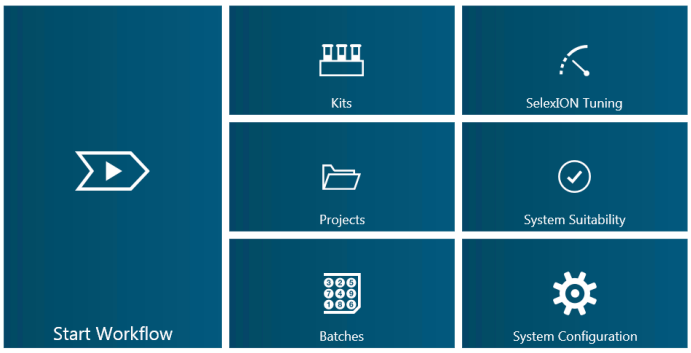


Figure 1. Lipidomics Workflow Manager. The software controlling the Lipidyzer™ Platform is the Lipidomics Workflow Manager (LWM) This software provides LIMS capabilities for sample-tracking and workflow management, complete control of the LC/MS system as well as the entire workflow. This includes automated SelexION® tuning and system suitability tests that can be run as daily or monthly checks 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.

Available Kits for Lipidyzer™ Platform

The following kits are available to use with the Lipidyzer platform:

1. Getting Started Kit, this includes the following components:
   - Internal Standards Kit – containing labelled internal standards for 13 lipid classes
   - Control Plasma
   - SelexION Tuning Kit
   - System Suitability Kit
   - QC Spike Standards Kit
2. The Internal Standards Kit - a 13 lipid class kit which contains all labeled molecular species for all classes
3. Individual internal standard kits per class
4. SelexION Tuning Kit
5. System Suitability Kit
6. QC Spike Standards Kit

Overview of the Workflow Steps

The Lipidyzer™ Platform involves a four-step process before samples can be acquired:

1. Kit registration
2. Tune the DMS cell automatically for optimized class-specific compensation voltages (COV) allowing for maximum specificity
3. Run a system suitability test to assess performance or the platform and the assay
4. Start a workflow and submit samples.

All of these actions can be performed from the Lipidomics Workflow Manager (LWM) Software.

Workflow Details

Step 1: Kits Registration

The LWM software allows the user to register the internal standard kits from a certificate of analysis which extracts the concentration for every labeled species (per class) to be used for reporting accurate quantitative data (Figure 2). Actual concentrations are unique and vary by manufacturing lot (±10% to the specified concentration). After kit registration, the software calculates all volumes that a user needs to pipette (based on number of samples being analyzed) to prepare their samples before extraction.

Step 2: SelexION® Technology Tuning

Tuning of the DMS cell is key to ensure maximum specificity. Any time the cell is cleaned, taken off or not used for a period of one month, the compensation voltages (COVs) optimal for the lipids classes measured must be re-tuned. The Lipidomics Workflow Manager Software automates all tuning and tests for "plug and play" workflows. The SelexION Tuning Kit contains ampoules with unlabeled lipid molecular species. An ampoule is opened and diluted to the appropriate concentration as instructed in the embedded protocol and infused to tune the voltages. The COV ramped from -25V to 10V separates the lipid classes (Figure 2). The optimized COV per class is automatically updated in the MRM tables in the method.

Kit Contents:

Lot Name	Lot Number	Type
Ceramides (CER)	CERISTLPV-100	IS
Cholesterol Ester (CE)	CHEISTLPV-100	IS
Diacylglycerol (DAG)	DAGISTLPV-100	IS
Dihydroceramides (DCER)	DCERISLPV-100	IS
Free Fatty Acids (FFA)	FFAISTLPV-100	IS
Hexosylceramides (HCER)	HCERISLPV-100	IS
Lactosylceramide (LCER)	LCERISLPV-100	IS
Lysophosphatidylcholine (LPC)	LPCISTLPV-100	IS
Lysophosphatidylethanolamine (LPE)	LPEISTLPV-100	IS
Phosphatidylcholine (PC)	PCISTLPV-100	IS
Phosphatidylethanolamine (PE)	PEISTLPV-100	IS
Sphingomyelin (SM)	SMISTLPV-100	IS
Triacylglycerol (TAG)	TAGISTLPV-100	IS

Phosphatidylcholine (PC) Details:

Chemical Name	Concentration
dPC(16:0/16:1)	0.0575
dPC(16:0/18:1)	0.2525
dPC(16:0/18:2)	0.255
dPC(16:0/18:3)	0.065
dPC(16:0/20:3)	0.0725
dPC(16:0/20:4)	0.2775
dPC(16:0/20:5)	0.07
dPC(16:0/22:4)	0.075
dPC(16:0/22:5)	0.0775
dPC(16:0/22:6)	0.145

Figure 2. Kit Registration. The Lipidomics Workflow Manager (LWM) allows automatic registration of each kit of internal standards by using the electronic certificate of analysis (CoA). Once registered the actual concentrations are logged and then used in the volume calculation in the Internal Standard Assembler tool during the Workflow stage. This eliminates user error from calculating concentrations manually. Once a batch of data is acquired, LWM automatically corrects the concentrations to the appropriate internal standard.

