

# Lipidomics Workflow Guide

Agilent 6560 Ion Mobility LC/Q-TOF



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#### **Manual Part Number**

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## Content

#### Introduction 5

#### Before You Begin 5

What are lipids? 6
What is lipidomics? 7
Why ion mobility mass spectrometry for lipids? 7
What will you learn from this guide? 7

#### Primary Lipidomics Workflow 9

Workflow Steps 9 References 10 Uses of the lipidomics workflow 10

#### Required Items 11

Computer Equipment 11 Software 11 Example Data Files 11

#### Calibrating Data 13

#### Correct Mass Calibration 15

Step 1. Start IM-MS Reprocessor 15
Step 2. Load selected file 16
Step 3. Set up recalibration 17
Step 4. Run recalibration 19
Step 5. Recalibrate remaining files 20
To restore original file 21

#### Apply Single-Field CCS Calibration 22

Step 1. Load the All Ions tune-mix file 22
Step 2. Sum the signals 24
Step 3. Calculate the Beta and TFix values 25
Step 4. Save the values to the tune-mix and sample files
Step 5. Repeat steps for MS1 tune-mix and sample files

#### Processing Data 29

#### Create Lipid PCDL 31

Step 1. Create new project**31**Step 2.Edit and run method**34**Step 3.Export to PCDL**39** 

26

28

Content

To export to CSV for Skyline 41

#### Find Features 44

Step 1. Add samples 44Step 2. Edit method 48Step 3. Extract and review features 51

#### Identify Compounds 52

Step 1. Set up method 52
Step 2. Specify PCDL 56
Step 3. Identify compounds 57
Step 4. Create CEF files for export 60

#### Analyzing Lipids 61

#### Create Lipidomics Experiment 63

Step 1. Create new project **63** Step 2. Create new experiment **64** 

#### Import and Group CEF Files 67

Step 1. Select data to import 67Step 2. Group samples 68Step 3. Filter data 70

#### Review Lipid Information 72

Step 1. View lipid ion mobility data 72
Step 2. Create interpretations 73
Step 3. Create a lipid matrix 76
Step 4. Plot CCS vs Mass 82

#### **Reference Information** 85

Definitions Resource Apps Agilent Web Site Agilent Community

## 1 Before You Begin

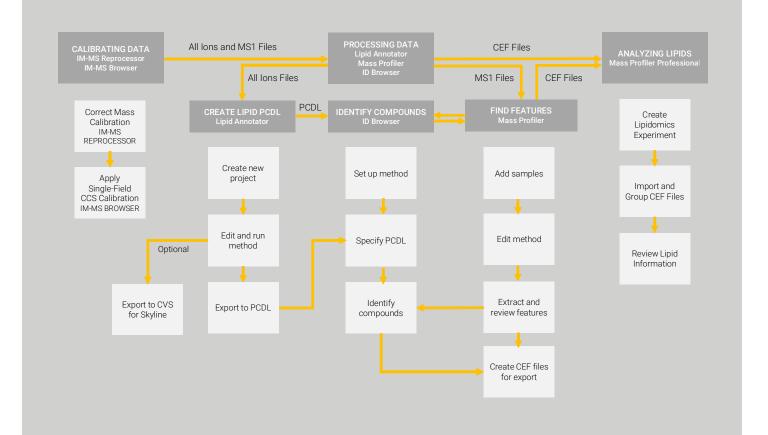
Make sure you read and understand the information in this chapter and have the necessary computer equipment, software and example data files before you start your analysis.

## Introduction

#### Introduction

What are lipids?6What is lipidomics?7Why ion mobility mass spectrometry for lipids?7What will you learn from this guide?7

5	Primary Lipidomics Workflow	9
6	Workflow Steps	9
7	References	10
7	Uses of the lipidomics workflow	10
7	·	



Required Items	11
Computer Equipment	11
Software	11
Example Data Files	11

To understand the specifics of the Primary Lipidomics Workflow, first familiarize yourself with lipids, lipidomics and the advantage of ion mobility mass spectrometry for lipidomics studies.

# What are lipids? Lipids are a very broad, diversified group of hydrophobic or amphipathic (both hydrophobic and hydrophilic) small molecules including fats, oils, hormones and certain components of membranes grouped together because they do not interact appreciably with water. One type of lipid, the triglycerides, is sequestered as fat in adipose cells, which serve as the energy-storage depot for organisms and also provide thermal insulation. Some lipids such as steroid hormones serve as chemical messengers between cells, tissues and organs. Others communicate signals between biochemical systems within a single cell.

The amphipathic nature of some lipids lets them form structures such as vesicles, micelles, liposomes, or membranes in an aqueous environment. Triglycerides, phospholipids and sphingolipids are amphipathic and consist of a polar "head" and a non-polar acyl, or fatty acid, tail or tails attached via an ester or amide bond to the head group. See an example below:

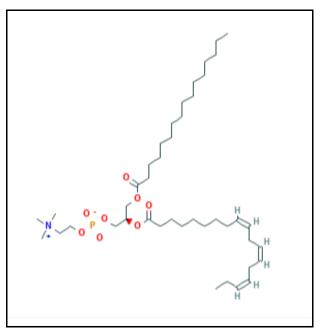


Figure 1. Example of an amphipathic lipid - phosphatidylcholine 34:3

#### https://pubchem.ncbi.nlm.nih.gov/compound/24778702#section=2D-Structure

Sterol lipids and prenol lipids, on the other hand, are hydrophobic and are derived from isoprene units. Cholesterol is an example of a sterol lipid.

	See <b>"Lipids"</b> on page 89 to understand how to name the lipids with fatty acid chains. See also <b>www.lipidmaps.org</b> .
What is lipidomics?	Lipidomics is one of the four components that make up the field of "metabolomics" research. Metabolites are the end products of all biological/cellular processes, and "metabolome" refers to the collective set of all metabolites generated in a biological system (cell, tissue, organ or organism). Metabolomics is the scientific study to characterize and identify the metabolome. The metabolome is comprised of four major classes of biological molecules: sugars, amino acids, nucleotides, and lipids.
	The systematic study of the entire lipid profile of a cell/tissue/organ/organism is referred to as "lipidomics". Mass spectrometry is one of the most widely used technologies in lipidomics research for the identification as well as quantitation of thousands of lipid molecules that constitute the cellular "lipidome", the full lipid complement of a biological system.
Why ion mobility mass spectrometry for lipids?	Resolving isomeric species in lipidomics workflows is a huge challenge. For example, the possible permutations of fatty acid tail length and double bond locations that could describe a TG 49:1 sum composition are quite large, and several isomers could be present within a sample. These permutations have the same mass and potentially the same RT, making it difficult to differentiate them with traditional LC-MS methods. Performing LC-MS with ion mobility provides an additional dimension of separation to these experiments.
	Following LC separation and before MS detection, ions are separated based on their size and shape during an ion mobility experiment, resulting in different drift times. These drift times are used to calculate a Collision Cross Section (CCS) value or a rotationally averaged surface area for the ion. Adding CCS values to accurate mass and RT values, which are both available from traditional LC-MS experiments, provides better specification and more confidence when identifying lipids.
	Lipidomics workflows also require MS/MS fragmentation to annotate lipids confidently. Detecting specific fragment ions can tell you that 15:0, 16:1, and 18:0 are the three fatty acid tails for the TG 49:1 lipid. All lons fragmentation is a data- independent acquisition mode where all precursor ions are fragmented. When All lons fragmentation is used without ion mobility separation, the resulting data can be hard to interpret because of the difficulty of linking precursor and fragment ions. When the technique is used with ion mobility, the fragments from the All lons experiment are drift-aligned with their precursor ion, which allows for easier data interpretation.
	See <b>"Lipid sum composition"</b> on page 89.
What will you learn from this guide?	Agilent has several software packages commonly used for compound discovery and identification. The newly developed Lipid Annotator generates a lipid database specific to your data set, which you can use in these existing software packages.

Additional tools have been added to Mass Profiler Professional (MPP) that allow you to evaluate the distribution of lipid classes, types, and abundances in different samples. See **Chapter 4**, "Analyzing Lipids".

Using a sequence of these software tools, you can execute the Primary Lipidomics Workflow, described on the next pages. Agilent calls this the primary workflow because, although other lipidomics workflows do exist, their instructions do not appear in this guide.

## Primary Lipidomics Workflow

	This guide takes you through the Primary Lipidomics Workflow, which uses both All lons and MS1 data files. All lons data is collected for a pooled QC of all your samples, and this data file is used to create a custom lipid database with Lipid Annotator. Mass Profiler finds features in each individual MS1 sample data file. These features are then identified in ID Browser using the lipids database created with Lipid Annotator based on the pooled QC sample. Or you can export the lipid database to a CSV file for import into Skyline for a targeted data analysis workflow.
Workflow Steps	After acquiring the data, follow these Major Steps to eventually assess the lipid composition and abundance differences between sample sets. Each chapter gives you additional instructions to complete a primary task or tasks in the workflow.
Step 1. Calibrate Data	Step 1. Calibrate data
(Chapter 2)	First, correct the mass calibration with IM-MS Reprocessor. Next, apply a single- field CCS calibration in IM-MS Browser.
Steps 2-4. Process Data	Step 2. Create lipid PCDL
(Chapter 3)	Use Lipid Annotator to annotate lipids in the All Ions data files when fragment ions match the theoretical library, generating a database that contains accurate mass, retention time and CCS values. You can either export the database to a PCDL file for use with ID Browser or to a CSV file for import into Skyline.
	Step 3. Find features
	Using Mass Profiler on MS1 data files, perform feature finding, which results in a feature list that includes a mass, retention time and CCS value for each feature.
	Step 4. Identify compounds
	Using the PCDL from Lipid Annotator, identify the MS1 features as lipids with ID Browser based on accurate mass, RT and CCS values. Then return the feature list to Mass Profiler, where you create CEF files for export.
Step 5. Analyze Lipids	Step 5. Analyze lipids
(Chapter 4)	With Mass Profiler Professional (MPP), lipid assessment tools are provided to compare the compound and abundance distribution between samples (exported CEF files imported to MPP).
	You can also use MPP to filter out features, leaving only the most relevant features. Normalizing the data in MPP corrects data for changes in RT or response so a single feature common to several samples is not treated. MPP also provides you with statistical data analysis tools for discovering significant differences between sample sets, although instructions are not provided in this guide. See <b>"Resource Apps"</b> on page 94.

#### 1 Before You Begin References

References	Chapter 5 gives you distinctions for the important terms in chapters 1-4 and a listing of guides, interactive tutorials and video tutorials to supplement your knowledge.
Uses of the lipidomics workflow	<ul> <li>The lipidomics workflow may be used to do the following analyses:</li> <li>Compare two or more biological groups</li> <li>Find and identify potential biomarkers</li> <li>Look for biomarkers of toxicology</li> <li>Integrate the lipidomics data with other multi-omics data sets to better understand the systems biology</li> <li>Discover new lipids</li> </ul>
	<ul> <li>Develop data mining and data processing procedures that produce characteristic markers for a set of samples</li> </ul>
	<ul> <li>Construct statistical models for sample classification</li> </ul>

Agilent enables lipidomics research for a variety of applications:

- Basic research Identify and validate lipid biomarkers that correlate with disease states, as well as provide fundamental insights into biology
- Pharmaceuticals Identify lipids and markers of toxicity for drug discovery and development
- Agriculture Identify and understand metabolic pathways to optimize crop development, improve yields, and avoid pesticide/herbicide resistance
- Environmental studies Identify lipids that relate to the effects of chemicals and other stressors in the environment on a biological system
- Biofuels Identify lipid profiles to optimize fermentation processes and biofuel production
- Food/Nutrition Identify the presence or absence of lipids that correlate with major food traits, such as quality, authenticity, taste, and nutritional value, and aid in the development of nutraceuticals

## **Required Items**

	You need this computer equipment, software and the specified data files to implement the Primary Lipidomics Workflow:
Computer Equipment	4 Physical Cores, 32-64 GB memory and 3.5 GHz processor speed
Software	Use these software programs and utilities for each Major Step of the Primary Lipidomics Workflow.
Step 1. Calibrate data	IM-MS Reprocessor 10.0 and IM-MS Browser 10.0
Step 2. Create lipid PCDL	Lipid Annotator 1.0 and PCDL Manager 8.0
Step 3. Find features	Mass Profiler 10.0
Step 4. Identify compounds	ID Browser 10.0

#### **Example Data Files**

Step 5. Analyze lipids

The data sets that you can use to work through the instructions in this guide are positive ion mobility LC/Q-TOF lipid data (both MS1 and All Ions data). You can find the files at **Agilent SubscribeNet** in the IM Lipidomics Data folder. See the part numbers and file names/descriptions in **Table 1**.

