Software: Nexus Point of a Targeted Lipid Analyzer

Pauline J. Vollmerhaus[1]; Baljit K. Ubhi[2]; Sarada Tanikella[3]; Peter Zhu[3]; Corey D. DeHaven[3];
[1]SCIEX, Concord ON, Canada; [2]SCIEX, Redwood City, CA; [3]Metabolon, Durham, NC

INTRODUCTION

Software is the primary interaction for a user with a mass spectrometer for both data acquisition and results generation. The challenge of any sophisticated technology is the reduction of analytical complexity so as to maintain focus on the biological problem. The intricacy of quantitative lipidomics with isobaric interference and complicated sample preparation adds another layer for consideration. There is a need for a software-driven solution that frees the user to focus on the biological problem at hand rather than the generation and processing of data. Lipidomics workflow software was developed to manage sample receiving, sample preparation protocols, mass spectrometry methods, and data processing of hundreds of MRM's for facile, quantitative lipid analysis. The software and its application are described herein.

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MATERIALS AND METHODS

Lipidomics Workflow Manager (LWM) Software is used to facilitate the targeted quantitation of 13 lipid classes covering over 1100 species . The software utilizes inputs from certificates of analysis from commercially available internal standard kits (SCIEX, USA) to generate accurate preparation protocols for quantitative analysis. The software with built-in acquisition methods was used to control the Lipidyzer™ Platform for targeted profiling.

Samples: Replicate control plasma samples were extracted to demonstrate workflow.

Chromatography: Flow injection (FIA) on a Shimadzu Nexera X2 system was used to introduce the extracted lipid samples to the mass spectrometer.

Mass Spectrometry: The MS analysis was performed on a Lipidyzer™ Platform, a QTRAP® 5500 system equipped with SelexION® Technology (Differential Mobility Separation – DMS). Multiple Reaction Monitoring (MRM) was used to target and quantify ~1000 lipid molecular species of the plasma lipidome. Acquisition was performed in positive and negative polarity and with and without DMS on, taking around 20 minutes per sample for acquisition.

Data Processing: All data was acquired and processed automatically using the Lipidomics Workflow Manager software. The data is automatically processed to yield 1. Quantitative results for each lipid class as a sum of individual lipid species (nmol/g); 2. Mole percent composition (%); and 3. Accurate lipid species concentrations (nmol/g). As well, heat maps, QC charts and box and whisker plots are generated.

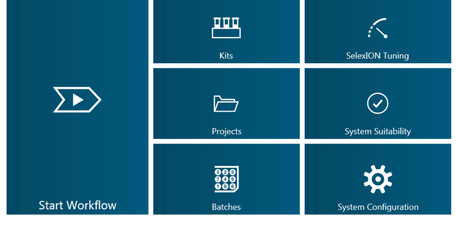
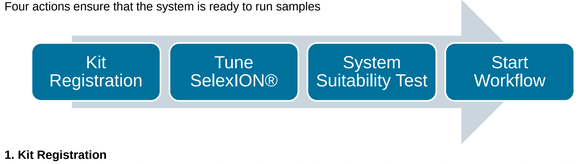


Figure 1. Lipidomics Workflow Manager. The software controlling the Lipidyzer™ Platform is the Lipidomics Workflow Manager (LWM)

RESULTS

Figure 2. Overview of the Workflow Steps
Four actions ensure that the system is ready to run samples



1. Kit Registration
To minimize error, the software enables the user to register the internal standard kits from an imported certificate of analysis (Figure 3). Actual concentrations are unique and vary by manufacturing lot (+10% to the specified concentration).
The actual concentrations are stored and then used in the volume calculation in the Internal Standard Assembler tool during the Workflow stage. This eliminates user error from calculating chemistries manually. As well, when data is acquired, the software extracts the concentration for every labeled species (per class) to be used for reporting accurate quantitative data and automatically corrects the concentrations to the appropriate internal standard.

Figure 3. Kit Registration. The Lipidomics Workflow Manager (LWM) allows automatic registration of each kit of internal standards by using the electronic certificate of analysis (CoA).