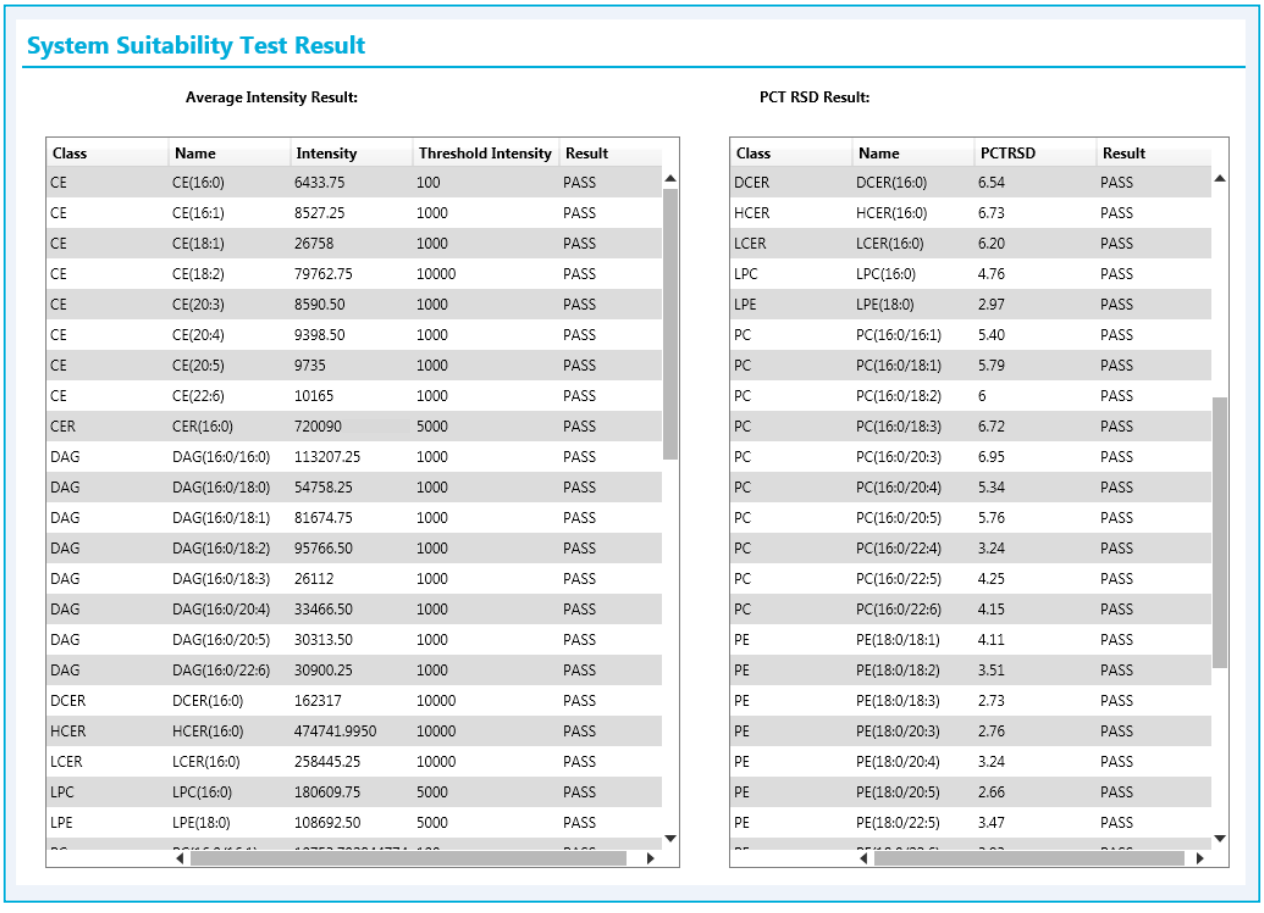
Step 3: System Suitability

The LWM software allows the user to perform the entire quantitative lipid analysis using an easy, guided workflow. The next step is to perform the system suitability test to confirm the platform performance is reproducible and robust, that the assay is performing to specification, and the limit of detection is being met. The System Suitability kits have been designed to allow the user to measure all of these attributes. The kit contains ampoules with unlabeled molecular species. The user can run one of two tests; a quick test or a comprehensive test.

The Quick System Suitability test is to be run weekly for a lab operating the Lipidyzer Platform daily and takes only 20 minutes to complete. An ampoule is opened and diluted to the appropriate concentration as instructed in the embedded protocol for “Quick Test”. One injection of a blank sample and an injection of the “LOD” sample are made (with the DMS ON method only) and the software reports a simple PASS or FAIL based on meeting a threshold cutoff in counts per second (cps) for 18/20 scans collected.

The Comprehensive System Suitability test is more extensive and runs for 3.5 hours. This is directed at users running the Lipidyzer™ Platform in between other LC-MS/MS assays or after a period of inactivity (i.e. more than 10 days). An ampoule is opened and diluted to the appropriate concentration as instructed in the embedded protocol for “Comprehensive Test”. Two injections of a blank sample, two injections of the “LOD” sample and three injections of the “RSD” sample are made with both methods (DMS On and Off).