Table 1. IM Lipidomics Data Files on Agilent SubscribeNet

Mass Profiler Professional 15.1 (MPP)

Agilent Part Number	File Name in SubscribeNet	Description	Data File Folder Name
G3335-10004	All lons.zip	Fragmentation Sample Data	All lons
G3335-10005	Stnd0_1017.zip	MS1 Sample Data with no standards	Nist0_1017
G3335-10006	Stnd2_1017.zip	MS1 Sample Data with standards	Nist2_1017
G3335-10007	Stnd4_1017.zip	MS1 Sample Data with twice the amount of standards as in previous folder	Nist4_1017
G3335-10008	Tune Mix.zip	One All lons file with tuning data One MS1 file with tuning data	Tune Mix

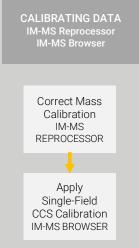
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## 2 Calibrating Data

Calibrating data is the first Major Step in the Ion Mobility Primary Lipidomics Workflow. This chapter takes you through the steps to calibrate the raw data for creating a PCDL and finding features. This chapter focuses on correcting mass calibration and applying a single-field CCS calibration to the corrected data.

Before you analytically process the data -- create a custom PCDL, extract features and identify compounds -- you must first calibrate the raw data. To do this, you correct mass calibration and then apply single-field CCS calibration.

15
15
16
17
19
20
21



#### Apply Single-Field CCS Calibration

Step 1. Load the All lons tune-mix file

Step 2. Sum the signals

Step 3. Calculate the Beta and TFix values

Step 4. Save the values to the tune-mix and sample files 26

Step 5. Repeat steps for MS1 tune-mix and sample files28

For standard-mode data (All Ions and MS1 non-multiplexed data) you can use the PNNL PreProcessor (https://omics.pnl.gov/software/pnnl-preprocessor) to smooth and repair saturated data, resulting in improved performance of the data analysis carried out in this guide. For simplicity, this guide processes the raw data files with mass and CCS calibration and does no preprocessing with the PNNL Preprocessor.

22

22

24 25

Additionally, if you have acquired multiplexed data, the lipid workflow in this guide cannot be completed until you use the PNNL Preprocessor for demultiplexing the data before CCS calibration.

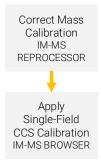
Calibrating standard-mode data involves two steps:

• Step 1. Correct mass calibration using IM-MS Reprocessor

The Reference Mass Calibration cannot be applied to IM-MS data during data acquisition as it can be for regular QTOF experiments. To recalibrate IM-MS data you use the IM-MS Reprocessor. Reference mass ions must be present and elute throughout the entire data file for this utility to apply a mass recalibration.

• Step 2. Apply single-field CCS calibration

After loading a tune-mix data file into the IM-MS Browser, you determine the Beta and TFix values and save them to the tune-mix file and to the sample data files acquired under the same settings as the tune-mix data file. During custom PCDL creation in Lipid Annotator and feature finding in Mass Profiler the CCS values are calculated using these Beta and TFix values.



## **Correct Mass Calibration**

Correcting mass calibration involves five major steps and an optional one:

- Step 1. Start IM-MS Reprocessor
- Step 2. Load selected file
- Step 3. mSet up recalibration
- Step 4. Run recalibration
- Step 5. Recalibrate remaining files
- Optional Step. Restore original file

NOTE You may not need to correct mass calibration if your data file already recalibrated with good results (fewer than half the scans are missing). To find out, do **step 4** page 21. To learn how to correct mass calibration using these instructions, assume you need to recalibrate.

# Step 1. Start IM-MSIM-MS Reprocessor is installed during the installation of IM-MS Browser. To access<br/>the reprocessor for the first time, follow these instructions:ReprocessorIM-MS Reprocessor for the first time, follow these instructions:

- 1 In the Windows Search field, type reprocess.
- 2 Right-click the IM-MS Reprocessor "Best Match" app.
- 3 Select Open.

OR

- 1 In the folder **\Program Files\Agilent\MassHunter\Workstation\IMS**, open the **Bin** folder.
- 2 Right-click the imsreprocess application and select Create shortcut.
- **3** Make sure you place the shortcut icon on the Desktop.
- 4 Double-click the IM-MS Reprocessor icon,

You can now double-click the IM-MS Reprocessor icon whenever you want to open the Reprocessor. The IM-MS Data File Reprocessing Utility main window appears with the Operations tab open.

IM-MS Data File Reproces	ssing Utility			-		×
File Help						
Operations Recentroid R	Recalibrate					
Data file(s) to process						
Only this file:						
<ul> <li>All file(s) in the sam</li> </ul>	e directory <u>See Lis</u>	t				
Reprocessing Operations	Special Operations					
Any of these oper						
	Calibration Results	Last File	All Files			
	Scans processed:					
	Missed some ions:					
	Missed all ions:					
	Start	Stop				

Figure 2. Operations tab for the Reprocessing Utility

Step 2. Load selected file

You can recalibrate the file sets in a major folder in any order -- tune mix, All lons, MS1. Follow the instructions below to load the files from the data folder.

- 1 Click the ... button (Browse button) next to the Only this file text field.
- 2 From your file folder, select any file in the folder you intend to reprocess.

This guide uses the All lons tune-mix file, but you can select any file in any folder. You now see the file name as the Data file(s) to process, and you see another selection appear, letting you reprocess all the files in the folder.

IM-MS Data File Reprocess	ing Utility			_		×		
File Help								
Operations Recentroid Re	ecalibrate							
Data file(s) to process								
	Only this file: C:\Agilent\MPP\lon Mobility\Data\Tune Mix\101719_TM_allions_0006.d      All 2 IM-MS files in the same folder See List							
Reprocessing Operations	Special Operations							
Any of these operations can be undone with the "Restore original file" special operation.  Recentroid Recalibrate Recalibrate Recol IRM calibration Calibration Recults								
		Last File	All Files					
	Scans processed:							
	Missed some ions:							
	Missed all ions:							
	Start	Stop						
						.::		

Figure 3. Operations tab with only one file in the folder loaded

In setting up the Recalibrate operation, make sure you have selected the correct masses for the tune mix or the samples.

#### Set up the operation

Step 3. Set up

recalibration

- 1 To recalibrate more than one file at a time, select **All 2 IM-MS files in the same folder**, or the selection that specifies how many files are in the folder.
- 2 Mark the **Recalibrate** check box.
- 3 Clear the Recentroid check box.

## Select the recalibration values

#### 1 Click the **Recalibrate** tab.

The Recalibrate dialog box appears. See Figure 4.

	×						
Negative m/z							
112.985587							
119.036320							
120.038418							
301.998139							
680.035536							
1033.988109							
1333.968947							
1633.949786							
Default Lists							
	<ul> <li>✓ 112.985587</li> <li>✓ 119.036320</li> <li>☐ 120.038418</li> <li>☐ 301.998139</li> <li>☐ 680.035536</li> <li>✓ 1033.988109</li> <li>✓ 1333.968947</li> <li>✓ 1633.949786</li> </ul>						

Figure 4. Recalibrate tab with positive ions selected for the sample files (default)

- 2 Keep the Minimum abundance and m/z tolerance values the same.
- 3 Mark the **Positive m/z** check boxes for the values you want as Reference Masses for either the tune-mix files or the sample files. (See Note on **page 15**.)

Step 4. Run recalibration

ΝΟΤΕ

Make sure to edit the positive mass values when switching between a tune-mix file (118, 322, 622, 922,1221 and 1521) and a sample data file, which likely has only 121 and 922 present. Replace the 121 value with 322.04873 for the tune-mix files. Don't worry about marking the Negative m/z check boxes at this time. Use **Figure 4** on page 18 for the sample files and **Figure 5** for the tune-mix files.

IM-MS Data File Reprocessing Utility		Х				
File Help						
Operations Recentroid Recalibrate						
Rules     250     counts       Minimum abundance     250     counts       m/z tolerance:     +/-     100     ppm						
Reference Masses						
Positive m/z	Negative m/z					
118.086255	112.985587					
322.04873	119.036320					
622.028960	120.038418					
922.009798	301.998139					
1221.990636	680.035536					
1521.971480	1033.988109					
1821.952310	1333.968947					
2121.933150	1633.949786					
Default Lists						
		.::				

Figure 5. Recalibrate tab with positive ions selected for the tune-mix files

Step 4. Run recalibration

Your new recalibration results will overwrite the previous ones.

#### Start the recalibration

- 1 Select the **Operations** tab.
- 2 Click Start.

Step 5. Recalibrate remaining files

When you see the Done message at the bottom of the screen, the masses in the data files have been recalibrated.

IM-MS Data File Reprocessing Utility					
File Help					
Operations Recentroid Recalibrate					
Data file(s) to process					
	101		0000.1		1
Only this file: bility\Data\Tune Mix\e	me_reprocessed files	(101719_1M_allio	ns_0006.d		
All 2 IM-MS files in the same folder	<u>See List</u>				
Reprocessing Operations Special Operations	s				
Any of these operations can be und	lone with the "Rest	ore original file'	special op	eration.	
_		-	'special op	eration.	
_	roid spectra (Total Fra	-	special op	eration.	
Recentroid - Recompute centr	roid spectra (Total Fra ation	-	'special op	eration.	
<ul> <li>Recentroid</li> <li>Recompute centroid</li> <li>Recalibrate</li> <li>Redo IRM calibrate</li> </ul>	roid spectra (Total Fra ation	-	' special op	peration.	
☐ Recentroid - Recompute centr	roid spectra (Total Fra ation Its Last File	me Spectra only)	'special op	eration.	
<ul> <li>Recentroid</li> <li>Recompute centr</li> <li>Recalibrate</li> <li>Redo IRM calibration</li> <li>Calibration Result</li> </ul>	roid spectra (Total Fra ation Its Last File sed: 27	me Spectra only) All Files	'special op	eration.	
<ul> <li>Recentroid</li> <li>Recompute centroid</li> <li>Recalibrate</li> <li>Redo IRM calibration Result</li> <li>Calibration Result</li> <li>Scans process</li> </ul>	roid spectra (Total Fra ation Its Last File sed: 27 ons: 0	me Spectra only) All Files 50	'special op	veration.	
<ul> <li>Recentroid</li> <li>Recompute centroid</li> <li>Recalibrate</li> <li>Redo IRM calibration Result</li> <li>Calibration Result</li> <li>Scans process</li> <li>Missed some in</li> </ul>	roid spectra (Total Fra ation Its Last File sed: 27 ons: 0	me Spectra only) All Files 50 0	special op	veration.	
<ul> <li>Recentroid</li> <li>Recompute centroid</li> <li>Recalibrate</li> <li>Redo IRM calibration Result</li> <li>Calibration Result</li> <li>Scans process</li> <li>Missed some in</li> </ul>	roid spectra (Total Fra ation Last File sed: 27 ons: 0 s: 0	me Spectra only) All Files 50 0 0	special op	peration.	
<ul> <li>Recentroid</li> <li>Recompute centroid</li> <li>Recolibrate</li> <li>Redo IRM calibration Result</li> <li>Calibration Result</li> <li>Scans process</li> <li>Missed some in</li> <li>Missed all ions</li> </ul>	roid spectra (Total Fra ation Last File sed: 27 ons: 0 s: 0	me Spectra only) All Files 50 0	special op	peration.	
<ul> <li>Recentroid</li> <li>Recompute centroid</li> <li>Recolibrate</li> <li>Redo IRM calibration Result</li> <li>Calibration Result</li> <li>Scans process</li> <li>Missed some in</li> <li>Missed all ions</li> </ul>	roid spectra (Total Fra ation Last File sed: 27 ons: 0 s: 0	me Spectra only) All Files 50 0 0	special op	peration.	