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

Lipidyzer Internal Standard Mixture Preparation
Example IS Mixture

Number of samples:	40
Number of batches/vials:	1

Lipid Class Mixes	Mix Lot #	Vol Lot of Mix
CER	ERISTLPV-100	520
CE	HEISTLPV-100	522
DAG	AGISTLPV-1(	502
DCER	CERISLPV-10(	520
FFA	FAISTLPV-10(	520
HCER	CERISLPV-1(	520
LCER	CERISLPV-10	520
LPC	PCISTLPV-10	520
LPE	PEISTLPV-10	520
PC	?CISTLPV-10(	482
PE	?EISTLPV-10(	526
SM	MISTLPV-10(	520
TAG	AGISTLPV-10	514
Final Volume (ml) Super Pool:		5.29

Species Concentration (nmol/g)

Name	CE(12:0)	CE(14:0)	CE(14:1)	CE(15:0)	CE(16:0)
QC_SPIKE-00182 .		24.034	1.7657	7.0675	325.7019
QC_SPIKE-00183	0.7057	23.5695	1.3589	7.2908	317.026
QC_SPIKE-00184	0.8839	26.4145	1.4236	7.1828	325.9986
QC-05839	0.8828	25.4126	1.5483	7.1907	305.8133

2. SelexION® Technology tuning for Optimal Methods
Tuning of the DMS cell ensures maximum specificity and definitive identification. Any time the cell is cleaned, taken off or not used, 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 compensation voltage (COV) is ramped from -25V to 10V to separate the lipid classes. The optimized COV per class is automatically updated in the MRM tables in the method.

3. System Suitability Test
The system suitability test confirms the platform performance is reproducible and robust, that the assay is performing to specification, and the sensitivity is appropriate. 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. One injection of a blank sample and an injection of the "LOD" sample are made.

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). 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 4, both tables reported).

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)	8596.50	1000	PASS
CE	CE(20:4)	9388.50	1000	PASS
CE	CE(20:5)	9735	1000	PASS
CE	CE(22:6)	10185	1000	PASS
CER	CER(d18)	720090.9650	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)	81474.75	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)	33486.50	1000	PASS
DAG	DAG(16:0/20:5)	30313.50	1000	PASS
DAG	DAG(16:0/22:6)	30900.25	1000	PASS
DCER	DCER(d18)	142317	10000	PASS
HCER	HCER(d18)	474741.9950	10000	PASS
LCER	LCER(d18)	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(d18:0)	6.54	PASS
HCER	HCER(d18:0)	6.73	PASS
LCER	LCER(d18:0)	6.29	PASS
LPC	LPC(16:0)	4.76	PASS
LPE	LPE(18:0)	2.87	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)	5.79	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)	4.13	PASS
PE	PE(18:0/18:1)	4.11	PASS
PE	PE(18:0/18:2)	2.92	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 4. Comprehensive System Suitability Test 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). PCT RSD = processed control relative standard deviation or better known as %CV. A sample must have a CV of 15% or less to pass the test

4. Start Workflow
The software workflow (Figure 5) minimizes the use of mass spectrometry terminology. The primary interaction of the user with the software is that of data entry related to the lipidomics study. Once samples are logged , standard sample preparation protocols are generated. The internal standard volume calculations are based on the registered kit concentrations from the chosen classes selected for analysis. 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. Sample extraction protocols and randomized tray-layouts (Figure 6) reduce the complexity of the experiment. Acquisition methods are built in. Results are automatically generated post-acquisition (Figures 7 & 8)

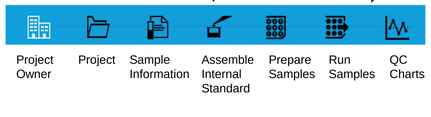
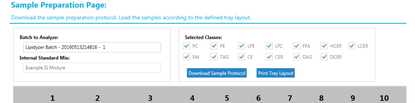


Figure 5. Workflow. The primary interaction of the user is to enter the study sample data.

Figure 6. Sample preparation page. Randomized tray layout and embedded step-by-step protocols simplify the experiment.



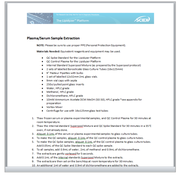
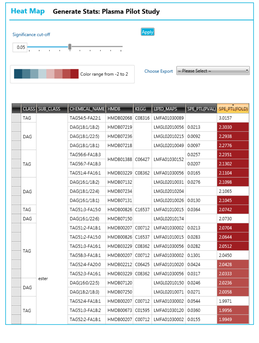


Figure 7. Raw Data View. The box and whisker plots provide an overview of the cycle-to-cycle variability in the measurement of the intensity of the specific lipid species selected per injection in a batch. Each vertical orange bar is the measured ion intensity per scan in the injection. No traditional mass spectra is shown to the user.

Figure 8. Automatically Generated Statistics based on Metadata. An unpaired t-test between two groups of samples acquired by the Lipidyzer Platform. The heat map highlights an significance set by a p value cutoff and the mean fold change between the two groups..



CONCLUSIONS

- Lipidomics workflow software was developed to manage sample receiving, sample preparation protocols, mass spectrometry methods, and data processing of hundreds of MRM's for facile, quantitative lipid analysis.

TRADEMARKS/LICENSING

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