The software reports a simple PASS or FAIL based on meeting a threshold cutoff in counts per second (cps) for 18/20 scans collected for the LOD sample and a coefficient of variation (%CV) value for the RSD sample (Figure 3, both tables reported). A sample must have a CV of 15% or less to pass the comprehensive test.



System Suitability Test Result

Average Intensity Result:

Class	Name	Intensity	Threshold Intensity	Result
CE	CE(16:0)	6433.75	100	PASS
CE	CE(16:1)	8527.25	1000	PASS
CE	CE(18:1)	26758	1000	PASS
CE	CE(18:2)	79762.75	10000	PASS
CE	CE(20:3)	8590.50	1000	PASS
CE	CE(20:4)	9398.50	1000	PASS
CE	CE(20:5)	9735	1000	PASS
CE	CE(22:6)	10165	1000	PASS
CER	CER(16:0)	720090	5000	PASS
DAG	DAG(16:0/16:0)	113207.25	1000	PASS
DAG	DAG(16:0/18:0)	54758.25	1000	PASS
DAG	DAG(16:0/18:1)	81674.75	1000	PASS
DAG	DAG(16:0/18:2)	95766.50	1000	PASS
DAG	DAG(16:0/18:3)	26112	1000	PASS
DAG	DAG(16:0/20:4)	33466.50	1000	PASS
DAG	DAG(16:0/20:5)	30313.50	1000	PASS
DAG	DAG(16:0/22:6)	30900.25	1000	PASS
DCER	DCER(16:0)	162317	10000	PASS
HCER	HCER(16:0)	474741.9950	10000	PASS
LCER	LCER(16:0)	258445.25	10000	PASS
LPC	LPC(16:0)	180609.75	5000	PASS
LPE	LPE(18:0)	108692.50	5000	PASS

PCT RSD Result:

Class	Name	PCTRSD	Result
DCER	DCER(16:0)	6.54	PASS
HCER	HCER(16:0)	6.73	PASS
LCER	LCER(16:0)	6.20	PASS
LPC	LPC(16:0)	4.76	PASS
LPE	LPE(18:0)	2.97	PASS
PC	PC(16:0/16:1)	5.40	PASS
PC	PC(16:0/18:1)	5.79	PASS
PC	PC(16:0/18:2)	6	PASS
PC	PC(16:0/18:3)	6.72	PASS
PC	PC(16:0/20:3)	6.95	PASS
PC	PC(16:0/20:4)	5.34	PASS
PC	PC(16:0/20:5)	5.76	PASS
PC	PC(16:0/22:4)	3.24	PASS
PC	PC(16:0/22:5)	4.25	PASS
PC	PC(16:0/22:6)	4.15	PASS
PE	PE(18:0/18:1)	4.11	PASS
PE	PE(18:0/18:2)	3.51	PASS
PE	PE(18:0/18:3)	2.73	PASS
PE	PE(18:0/20:3)	2.76	PASS
PE	PE(18:0/20:4)	3.24	PASS
PE	PE(18:0/20:5)	2.66	PASS
PE	PE(18:0/22:5)	3.47	PASS

Figure 3. Comprehensive System Suitability Test Results for the Lipidyzer™ Platform Ensures High Quality Results. The integrated comprehensive system suitability tests allow a user to assess the performance of the assay (left table) as well as the performance of the platform (right table). PCTRSD = processed control relative standard deviation or better known as %CV. A sample must have a CV of 15% or less to pass the test.

Step 4: Start Workflow

The Lipidyzer™ Platform has been tuned for specificity and the user has determined the system and assay are meeting the performance requirements, so samples can now be submitted for analysis. The LWM software allows the user to log the samples per study as a Project. Once samples are logged a user then must make up the internal standard mixture to add to all the samples that need analyzing. The software automatically calculates this using the Internal Standard Assembler. By taking the concentrations from the kit registration the software is able to then calculate automatically the volumes required of each class selected for analysis.

Conclusions

The Lipidyzer™ Platform offers the following benefits:

- **Comprehensive coverage** across thirteen lipid classes employing over 50 internal standards, a complete and novel approach to lipid quantitation.
- **Specificity** of the SelexION® Technology to eliminate isobaric interferences from overlapping lipid species within the same m/z range.
- **Quantitation** that has less bias (from 110% to 8.9%) through the application of the novel multiple labeled internal standards per class strategy[1]. This provides much more accurate and precise data than ever before.

Figure 4. The Lipidyzer™ Platform Chemical Standard Kits. Top: The Internal Standards Kit for Lipidyzer Platform, SelexION Tuning Kit, QC Spike Kit and the System Suitability Kit. Bottom: The Internal Standards Kit for Lipidyzer Platform containing 13 ampoules of labeled material for each lipid class.

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References

1. Connor et al (2015). A Novel Lipid Screening Platform that Provides a Complete Solution for Lipidomics Research. SCIEX Technical Application Note, RUO-MKT-02-2871A.
2. Lintonen et al. (2014). Differential mobility spectrometry-driven shotgun lipidomics. *Analytical Chemistry* **86(19)**: 9662-9.

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