Figure 6. Completed recalibration for both tune-mix files

3 If you applied the mass calibration to sample files, check them for missed ions.

For both tune-mix files and sample files, the zeros indicate all the marked ions are present in all the scans. Missing ions are not likely to appear for tune-mix files. For a sample file, if more than half the scans are missing ions, you likely have a bad recalibration.

- 4 (optional) For a bad recalibration, do these steps:
  - **a** Restore the original data file, which you can do with only one data file at a time, and then recalibrate. See **"To restore original file"** on page 21.
  - **b** If more than half the scans are still missing ions, check the data file in IM-MS Browser to verify that the reference mass ions meet the Rules values specified on the Recalibrate tab. See **Figure 4** on page 18.

Step 5. Recalibrate remaining files

• Repeat Steps 2-4 for the files in other folders.

Remember that for sample files you mark the positive ions in **Figure 4** on page 18 and for tune-mix files you mark those in **Figure 5** on page 19.

To restore original file

Follow these steps if you think you need to revert back to the original data file after a bad mass recalibration.

#### 1 Click the Special Operations tab.

#### Figure 7 appears.

Reprocessing Operations Special	Operations
Restore original file	Restore backup data from all operations that can be undone
These operations can be	undone with "Restore original file"
O Make file "peak only"	Remove profile Total Frame Spectra
Remove IRM calibration	Remove internal reference mass calibration
These operations can NO C Remove user calibration Remove backup data	<i>T be undone!</i> Remove calibration done via MassHunter Qual (with backup) Remove backup data (makes all changes permanent) Do Operation

Figure 7. Special Operations Tab

2 Make sure **Restore original file** is marked, or mark any of the other operations you want to do.

#### 3 Click Do Operation.

NOTE

These special operations will work only on the data file loaded into the Only this file field. If you mark the description for all the files in the folder, the operations will not be performed on the other files.

4 (optional) To see all the reprocessing and restoration operations that have been applied to the data file, open the ReprocessLog.txt file in the AcqData folder under the .d folder. See Figure 8.

7/19/2020 9:42:45 AM	Reprocessing started.
7/19/2020 9:42:45 AM	Recalibration requested
7/19/2020 9:42:45 AM	Positive ion reference m/z values = 118.086255, 322.048730, 622.028960, 922.009798, 1221.990636, 15
7/19/2020 9:42:45 AM	m/z tolerance (ppm) = 100
7/19/2020 9:42:45 AM	Minimum abundance = 250
7/19/2020 9:42:45 AM	Peak min valley = 0.70
7/19/2020 9:42:45 AM	IRM statistics: 23 scans 0 missing some ions 0 missing all ions
7/19/2020 9:42:45 AM	Reprocessing finished.
12/20/2020 9:16:32 AM	Reprocessing started.
12/20/2020 9:16:32 AM	Recalibration requested
12/20/2020 9:16:32 AM	Positive ion reference m/z values = 118.086255, 322.048730, 622.028960, 922.009798, 1221.990636, 15
12/20/2020 9:16:32 AM	m/z tolerance (ppm) = 100
12/20/2020 9:16:32 AM	Minimum abundance = 250
12/20/2020 9:16:32 AM	Peak min valley = 0.70
12/20/2020 9:16:33 AM	IRM statistics: 23 scans 0 missing some ions 0 missing all ions
12/20/2020 9:16:33 AM	Reprocessing finished.
12/20/2020 9:31:21 AM	Restore original data - begin
12/20/2020 9:31:21 AM	Operation succeeded
12/20/2020 9:31:21 AM	Restore original data - end

Figure 8. ReprocessLog.txt file in Notepad

## Apply Single-Field CCS Calibration

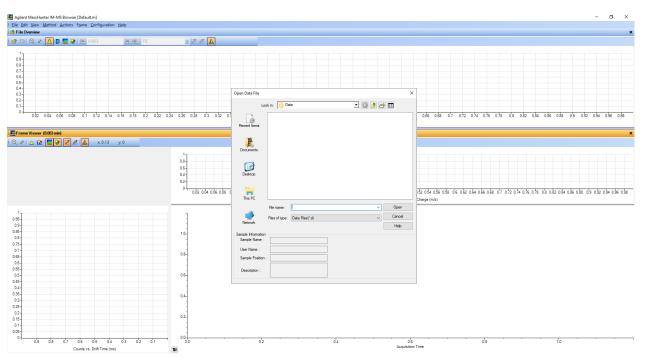
	In this step you take the mass-calibrated tune-mix data into IM-MS Browser to determine the single-field collision cross section (CCS) constants, Beta and TFix, which the Lipid Annotator and Mass Profiler programs use to calculate CCS values. You apply the constants to the tune-mix files and the sample files corresponding to each tune-mix file. Follow these instructions for the All Ions and MS1 tune-mix and sample files.
	Step 1. Load the All lons tune-mix file
	Step 2. Sum the signals
	Step 3. Calculate the Beta and TFix values
	Step 4. Save the values to the tune-mix and sample files
	Step 5. Repeat steps for MS1 tune-mix and sample files
NOTE	If you have multiplexed data you must demultiplex it before doing the single-field calibration. You can demultiplex it in the PNNL Preprocessor.
Step 1. Load the All Ions tune-mix file	Be sure you have downloaded Agilent IM-MS Browser 10.0.

Start IM-MS Browser

Click the IM-MS Browser icon



Step 1. Load the All lons tune-mix file



#### The Open Data File dialog box opens on top of the IM-MS Browser main screen.

Figure 9. Open Data File dialog box on top of IM-MS Browser main window

#### Open a tune-mix file

In the tune-mix files folder, open the All Ions mass-calibrated tune-mix file.

#### 1 Select 101719\_TM\_allions\_0006.d.

Open Data File						×
	Look in: eme_	calibrated files_no PN	INL	- 0	1	
Recent Items		719_TM_allions_000 719_TM_standard_0				
Documents						
Desktop						
This PC						
	File name:				~	Open
- <b></b>	Files of type: [	)ata Files(*.d)			~	Cancel
Network						Help
- Sample Information	ion					
Sample Name			]			
User Name :			j			
Sample Posit	on :		]			
Description :						



2 Click **Open** to add the file to IM-MS Browser.

IM-MS Browser now contains the All lons tune-mix data file that has been masscalibrated.

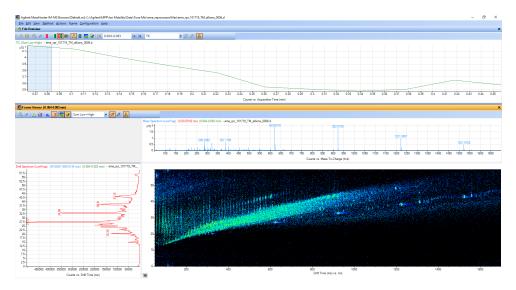


Figure 11. IM-MS Browser with All lons mass-calibrated tune-mix data file

# Step 2. Sum the signals

To sum the signals you must first select the entire TIC, then extract the frame.

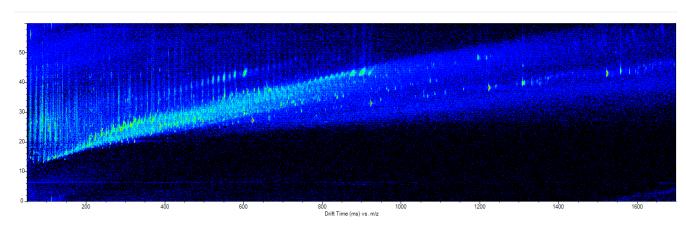
- 1 In the File Overview pane, select the entire TIC.
- 2 To sum all the signals together, right-click the selected TIC.

🕌 Agilent MassHunter IM-MS Browser [Default.m]: C:\Agilent\MPP\lon Mobility\Data\Tune Mix\eme\_reprocessed files\eme\_rpr\_101719\_TM\_allions\_0006.d File Edit View Method Actions Frame Configuration Help 🖻 File Overview A <u> 1</u> 🔤 🍞 🔄 0.064-0.083 - 2 2 🖻 🖻 🔍 🗷 📗 🛋 🖶 🛛 TIC TIC (Sum Low+High): - eme\_rpr\_101719\_TM\_allions\_0006.d x107 4.1 Extract Frame 4. User Chromatograms Δ 3.9 Clear Selection 3.8-Q Unzoom Ctrl+Z 3.7 2 Unzoom Completely 3.6 Copy to Clipboard Ctrl+C 3.5 Copy to User Chromatograms Window Ctrl+U 0.07 0.08 0.09 0.1 0.11 0.12 0.13 0.14 0.22 0.2 Export...

Figure 12. Selected All Ions TIC with short-cut menu

Step 3. Calculate the Beta and TFix values

#### 3 Select Extract Frame.



You can now see the summed signal in the Drift Time vs m/z pane.



See Agilent 6560 Ion Mobility LC-QTOF Fundamentals Guide to learn about "frames".

# Step 3. Calculate the Beta and TFix values

You calculate these values through the CCS Calibration (Single-Field) pane.

## Display the CCS Calibration (Single-Field) pane

- 1 If the pane is visible when you first open IM-MS Browser, hide it and then follow the next instructions.
- 2 Click the View menu.

Agilent MassHunter IM-MS Browser [Default.m]: C:\Agilent\MPP\\on Mobility\Data\Tune Mix\eme\_reprocessed files\eme\_rpr\_101719\_TM\_allions\_0006.d

E File E	dit	View	Method Actions Frame Config	urati	on He	lp											
🛃 🔁 File	: Ov	ø	File Overview														
8	3	<b>!</b>	Frame Viewer	•		0.064-0	476		<b>I</b>	TIC			-	1	A		
TIC (Sum	1 Lov	1	Frame Information	d													
×10 7 4.1-		止	Drift Spectrum Peak List														
4.1-		止	Mass Spectrum Peak List														
3.9-		<b>@</b>	Feature List														
3.8-		Q	CCS Calibration (Single-Field)														
3.7-		ቤ	CCS Calculator (Multi-Field)														
3.6-		Ω	CCS Plot (Multi-Field)														
3.5-		Δ	User Chromatograms														
		Щ	User Drift Spectra	0.	12	0.13	0.14	0.	5 (	).16	0.17	0.18	0.19	0.2	0.21	0.22	0.2
		Щ	User Mass Spectra														
🛃 Fra	me'		Restore Default Window Layout														



3 Select CCS Calibration (Single-Field).

Step 4. Save the values to the tune-mix and sample files

The CCS Calibration (Single-Field) pane appears. Note the bottom part of the pane.

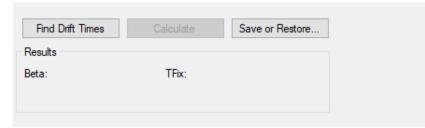


Figure 15. Location for calculating Beta and TFix values for the CCS Calibration

#### Find the drift times

#### • Click Find Drift Times. See the results below.

Find Drift Times	Calculate	Save or Restore
Results		
Beta: 0.137863	TFix: -0.04	48523
	<i>SE</i> : 0.03	7860

Figure 16. Calculated Beta and TFix values

## Step 4. Save the values to the tunemix and sample files

Now you save these values to the current tune-mix file and its corresponding sample files, in this case, the All Ions sample files.

#### 1 Click Save or Restore.

The Save or Restore dialog box appears.

CCS Calibration (Single-Field)	×
Save To file(s)	
Beta: 0.137863	
TFix: -0.048523	
Save to Current File	
Save to Multiple Files	
Save as Instrument Calibration	
Restore previous	
Restore Current File	
ОК	

Figure 17. Save or Restore dialog box - Save to Current File highlighted

Step 4. Save the values to the tune-mix and sample files

#### 2 Click Save to Current File.

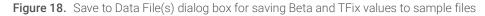
The Beta and TFix values are saved to the tune-mix file, in this case the All Ions tune-mix file.

#### 3 Click Save to Multiple Files.

The Open Data Files dialog box appears.

**a** Open the folder holding sample files corresponding to the tune-mix file used for calculating the constants, in this first instance, the All Ions sample folder.

Save to Data File(s)						×
Loo	ok in: 🔁 All	lons		- 🗿 🤌	<b>e</b>	
Recent Items	<u> 101719</u> P	M_nist_6_allions_0001.	4			
Documents						
Desktop						
This PC						
	File name:				~	Open
1	Files of type:	Data Files(*.d)			~	Cancel
Network						Help
Sample Information						
Sample Name :			]			
User Name :			]			
Sample Position :			]			
Description :						
			-			



- **b** Select all the sample files that were acquired under the exact same experimental conditions as the tune-mix data file you used to calculate the drift times and constants. (If you used the All lons tune mix, then select the All lons sample files. Only one file is present in this example All lons sample folder.)
- c Click Open.
- 4 If more than one folder contains sample files you intend to save with the tunemix file, repeat **step 3** for each folder.
- 5 To close the Save or Restore dialog box, click OK.

#### Step 5. Repeat steps for MS1 tune-mix and sample files

• If you began these instructions with the All lons tune-mix and sample files, repeat Steps 1-4 outlined on the previous pages with the MS1 files.

The MS1 tune-mix file is the remaining file in the Tune Mix folder. The MS1 sample files reside in the three folders with the NIST prefix.

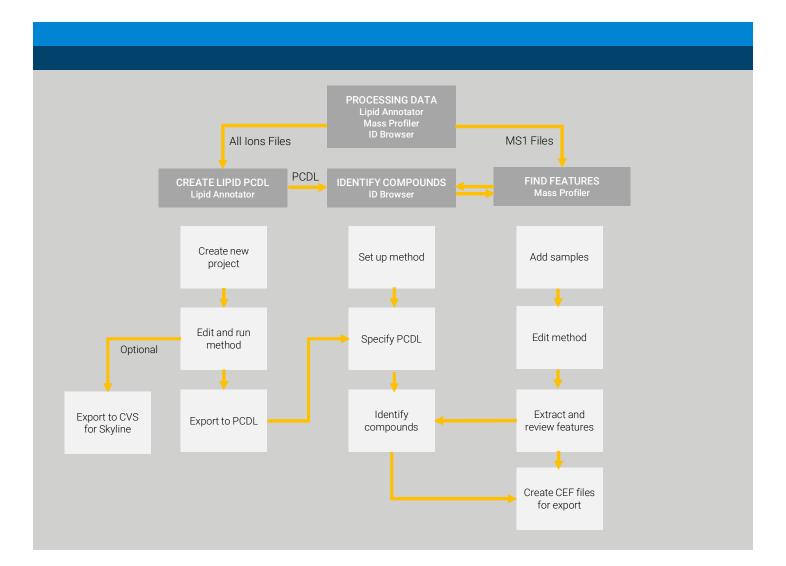
You are now ready to use the calibrated All lons sample file for creating a custom lipid database and the calibrated MS1 sample files for finding features and identifying compounds.

## 3 Processing Data

This chapter takes you through the steps for processing already calibrated data. This chapter focuses on using All Ions data and Lipid Annotator to create a custom lipids PCDL (Personal Compound Database and Library), and using MS1 data and Mass Profiler to extract features, which are identified with the custom lipids database and ID Browser.

This chapter presents the Major Steps and substeps in the processing section of the lipidomics workflow. It assumes you have already calibrated the raw data for Step 1, following the instructions in **Chapter 2**, "Calibrating Data".

Create Lipid PCDL	31
Step 1. Create new project	31
Step 2.Edit and run method	34
Step 3.Export to PCDL	39
To export to CSV for Skyline	41
Find Features	44
Step 1. Add samples	44
Step 2. Edit method	48
Step 3. Extract and review features	51



	Identify Compounds Step 1. Set up method Step 2. Specify PCDL Step 3. Identify compounds Step 4. Create CEF files for export	52 52 56 57 60
	After acquiring and calibrating the data, you for Primary Lipidomics Workflow to identify the lip MPP to assess the lipid composition difference	pids, which data you then pass on to
	Each section gives you additional instructions processing part of the workflow.	to complete the primary tasks in the
Step 2. Create lipid PCDL	Use Lipid Annotator to annotate lipids in the A match the theoretical library, generating a PCL retention time and CCS values. The CCS value TFix values determined in <b>Chapter 2</b> . You can Skyline for targeted data analysis.	DL that contains accurate mass, are calculated from the Beta and
Step 3. Find features	Find features according to mass, retention tim on MS1 data files.	ne and CCS values using Mass Profiler
Step 4. Identify compounds	Use the PCDL from Lipid Annotator with ID Bro lipids and return the compound information to files for export.	
	In <b>Chapter 4</b> you learn how to analyze the lipic Professional (MPP), which provides lipid asset the compound distribution between samples MPP).	ssment tools with which to compare

## Create Lipid PCDL



The second Major Step in the Primary Lipidomics Workflow, Create Lipid PCDL (Personal Compound Database and Library), includes three sub-steps:

- Step 1. Create new project
- Step 2. Edit and run method
- Step 3. Export to PCDL
- Optional Step. Export to CVS for Skyline

With these steps you create a custom lipid database used to identify the compounds in the feature extraction list produced in Mass Profiler (Export to PCDL) or used to further analyze ion mobility data in Skyline (Export to CSV for Skyline).

# Step 1. Create new project

You must use calibrated All lons data to create your custom lipid database.

Project Wethod Tools Help

 Project Wethod Tools Help
 Ipid Classes

 Feature Plot

 Imid Classes

 Imid Classes

</tabl

#### **1** Open Lipid Annotator.

Figure 19. Lipid Annotator main window

#### Name the new project

0== 2=+	Create a Project	×
Create Project		
Project Name		
File	Fragmentation Type	\$
	Add	emove
	Create	ancel

2 Select **Project > Create** to bring up the Create a Project dialog box.

Figure 20. Create a Project dialog box

**3** Type any name you want.

## Add data files and create the project

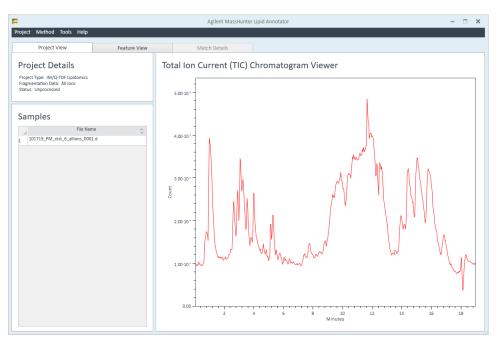
1 Click **Add** and open the All lons calibrated data file.

If you have multiple All lons data files that contain different lipids you will need to process these separately in Lipid Annotator and then manually merge the PCDLs together in PCDL Manager.

0== }	Crea	te a Project		×
Create Project				
Project Name	eme_create	ipid PCDL		
F	ile	\$	Fragmentation Type	0
1 101719_PM_nist_6_all	ions_0001.d	All lons		
			Add	emove
			Create	Cancel

Figure 21. File added to custom lipid database project

2 Click Create.



The Project View - TIC Chromatogram Viewer appears with the data files listed.

Figure 22. Project View - TIC Chromatogram Viewer

You are now ready to edit and run the method.

# Step 2.Edit and run method

This exercise shows you how to edit the method should you choose to change parameter values, but you will keep the default values for this exercise. If possible, use the Default Method that comes with the program because these parameters were selected after analyzing over 60 lipid standards and achieved good results.

Edit the method

- 1 To make sure the default method is loaded, select **Method > Reset Method to Default**.
- 2 Select **Method > Edit Method** from the top menu.

3 Processing Data Step 2.Edit and run method

The Method Parameters tabs appear with the default parameter values. The first tab contains values for filtering features.

<b>4</b> 4	Method Parameters		×
Feature Finding Parameters	Library Parameters	Identification Parameters	
Feature Filter			
Q-Score ≥ 30.0			
Abundance ≥			
Restrict RT to	min.		
Restrict DT to	ms		
Restrict m/z to	m/z		
		OK Cancel	Run

Figure 23. Feature Finding Parameters (default settings)

- **3** For this exercise keep the default values for Feature Finding Parameters.
- 4 Click Library Parameters and view the parameter values.

		od Parameters		
Feature Finding Parameters	Library Para	meters	Identification Parameters	
Positive lons	Lipid Classes			
I H4N				
✓ H4N ;	Select All	Deselect Al	1	
✓ Na	Ac2PIM1		^	
NI 11 I	Ac3PIM2			
Negative lons	Ac4PIM2			
CHO2	✓ ACar ✓ AcylGlcADG			
✓ C2H3O2 ✓ -H	BMP			
Y -H	CE			
	Cer CerADS			
	Cer <u>A</u> P			
	Cer <u>A</u> S			
	Cer <u>B</u> DS Cer <u>B</u> S			
	Cer <u>E</u> ODS			
	Cer <u>E</u> OS			
	Cer <u>N</u> DS Cer <u>N</u> P			
	✓ Cer <u>N</u> S			
	✓ CerP ✓ Cholesterol			
	Cholesteroi			
	✓ DG			
	✓ DGDG ✓ DGTS			
	EtherOxPC			
	EtherOxPE			
	✓ EtherPC ✓ EtherPE			
	✓ FA			
$\checkmark$	✓ FAHFA ✓ GICADG			
	GICADG			
	HBMP			
	HexCer <u>A</u> P HexCer <u>N</u> DS			
	✓ HexCerNS			

This tab lets you set the positive/negative ion filters and the lipid class filters.

Figure 24. Library Parameters (default settings)

- **5** For this exercise keep the default values for the Library Parameters.
- 6 Click Identification Parameters and view the parameter values.

This tab lets you choose which lipids to report.

	Method Parameters		
Feature Finding Parameters	Library Parameters	Identification Parameters	
Sum Composition Le	evel		
Mass Threshold			
Mass deviation ≤ 10.0 ppm	1		
Multiple Lipid IDs for Sa	me Feature		
Report top candidate only			
Score Threshold			
✓ Fragment score ≥ 30			
✓ Total score ≥ 60			
Constituent Level			
Report dominant constituent if re	lative abundance differential ≥ 10	96	
		OK Cancel	Run

Figure 25. Identification Parameters tab (default settings)

NOTE

Amphipathic lipids are identified by lipid class, sum composition and lipid constituents, if fragmentation information confirms specific fatty acid tail fragments. See **"Lipids"** on page 89 for the definitions of these terms and for lipid naming protocols.

7 For this exercise keep the default values for the Identification Parameters tab.

If you intend to save the values you change, then save the method. If you intend to bring up the new method when you open a project, then save the project to a new name before you exit Lipid Annotator.

Run the method

Notice every tab has a Run button.

• Click **Run** after you complete editing the method.

Processing time should be a minute or so with the example file. With your own All lons file, the time will be longer because your file contains no results folder from the previous user's run.

#### The Processing Data message appears.

9===  +=+	Processing Data
18%	complete
	Cancel

Figure 26. Processing Data message

When processing completes, the results appear in the Feature View.

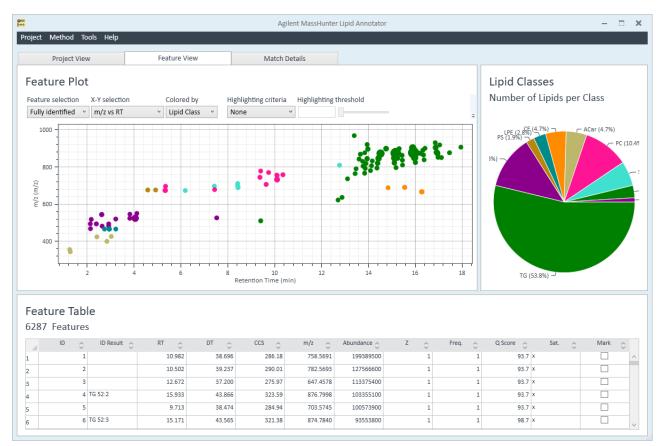


Figure 27. Feature View after run completion

To learn how to change the display and use the Lipid Annotator Tools, see **"Resource Apps"** on page 94 and find the tutorial for Lipid Annotator.

#### **Review CCS values**

Notice the calculated CCS values in the Feature Table CCS column. The program calculated the values based on the Beta and TFix constants determined in **"Apply Single-Field CCS Calibration"** on page 22 of **Chapter 2**.

# Step 3.Export to PCDL

At this point you export the data as a custom lipid PCDL file.

#### Export to PCDL

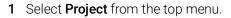




Figure 28. Project menu

#### 2 Select Export Match Result to PCDL...

The dialog box of the same name appears.



Figure 29. Export Match Result to PCDL dialog box

3 Click OK.

The Save As window appears. You now have the ability to name and save the database file.

	rogram Files > Agilent > MassHunter >			v Č	Search PCDL	
rganize 👻 🛛 New fold	der					
<ul> <li>OneDrive</li> </ul>	Name	Date modified	Туре	Size		
71: 00	B.07.00	5/3/2019 10:00 AM	File folder			
This PC	B.08.00	2/26/2019 12:31 PM	File folder			
3D Objects	Import Files	10/26/2019 1:22 PM	File folder			
📃 Desktop	Mass List Files	3/25/2020 10:11 AM	File folder			
🗎 Documents	Metlin DB_G3874-60007	5/2/2019 7:28 AM	File folder			
🖶 Downloads	Pesticide_Example	11/30/2016 6:20 AM	Agilent Compoun	252 K	В	
Music	Sulfas_AM_PCDL	5/1/2019 7:05 AM	Agilent Compoun	188 K	В	
Pictures	Target01	5/7/2019 8:44 AM	Agilent Compoun	380 K	В	
Videos	Target02	5/7/2019 8:54 AM	Agilent Compoun	444 K	В	
	Target03	1/13/2020 6:45 PM	Agilent Compoun	508 K	В	
Windows (C:)	Target04	1/13/2020 6:50 PM	Agilent Compoun	508 K	В	
RECOVERY (D:)	Test_AM_PCDL	1/29/2018 5:22 AM	Agilent Compoun	252 K	В	
network	TimeAlignmentExample	6/9/2017 11:23 AM	Agilent Compoun	124 K	В	
File name: eme	e_custom lipid PCDL					-
Save as type: PCD						-

**4** Type *iii* custom lipid PCDL, where *iii* refers to your initials.

Figure 30. Save As window

#### 5 Click Save.

The Export Successful message appears.

E	xport su	ccessfu	I			
Ex	port compl	eted succe	ssfully!			
				[	OK	]

Figure 31. Export successful message

#### 6 Click OK.

#### NOTE

If you want to add or delete compounds from the database you just created, load the file into PCDL Manager, which is included in your MassHunter software kit.

You are now ready to use this new custom lipid PCDL as a means to identify the features extracted with Mass Profiler in the next section.

# To export to CSV for Skyline

If you want to use Skyline for targeted feature extraction instead of Mass Profiler for untargeted feature extraction, export the file to CSV.

#### Export to CSV

1 Select **Project** from the top menu.

See Figure 28 on page 39.

#### 2 Select Export Features to CSV...

The dialog box of the same name appears.



Figure 32. Export Features to CSV dialog box

3 Click OK.

The Save As window appears. You now have the ability to name and save the database file.

4 Type *iii* custom lipid CSV, where *iii* refers to your initials.

rganize 🔻 👘 New fold	er				
OneDrive	Name	Date modified	Туре	Size	
This PC	B.07.00	5/3/2019 10:00 AM	File folder		
-	B.08.00	2/26/2019 12:31 PM	File folder		
3D Objects	Import Files	10/26/2019 1:22 PM	File folder		
E Desktop	Mass List Files	3/25/2020 10:11 AM	File folder		
Documents	Metlin DB_G3874-60007	5/2/2019 7:28 AM	File folder		
Downloads	Pesticide_Example	11/30/2016 6:20 AM	Agilent Compoun	252 KB	
Music	Sulfas_AM_PCDL	5/1/2019 7:05 AM	Agilent Compoun	188 KB	
Pictures	Target01	5/7/2019 8:44 AM	Agilent Compoun	380 KB	
Videos	Target02	5/7/2019 8:54 AM	Agilent Compoun	444 KB	
	Target03	1/13/2020 6:45 PM	Agilent Compoun	508 KB	
L Windows (C:)	Target04	1/13/2020 6:50 PM	Agilent Compoun	508 KB	
RECOVERY (D:)	Test_AM_PCDL	1/29/2018 5:22 AM	Agilent Compoun	252 KB	
network	TimeAlignmentExample	6/9/2017 11:23 AM	Agilent Compoun	124 KB	
File name: eme_	custom lipid CSV				_
Save as type: PCDL	Eler (* edle)				

Figure 33. Save As window

5 Click Save.

#### The Export Successful message appears.

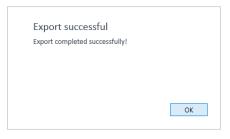


Figure 34. Export successful message

6 Click OK.

# Prepare CSV file for Skyline import

You cannot import the CSV file into Skyline unless you do the following steps.
Copy the CSV file as another name, say, forskyline.csv, and open it in Excel.
You must do the steps below to have the spreadsheet look like Figure 35.

A	utoSave 💽 🗄 り	∽ ে ় → Fors	kyline2 🗸 🛛 🔎 Search	h			Elaine Ea	arl 🔃 🖻	- 0	X
File	Home Insert	Page Layout For	mulas Data Review	View M	MASSHUNT	ER REPORTING	Help	ACROBAT	🖻 Share	$\nabla$
rê:		Δ <u>Δ</u> ΔΔ	≡ = _ ab Gene	eral 🗸 📕	Conditiona	al Formatting ~	🚝 Insert	~ <u>\</u> \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	· 4	
	Calibri	~ 11 ~ A^ A`	= = = = = , ( ,		Format as	Table ~	🗮 Delete	_	4/	
Pas	<sup>te</sup> <b>8</b> <i>I</i> <u>U</u> ~		↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓		Cell Styles		Format		Ideas	
Clip	board 🗔	Font 5	Alignment 🗔 Nu	mber 🗔	St	yles	Cells	Editing	Ideas	
43	• : ×	√ <i>f</i> <sub>x</sub> 489								
	А	В	с	D		E		F	:	
Ν	Nolecule List Name Pred	cursor Name	Precursor Formula	Precursor Ad	duct	Explicit Retent	ion Time	Collisional Cros	s Section (sq A)	
	1324 ACa	r 14:0	C21H41NO4	[M+H]+			1.896		206.96	í
	489 ACa	ir 16:0	C23H45NO4	[M+H]+			2.835		213.91	
	895 ACa	ir 18:0	C25H49NO4	[M+H]+			3.782		220.68	1
6	537 ACa	ir 18:1	C25H47NO4	[M+H]+			3.024		216.45	
	342 CE 1	18:1	C45H78O2	[M+H4N]+			17.343		289.86	í -
	81 CE 1	18:2	C45H76O2	[M+H4N]+			16.292		291.56	6
	132 CE 1	18:2	C45H76O2	[M+H4N]+			16.286		413.74	
	3952 CE 2	20:3	C47H78O2	[M+H4N]+			16.398		417.8	1
D	280 CE 2	20:4	C47H76O2	[M+H4N]+			15.548		295.27	/
1	553 CE 2	20:4	C47H76O2	[M+H4N]+			15.553		415.96	í.
2	1684 CE 2	20:4	C47H76O2	[M+H4N]+			16.055		295.25	
3	202 Cer	_NS d18:1_14:0	C32H63NO3	[M+H]+			9.408		248	1
4	1813 LPC	0:0/15:0	C23H48NO7P	[M+H]+			2.426		227.05	
5	136 LPC	0:0/16:1	C24H48NO7P	[M+H]+			2.386		225.61	
6	1307 LPC	0:0/16:1	C24H48NO7P	[M+H]+			2.931		226.07	/
7	39 LPC	0:0/17:0	C25H52NO7P	[M+H]+			3.586		234.51	
8	161 LPC	0:0/18:0	C26H54NO7P	[M+H]+			3.821		239.72	1
9	24 LPC	0:0/18:1	C26H52NO7P	[M+H]+			3.322		234.02	1
0	147 LPC	0:0/18:2	C26H50NO7P	[M+H]+			2.463		229.8	1
1	613 LPC	0:0/20:1	C28H56NO7P	[M+H]+			4.124		240.44	
2	782 LPC	0:0/20:5	C28H48NO7P	[M+H]+			2.071		227.18	1
3	389 LPC	14:0/0:0	C22H46NO7P	[M+H]+			2.136		222.44	
4	8 LPC	16:0/0:0	C24H50NO7P	[M+H]+			3.153		230.92	-
4	Forskyline2	+			:	4				•
ead	v						<b>=</b>	四	+	100

Figure 35. Excel .csv file with the correct entries.

- 2 Remove all the columns that are not included **Figure 35** on page 42.
- **3** Remove all rows that have no Molecule List Name and Precursor Name.
- **4** Rename columns to the names above.
- **5** Change "(" to "[" and ")" to "]".
- 6 If Deuterium is present in the sample, change the "[2H]" in the Precursor Formula to "D".
- 7 Import the file into Skyline and refer to its user guides to perform the tasks you want to do.

## **Find Features**

		This is the third Major Step in the Primary Lipidomics Workflow. It uses Mass Profiler and comprises three sub-steps.
Add samples		Step 1. Add samples
	1	After you launch Mass Profiler, you add the calibrated MS1 samples.
	<b>•</b>	Step 2. Edit method
	Edit method	This step gives you instructions for editing the method in Mass Profiler to extract features based on retention time, mass and CCS value.
		Step 3. Extract and review features
	+	Ion mobility features are unidentified compounds represented by retention time,
	Extract and review features	mass and CCS value. In this step you run the method you created in Step 2 to extract these features from the data. In the next section you identify the compounds with ID Browser.

## Step 1. Add samples

You must first create a project and add samples before you edit a default method for the feature extraction.

#### 1 Launch Mass Profiler.

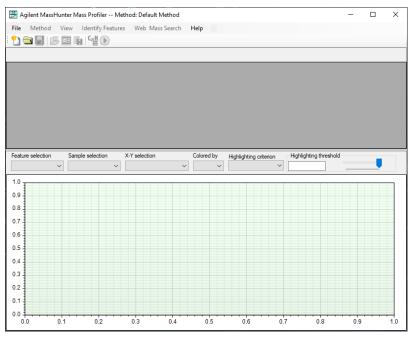


Figure 36. Agilent Mass Profiler main window

## Create new project

- 2 Note the method whose name is on the Title Bar.
- 3 Select File > Create Project.
- 4 For Number of groups, select 1 (Figure 37).

Create Project			×
Number of groups <ul> <li>1</li> <li>2</li> </ul> Input data type	Project name		
*.d (MFE)	ef (MFE, FbF)	○ *.csv (RT, Mass, Abund.)	
Samples Add	Delete		
Scalar File N	Name		
Open method on OK	ОК	Cancel	

Figure 37. Create Project dialog box

#### Add calibrated MS1 samples

Because there are no control samples in the MS1 files, use the following instructions:

- 1 Type the project name, *iii\_find lipids*, where *iii* refers to your initials.
- 2 Click Add.
- 3 Select the calibrated files in the Nist0\_1017 folder, and click Open.
- 4 Repeat step 2 and step 3 for the calibrated files in the other two Nist folders.

The Create Project dialog box now contains the sample files.

Numbe	er of groups	Project name eme_find lipids	
	lata type (MFE) (	*.cef (MFE, FbF) (*.csv (RT, Mass, Abund.)	
Sampl	es Add.	. Delete	
	Scalar	File Name	1
•	1.000	101719_PM_nist_0_std_0001.d	1
	1.000	101719_PM_nist_0_std_0002.d	
	1.000	101719_PM_nist_0_std_0003.d	
	1.000	101719_PM_nist_2_std_0001.d	
	1.000	101719_PM_nist_2_std_0002.d	
	1.000	101719_PM_nist_2_std_0003.d	
	1.000	101719_PM_nist_4_std_0001.d	
	1.000	101719_PM_nist_4_std_0002.d	
	1.000	101719_PM_nist_4_std_0003.d	J.

Figure 38. Mass Profiler Create Project dialog box with added files

- **5** If the default method's name is in the Title Bar of the main window:
  - a Make sure the **Open method on OK** check box is **marked**.
  - **b** Click **OK**.

At first, you see the dialog box with only commonly changed parameters (**Figure 39**).

Feature finding Input filters	
Chromatographic data	Infusion data
	n intensity >= 100.0 count
Isotope model Common organic molecules	$\sim$
✓ Limit charge states to a range of 1-1	
Report single-ion features with charge state :	 z=1
Alignment parameters	
RT tolerance = $\pm (0.0 \%)$	+ 0.30 min)
DT tolerance = ± 1.5 %	
Mass tolerance = ± ( 15.0 ppm	+ 2.0 mDa)
	Apply     Both Up Down
Sample occurrence	Both O Up O Down
	Fold change >=
☐ Frequency >= 50.0 % Group 1: 3/6	Fold change >=     [log2(A1/A2)]>=
Frequency >= 50.0 %	○  log2(A1/A2) >=
☐ Frequency >= 50.0 % Group 1: 3/6 Group 2: 2/3 ● in at least one group	
☐ Frequency >= 50.0 % Group 1: 3/6 Group 2: 2/3	O  log2(A1/A2) >=
<ul> <li>□ Frequency &gt;= 50.0 % Group 1: 3/6 Group 2: 2/3</li> <li>◎ in at least one group</li> <li>○ across all samples</li> </ul>	O  log2(A1/A2) >=
Group 1: 3/6 Group 2: 2/3 in at least one group across all samples	O  log2(A1/A2) >=
<ul> <li>□ Frequency &gt;= 50.0 % Group 1: 3/6 Group 2: 2/3</li> <li>◎ in at least one group</li> <li>○ across all samples</li> </ul>	O   log2(A1/A2)   >=       Differential score       Score >=
Group 1: 3/6 Group 2: 2/3 in at least one group across all samples	Ilog2(A1/A2)  >=       Differential score       Score >=

Figure 39. Default method with commonly changed parameters

c Go to "Step 2. Edit method" on page 48.

If a different method has been loaded:

- a Make sure the **Open method on OK** check box is **clear**.
- **b** Click **OK**.
- c Select Method > Reset Method to Default.
- d Select Method > Edit Method.

Figure 39 appears.

e Go to "Step 2. Edit method" on page 48.

## Step 2. Edit method

One of the parameters you are going to edit is not on **Figure 39** on page 47.

#### Edit parameters

#### 1 Select All parameters.

You now see the default method dialog box with three tabs.

Method Parameters - Default Method Feature Finding/Loading Alignment & Nomalization Statistics & Filters
Measure of abundance O Max ion intensity   Max ion volume  O Feature volume
Feature finding input filters     O Infusion data     Restrict RT to     min, Ion intensity >= 100.0 count
Sample chemistry and ionization Isotope model Common organic molecules ~
Limit charge states to a range of <u>1-1</u> Report single-ion features with charge state z=1     Positive ions in priority     Negative ions in priority     Negative ions in priority
Image: state of the state o
Commonly changed parameters     All parameters     OK Cancel Run

Figure 40. Default method with all parameters

#### 2 Click a tab, and for each tab and parameter take the action described in Table 2.

#### Table 2 Parameters

For This Tab and Parameter:	Take This Action:
Feature Finding/Loading Tab:	
Isotope Model	Select Common organic (no halogens).
Limit charge states to a range of	Type 1-3
Report single ion features with charge state z=1	Check box marked automatically when above Isotope Model selected
Alignment & Normalization Tab:	No edits
Statistics and Filters Tab:	
Abundance >	5000

The Feature Finding/Loading Tab now looks like this.

Method Parameters - D	efault Method		×
Feature Finding/Loading	Alignment & Normalization	Statistics & Filters	
Measure of abundance	-	ne O Feature	volume
Feature finding input fil <ul> <li>Chromatograph</li> <li>Restrict RT to</li> </ul>	ic data 🔿 Inf	usion data tensity >= 100.0	count
Sample chemistry and Isotope model Con	nmon organic (no halogens)	~	
Report single-ior	n features with charge state z	z=1	

Figure 41. Edited Feature Finding/Loading Tab

The Statistics and Filters Tab now looks like this.

eature Finding/Loading	Alignment & Normalization	Statistics & Filters
Missing appeals tracted		
Missing sample treater	_	
Assign 0 abunda	nce 🔘 Evolude from ana	hunin
0		lysis
0		iyaa
Statistics and filters		iyələ
		Group difference
Statistics and filters Feature filter		
Statistics and filters Feature filter	70.0	Group difference

Figure 42. Edited Statistics and Filters Tab

#### 3 Click OK.

NOTE

Changing a parameter value that is not commonly changed does not automatically place the parameter on the Commonly changed parameters dialog box. The Commonly changed parameters (not values) are always the same.

Save the method and project You c

You can start a run from any tab of the method dialog box, but it's wise to save both the method and project at this point.

- 1 Select Method > Save Method As.
- 2 Type the new method, *iii\_*find lipids.mpm., and click **Save**.
- 3 Select File > Save Project.

NOTE

Step 3. Extract and review features

Now you can run the method to extract features. Do not worry if the number of features varies a bit from run to run. The lipid matrix results in MPP should be the same as shown in the next chapter.

#### Run method

#### • Select Method > Run Current Method, or

Open the method and click **Run**.

For these example files, the run will take around a minute or so because Results folders already exist (from previous user's run).

#### **Review features**

When the run is complete, the Mass Profiler main window looks like this:

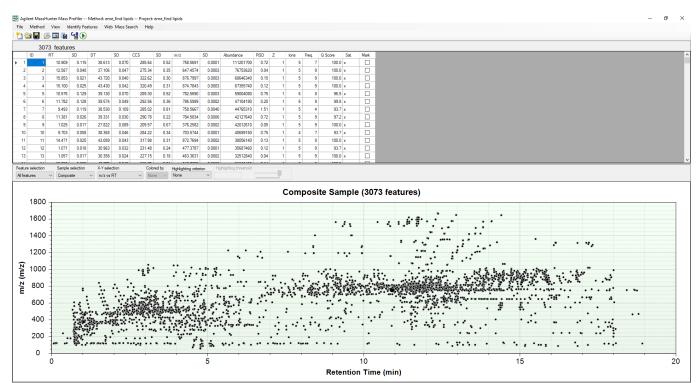
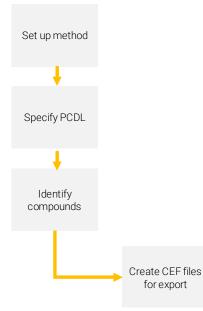


Figure 43. Mass Profiler main window with feature extraction results

## Identify Compounds



# Step 1. Set up method

# Enter database search criteria

In this fourth Major Step you identify features using the ID Browser within Mass Profiler and the custom lipid PCDL you created with Lipid Annotator. This Major Step comprises four sub-steps.

• Step 1. Set up method

After you launch ID Browser from Mass Profiler containing the extracted MS1 features, you edit the parameters in the ID Browser method.

- Step 2. Specify PCDL
  - This is where you enter the custom lipid PCDL file you created.
- Step 3. Identify compounds

You then run the method to match the MS1 features with the compounds in the PCDL and return the data to Mass Profiler, where the CEF files are created.

• Step 4. Create CEF files for export

In Mass Profiler you create the CEF files for export and then import them to Mass Profiler Professional (MPP) in the fifth Major Step, Analyze Lipids.

After launching ID Browser from Mass Profiler, you set up the method to identify the lipid compounds in the features you extracted.

- 1 Launch ID Browser from Mass Profiler.
  - a Click the Identify Features menu.

💹 Agilent MassHunter Mass Profiler -- Method: eme\_find lipids -- Project: eme\_find lipids

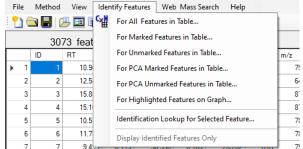


Figure 44. Identify Features menu for Mass Profiler

**b** Select For All Features in Table...

Agilent MassHunter ID Browser 10.0		- a ×
Eile Edit View Identification Method Configuration Help		
*ク・ペー ③ Run ID Wizard 山 神 灸 山 G Save and <u>R</u> eturn		
MS Spectrum Results	× j_h MS Peaks One: + MFE Spectrum (rt: 10.909 min) ×	Structure Viewer ×
	m/z / V + Abund V + Abund % (Norm) V + Z V + Sat V + Species V + Label V + Formula & Ion Species V + m/z (prod.) V + Z (prod.) V + Ion V +	
	12 * * + Pound & Pound & Pound & (norm) * + 2 * 3a * + 3pectes * + Later * + Portmale s on species * = miz (prod.) * + 2 (prod.) * + on * + 758,5592   55644464   1   (M-H)+   (M-H)+	No data to display.
x10 <sup>8</sup> Cpd 1: + MFE Spectrum (rt: 10.909 min)	759.5727 100799608 1 (M+H)+ (M+H)+	
1.9-	760.576 32173810 1 (M+H)+ (M+H)+	1
1.8-	761.5797 5443934.5 1 (M+H)+ (M+H)+	
1.7- (M+H)+	762.5834 875382.44 1 (M+H)+ (M+H)+	_
((n+n)+		
1.6-		
1.5-		
1.4-		
	Compound Identification Wizard X	
Ci Ci	sound Identification Browner e select the identification methods you wish to apply to the compounds in this .CEF file	
	mound selection	
	Identify only highlighted compounds	
1-	Identify only unidentified compounds	
	Identify all compounds	
08-	aentry ai compounds	
0.7-		
0.6-		
0.5-		
0.4- (M+H)+		
0.3		
0.2-		
201 5707		
0.1- (M+H)+		
758.5 759 759.5 760 760.5 761 761.5 Counts vs. Mass-to-Charge (m/z)		
III MS Spectrum Results Spectral Difference Results: Cpd 1: 10.909	>	
Compound List		×
Cpd ⊽+⊅ Label ⊽+⊅ Name ⊽+⊅ Formula ⊽+⊅ Score ⊽+⊅ Mass ⊽+⊅ Avg Mass ⊽+⊅ Std Dev ⊽+⊅ Mas	Min Z マキ Start マキ RT マキ End マキ RT (Lib/DB) マキ RT Diff (Li	
	Help << Back Next >> Brish Cancel 1 4.183	516 17589
3065 Cpd 3065: 3.246 488.2697	Positive 1 1 3246	553 6535
3066 Cpd 3066: 4.063 382.3074		677 15736
3067 Cpd 3067: 1.484 122.0843		1105 6687
3068 Cpd 3068: 1.696 751.5067	Positive 1 1 1666	487 6017
3069 Cpd 3069: 14.863 877.7199 3070 Cpd 3070: 16.222 87.1046	Positive 1 1 14.863 Positive 1 1 16.222	795 6878 596 8647
30/0 Cpd 30/0: 16.222 87,1046 3071 Cpd 3071: 15.111 522.4634	Positive 1 1 16222	308 10504
3071 Cpd 3071: 13.111 322.4634	Positive 1 1 15372	731 12830
3073 Cpd 3073: 3.172 495.3307	Positive 1 1 3.172	602 8127
<		``````````````````````````````````````

#### The ID Browser Wizard appears on top of the main window.

Figure 45. ID Browser Wizard on top of default identification results

#### 2 In the Compound Identification Wizard, click Next.

ī	Compound Identification Wize	ard						$\times$
F	Compound Identification Browser lease set parameters for identification Identification method							
	C:\Program Files\Agilent\MassHunte	r\Workstation\Met	hods\Method	s\10.0\Default	.m		2	
	Identification	Search Criteria	Peak Limits	Positive lons	Negative lons	Scoring	Search Results	
	Identification Workflow	Match tolerar	nce					
	Database Search Settings	Mass						
	Library Search Settings	Tolerance	+/- (	5.00	ppm + 2.00	m	Da)	
	Generate Formulas						, i i i i i i i i i i i i i i i i i i i	
	Combine Identification Res	Retention	time	Required				
		Tolerance	+/- [	0.100	minutes			
				Required				
		Tolerance	+/-	1.00	%			
l								
	Help			<< Back	<u>N</u> ext >>	Finish	Cancel	

Figure 46. Database Search Settings - Search Criteria Tab

**3** If the default method is listed as the Identification method, go to **step 4**.

If the default method is not listed as the Identification method:

- a Click the **Open** icon.
- **b** In the ...\Workstation\Methods\Methods\10.0 folder, select **Default.m**.
- c Click Open.
- 4 Select Database Search Settings and click the Search Criteria tab.
- 5 Mark the Retention Time check box and the Required check box next to it.
- 6 Mark the CCS check box and the Required check box next to it.
- 7 Retain default values for the other parameters.

	ter\Workstation\Methods\Methods\1		
Identification	Negative lons	Scoring Peak Limits	Search Results Positive Ions
dentification Workflow		Peak Limits	Positive ions
)atabase Search Settings 🛛 🛕	Match tolerance		
ibrary Search Settings	Mass		
enerate Formulas	Tolerance +/- ( 5.0	) ppm + 2.00	mDa)
ombine Identification Res			
	Retention time Rec	juired 🛕	
	Tolerance +/- 0.1	00 minutes	
	CCS Red	juired 🛕	
	Tolerance +/- 1.0	) %	
	Tolerance +/- 1.0	10	

Figure 47. Search Criteria selections made

#### Enter additional positive ion

1 In Database Search Settings, click the **Positive lons** tab.

	nter\Workstation\Methods\Methods\10	/.v.uberduit.in	6	
Identification	Negative lons	Scoring	Search Results	
dentification Workflow	A Search Criteria	Peak Limits	Positive lons	3
atabase Search Settings	Charge carriers	Neutral losses		1
ibrary Search Settings	-electron	H20		
ienerate Formulas	✓ +H ✓ +Na			
combine Identification Res	I +Na ⊢+K			
ombine Identification Res	+NH4			
	+ ×		+ 🗙	
	Charge states, if not known	Aggregates		
	Charge state range 1	Dimers	e.g., [2M+H]+	
		Trimers	e.g., [3M+H]+	
	DB ion type search mode			
	✓ Neutrals			

Figure 48. Positive lons tab

2 Type H4N and click + to add it to the list of Charge carriers.

Because the PCDL from Lipid Annotator writes H4N, you need to add that to ID Browser, which is looking only for NH4.

:\Program Files\Agilent\MassHunt	er\Workstation\Methods\Methods\10	).0\Default.m	<b>11</b>	
Identification	Negative lons	Scoring	Search Results	
dentification Workflow	🛕 Search Criteria	Peak Limits	A Positive lons	3
atabase Search Settings	Charge carriers	Neutral	losses	^
ibrary Search Settings Generate Formulas Combine Identification Res	Hetron H H HAN HAN Charge states, f not known Charge state range		ates ers e.g. [2M+H]+	
	DB ion type search mode ☑ Neutrals ☑ Cations (this type is not app	icable to CSV data	ibases)	

Figure 49. Positive lons tab modified



### Step 2. Specify PCDL

Before saving the method, as the last set-up step you link to the custom lipid PCDL you created previously.

#### 1 Select Identification Workflow.

	Database Search Settings Library Search Settings Generate Formulas Combine Identification Res.  Move Up Move Down Add Remove Search all libraries / databases Stop at first library / databases Stop at first library / database match Maximum hits per compound: 10	Database Search Settings 🛕 Library Search Settings Generate Formulas	Library / Database Sc	core (fwd) Score (rev)	
esMove UpMove DownAddRemove	Library Search Settings Generate Formulas Combine Identification Res Move Up Move Down Add Remove © Search all libraries / databases © Stop at first library / database match Maximum hits per compound: 10	ibrary Search Settings Generate Formulas			
Move Up         Move Down         Add         Remove <ul></ul>	Generate Formulas Combine Identification Res_  Move Up Move Down Add Remove  Search all libraries / databases Stop at first library / database match Maximum hts per compound: 10	Generate Formulas			
Move Up         Move Down         Add         Remove <ul></ul>	Combine Identification Res  Move Up Move Down Add Remove  Search all libraries / databases  Stop at first library / database match  Maximum hits per compound: 10				
Move Up         Move Down         Add         Remove <ul></ul>	Move Up     Move Down     Add     Remove <ul> <li>Search all libraries / databases</li> <li>Stop at first library / database match</li> <li>Maximum hits per compound:</li> <li>10</li> </ul> <ul> <li>Interview</li> </ul> <ul> <li>Interview</li> <li>Interview</li> <li>Interview</li> <li>Interview</li> <li>Interview</li> <li>Interview</li> </ul> <ul> <li>Interview</li> </ul>	Combine Identification Res			
Search all libraries / databases     Stop at first library / database match	Search all libraries / databases     Stop at first library / database match     Maximum hits per compound:		Maura Line Maura Daura Add	Remains	J
Stop at first library / database match	Stop at first library / database match Maximum hits per compound: 10			Nelliove	
	Maximum hits per compound: 10		-		
Maximum hits per compound: 10					
	Identify by - Formula generation		Maximum hits per compound:	10	
Identify by - Formula generation			🗖 Identific by Fermida conception		
	Always     When there are no Library / Database hits				
<ul> <li>Always</li> <li>When there are no Library / Database hits</li> </ul>				Library / Database hits	
Identify by - Formula generation			Maximum hits per compound:	10	
Always Always and the second and the second	Always when there are no Library / Database hits			Uhana / Databasa hita	
Identify by - Formula generation					

Figure 50. Identification Workflow without specified PCDL

- 2 To add the lipid database you created earlier in this chapter, click Add.
- 3 Select *iii\_custom lipid PCDL*, and click Open.

Compound Identification Wiza	rd	×
Compound Identification Browser Please set parameters for identification	lechniques	
C:\Program Files\Agilent\MassHunter	Workstation \Methods \10.0 \Default.m	^
Identification Workflow	Library / Database Score (fwd) Score (rev)	
Database Search Settings 🔺	C:\Program Files\Agilent\MassHunter\ 25.00 70.00	
Library Search Settings 🔒		
Generate Formulas		
Combine Identification Res	Move Up Move Down Add Remove	
	Search all libraries / databases	
	Stop at first library / databases	
	Maximum hits per compound: 10	
	Identify by - Formula generation	
	O Always ( ) When there are no Library / Database hits	
		~
Help	<< Back Mext >> Finish (	Cancel

Figure 51. Identification Workflow with custom lipid PCDL added

#### Save the method

- 1 Click the **Save** icon.
- 2 Type *iii*\_ID Method Lipids, and click **Save**.

Compound Identification Browser Please set parameters for identification t	techniques	
Identification method		
C:\Program Files\Agilent\MassHunter	\Workstation\Methods\Methods\10.0\ID Method - Lipids.m	
Identification	✓ Identify by - Library / Database search	^
Identification Workflow	Library / Database Score (fwd) Score (rev)	
Database Search Settings	C:\Program Files\Agilent\MassHunter\ 25.00 70.00	
Library Search Settings		
Generate Formulas		
Combine Identification Res	Move Up Move Down Add Remove	
	Search all libraries / databases	
	O Stop at first library / database match	
	Maximum hits per compound: 10	
	Identify by - Formula generation	
	O Always       When there are no Library / Database hits	
		~

Figure 52. Identification Workflow with new saved method.

# Step 3. Identify compounds

Run the method

Now you are ready to run the method to identify compounds.

#### 1 To run the ID method, click **Finish**.

After ID Browser completes the identification, you see the same main screen as in **Figure 45** on page 53 but with a few identified compounds in the table.

2 To view identified and unidentified features in Mass Profiler, click **Save and Return**.

3 **Processing Data** 

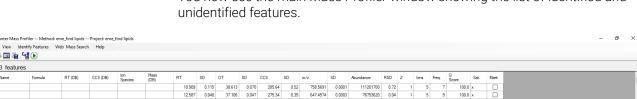
N.B

Method

🔁 📾 🖬 😕 🖬 🖬 🖬 🕑 3073 features

Step 3. Identify compounds

RT (DB)



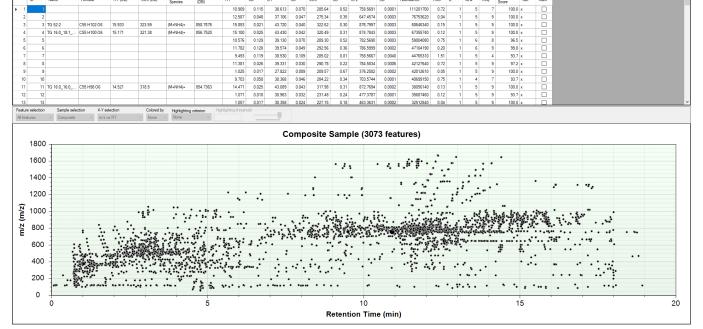
111201700

76753620

You now see the main Mass Profiler window showing the list of identified and

100.0 >

100.0 x



758.5691 647.4574

0.000

0.0003

Figure 53. Mass Profiler main window with table of identified and unidentified features

10.909

12.587

#### **Display identified** compounds only

#### 1 Click Identify Features.

🧱 Agilent MassHunter Mass Profiler -- Method: eme\_find lipids -- Project: eme\_find lipids File Method View Identify Features Web Mass Search Help 🎦 🚍 🔚 🕖 🖬 🕻 🛀 For All Features in Table... For Marked Features in Table... 3073 feat For Unmarked Features in Table... ID Name For PCA Marked Features in Table... For PCA Unmarked Features in Table... 2 2 For Highlighted Features on Graph... 3 3 TG 16:0\_ NH4)+ 4 TG 16:0\_ 4 +NH4)+ Identification Lookup for Selected Feature... 5 5 Display Identified Features Only 6 6

Figure 54. Identify Features menu

2 Select Display Identified Features Only.

3 Processing Data

Step 3. Identify compounds

The identified compounds appear in the table and graphic (I	Figure 55).
	· · · · · · · · · · · · · · · · · · ·

	4 features																							
ID	Name	Formula	RT (DB)	CCS (DB)	lon	Mass	RT	SD D	τ.	SD	ccs	SD m	(a.	SD	Abundance	RSD Z	lone	Freq.	Q Score	Sat.	Mark			
	1 TG 52:2	C55 H102 O6	15.933	323.59	Species (M+NH4)+	(DB) 858.7676	15.853	0.021	43.720	0.040	322.62	0.30	876.7997	0.0003	68646340	0.15	ions	5		.0 x				
2	2 TG 16:0_18:1		15.933	323.59	(M+NH4)+ (M+NH4)+	856.7520	15.853	0.021	43.430	0.040	322.62	0.30	874.7843	0.0003			1	5		.0 x				
			14.521	318.9	(M+NH4)+	854,7363	14.471	0.025	43.089	0.042	317.98	0.31	872.7694	0.0002	38056140		1	5		.0 x				
	4 TG 14:0_18:1		15.127	316.61	(M+NH4)+	830.7363	15.058	0.025	42.773	0.042	315.79	0.31	848.7695	0.0002			1	5		0 x				
	5 TG 16:0_16:0	C53 H100 O6	15.973	319.01	(M+NH4)+	832.7520	15.884	0.024	43.100	0.041	318.19	0.30	850.7848	0.0001	26167500	0.16	1	5		.0 ×				
	6 TG 18:1_18:1	C57 H104 O6	15.895	328.26	(M+NH4)+	884.7833	15.834	0.022	44.353	0.035	327.14	0.25	902.8158	0.0002		0.17	1	5	9 100	.0 ×				
7	7 TG 16:0_16:1	C53 H96 O6	14.437	314.2	(M+NH4)+	828.7207	14.394	0.027	42.445	0.039	313.38	0.29	846.7539	0.0002	17026970	0.15	1	5	9 100	.0 x				
8	8 LPC 18:0/0:0	C26 H54 N O7 P	4.049	238.8	(M+H)+	523.3638	4.074	0.117	31.890	0.056	237.81	0.42	524.3698	0.0001	15432480	0.61	1	5	9 100	.0 ×				
9	9 TG 16:0_18:0	C55 H104 O6	16.921	324.86	(M+NH4)+	860.7833	16.855	0.028	43.920	0.051	324.08	0.37	878.8156	0.0002	12241760	0.24	1	5	9 100	.0 ×				
	10 TG 54:2	C57 H106 O6	16.874	329.24	(M+NH4)+	886.7989	16.816	0.028	44.512	0.047	328.30	0.35	904.8316	0.0001	9898488	0.24	1	5	9 100	.0 × 0.				
	11 TG 54:5	C57 H100 O6	14.758	324.67	(M+NH4)+	880.7520	14.722	0.028	43.863	0.047	323.55	0.35	898.7854	0.0002		0.16	1	5		.3 x				
	12 TG 16:0_16:0		15.112	311.92	(M+NH4)+	804.7207	15.035	0.028	42.139	0.045	311.27	0.33	822.7538	0.0001	7760612		1	5						
3 ture select	13 TG 16:0_16:1 on Sample self	C51 H94 O6 ection X-Y sel	14.398	309.54 Colored b	(M+NH4)+ / Highlighting	802.7050	14.363 lighlighting thresh		41.818	0.040	308.92	0.30	820.7382	0.0002	6876815	0.22	1	5	9 100	.0				
									<b>(</b>	Comr	posite	Sam	le (84	featu	ures)									
100	00								C	Comp	osite	Samp	ole (84	featu	ures)									
100 90	-								(	Comp	posite	Samp	ole (84	featu	ures)			•			•			
	-								(	Comp	posite	Samp	ole (84	featu	ures)			•	••	• .	è			
90	0								(	Comp	osite	Samp	ole (84	featu	ures)					•	•	•	•	
	0								(	Comp	osite	Samp	ole (84	featu	ures)	•			•	•	•	::	•	
90	0								(	Comp	osite	Samp	ole (84	featu	ures)	•			•	•	•	•	•	
90 80	0									Comp		Samp	le (84	featu	ures)	•			•	•:	•	•	•	
90 80	0									Comp	oosite ∵	Samp	ile (84	featu	ures)	•	•		•	•	•	•	•	
90 80	0					•				Comp		Samı	· •	featu	ures)		•	•	•	•	•	: :	•	
90 80	0					•				Comp		Samı	ile (84	featu	ures)	•			•	•	•	: :	•	
90 80	0					•				Comp		Samı	ile (84	featu	ures)	•		••••	•	•	•	•	•	
90 80 70 60	0				::	•	•			Comp		Samı	ile (84	feat	ures)				•		•			
90 80	0				::	•				Comp		•	ile (84	feat	ures)	•				•	•	: :	•	
90 80 70 60	0		••••		::	•	-		·	Comp		•	ile (84	feat	ures)	•		••••		•	•	: :	•	
90 80 70 60	0		÷•••		::	•	•			Comp		•	le (84	feat	ures)	•			•••••••••••••••••••••••••••••••••••••••		•	•	•	
90 80 70 60 50	0		÷•••	· ·	::	•	•			Comp		•	• • •	feat	ures)		-		•	•	•	: :		
90 80 70 60 50	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Ÿ	÷•••	· · ·	::	•				Comp		•	le (84	featu	ures)	•	•		•		•	: .	•	

Figure 55. Identified features only in three sample sets

At this point you may want to save the project with the feature finding and identification results.

# Step 4. Create CEF files for export

If you intend to analyze the sample files with the 84 identified lipid compounds, you must convert the files to CEF files and import them into MPP. See **Chapter 4**.

1 To see the menu item for creating CEF files for export, click File.

File	Method View Identify Features	Web	Mass Searc	:h Help	
1	Create Project				
	Open Project				
	Edit Project		RT (DB)	CCS (DB)	lon Species
	Save Project		5.928	323.44	(M+NH4)+
	Save Project As		5.169	321.25	(M+NH4)+
	Export Feature Summary	•	.712	284.77	(M+H)+
		,	4.518	318.69	(M+NH4)+
	Export Target MS/MS Inclusion List	•	5.123	316.32	(M+NH4)+
	Export TSV File	•	.151	230.99	(M+H)+
	Export Composite-Compounds CEF	•	5.969	318.86	(M+NH4)+
	Export Each Sample to CEF		5.159	326.2	(M+NH4)+
			5.89	328.12	(M+NH4)+
	Export Each Sample to Spectrum Mill		.048	238.7	(M+H)+
	Launch Standalone ID Browser		6.915	324.68	(M+NH4)+
	Exit		4.478	323.73	(M+NH4)+
L			000	222.10	/M.10.

💹 Agilent MassHunter Mass Profiler -- Method: eme\_find lipids -- Project: eme\_find lipids

Figure 56. File menu

#### 2 Select Export Each Sample to CEF.

A dialog box appears asking if you want to average sample values.

Options	$\times$
In each export file, do you want to replace measured RT, DT, CCS, m/z values with average values across all the samples?	
<u>Y</u> es <u>N</u> o	

Figure 57. Dialog box for averaging sample values

3 Click No.

A dialog box appears locating the CEF files.

×
CEF files have been exported to C:\Agilent\MPP\Ion Mobility\Data\2_Copy for Testing\Nist0_1017 folder.
OK

Figure 58. Dialog box for locating the CEF files

4 Click OK.

You can now import and analyze the files in MPP, as described in Chapter 4.

## 4 Analyzing Lipids

This chapter takes you through the tools you need to compare lipid classes in different sample sets using Agilent Mass Profiler Professional.

#### **Create Lipidomics Experiment**

Step 1. Create new project Step 2. Create new experiment

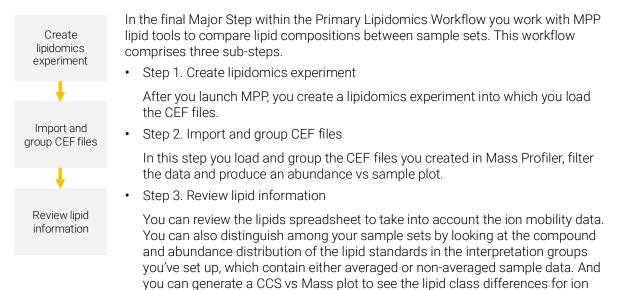
#### **Import and Group CEF Files**

Step 1. Select data to import Step 2. Group samples Step 3. Filter data

# 63Review Lipid Information7263Step 1. View lipid ion mobility data7264Step 2. Create interpretations7367Step 3. Create a lipid matrix7667Step 4. Plot CCS vs Mass82

68 70





mobility data.

This fifth Major Step, Analyzing Lipids, completes the Primary Lipidomics Workflow.

## Create Lipidomics Experiment

To create a lipidomics experiment you first create a new project and then a new experiment.

# Step 1. Create new project

After launching Mass Profiler Professional (MPP), you set up a project.

#### 1 Launch MPP.

The MPP main window appears with the Startup dialog box.

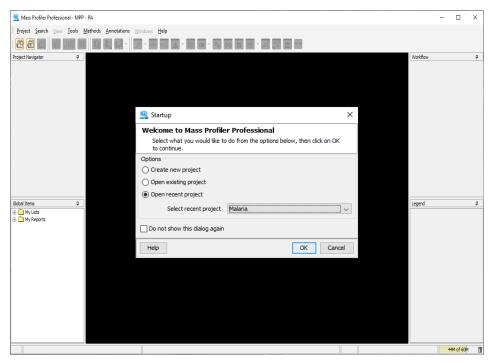


Figure 59. Startup dialog box in MPP main window

2 Select Create new project and click OK.

The Create New Project dialog box appears.

🖳 Create New Project	×
New Project Details	
Name	New Project
Notes	
Help	OK Cancel

Figure 60. Create New Project dialog box

- **3** Type the new project name, *iii\_*IM Lipid Project, where *iii* represents your initials.
- 4 Click OK.

# Step 2. Create new experiment

The Experiment Selection Dialog appears.

Experiment Selection Dialog	×
Choose whether you would like to be guided through the creation of a ner experiment or if you would like to open an existing experiment from a pre- project.	
Choose Experiment  Create new experiment  Open existing experiment	
Неір ОК	Cancel

Figure 61. Experiment Selection Dialog box

1 Click OK to create a new experiment.

The Experiment description page of the New Experiment dialog box appears with no entries.

- 2 Type the **Experiment name** as *iii*\_IM Lipid Expt.
- 3 For the Experiment type, select Lipidomics from the list.
- 4 For the Workflow type, select Data Import Wizard from the list.

You now see the Experiment description page with entries from **step 2** to **step 4** (Figure 62 on page 65).

If you intend to do any statistical analyses on the data, you can select Analysis: Significance Testing and Fold Change from the Workflow type list now, or wait to select it from the Workflow menu when the main window appears after completing the Data Import Wizard. 4 Analyzing Lipids

Step 2. Create new experiment

The instructions that follow assume you have selected Data Import Wizard as the Workflow type.

🖳 New Experiment		×
Experiment description		
a statistical significance test and fold char	type and a desired workflow type. "Analysis" will guide you through ige analysis. "Data Import" will guide you through experiment you through the creation and testing of a prediction model, using	
Experiment name	eme_IM Lipid Expt	
Analysis type	Mass Profiler Professional	~
Experiment type	Lipidomics	~
Workflow type	Data Import Wizard	] ~
Experiment notes		
Help	OK Cance	:

Figure 62. Experiment description with entries

5 Click OK.

4 Analyzing Lipids

Step 2. Create new experiment

The first step of the Experiment Creation Wizard appears.

MS Experiment Creation	1 Wizard (Step 1 of 11)	×
Choose a data source and Data can be imported from	sm and Data to Import d an organism. M files or samples from existing experiments. format, specify if the data is in log2 or log10 scale.	
MassHunter Profinder Archi     MassHunter Qual (.CEF)	ve (PFA)	
⊖ Generic		
Organism	None	
Туре	Selected files and samples	
	Select Data Files Select Samples Remove	
Help	< <back next="">&gt;&gt; Einish Can</back>	cel

Figure 63. Step 1 of MS Experiment Creation Wizard

## Import and Group CEF Files

After naming and describing the new project and experiment, you go through the Experiment Creation Wizard (Data Import Wizard) to import and group the CEF files you created in Mass Profiler. You then filter the data.

# Step 1. Select data to import

You now work with the Experiment Creation Wizard that appeared after you clicked OK in New Experiment dialog box. You first select the data source, the organism and the data to import, as shown below.

- 1 Select Masshunter Qual (.CEF) as the Data Source.
- 2 For the Organism, select Homo sapiens.
- 3 Click Select Data Files, select the MS1 files in the Nist0\_1017 folder and click Open.

🖳 MS Experiment C	Creation Wizard (Step 1 of 11)	×
Choose a data so Data can be impor	rganism and Data to Import urce and an organism. rted from files or samples from existing experiments. generic format, specify if the data is in log2 or log10 scale.	
MassHunter Profind	er Archive (.PFA)	
MassHunter Qual (.)	CEF)	
Generic		
Organism	Homo sapiens V	
Туре	Selected files and samples	
	101719_PM_nist_0_std_0001.cef	
	101719_PM_nist_0_std_0002.cef	
	101719_PM_nist_0_std_0003.cef	
	101719_PM_nist_2_std_0001.cef	
	101719_PM_nist_2_std_0002.cef	
	101719_PM_nist_2_std_0003.cef	
	101719_PM_nist_4_std_0001.cef	
	101719_PM_nist_4_std_0002.cef	
	101719_PM_nist_4_std_0003.cef	
	Select Data Files Select Samples Remove	
Help	< <back <u="">Next &gt;&gt; <u>Ei</u>nish <u>C</u>an</back>	cel

Figure 64. Step 1 of MS Experiment Creation Wizard with data files

4 Click Next twice.

The Experiment Grouping dialog box appears.

ample Reordering		
	es, select the samples and use the appropriate buttons on the right to move samples up or down. This sample order will be used	
throught out the expe		
	Sample Name	1
	101719_PM_nist_0_std_0001	
	101719_PM_init_0_std_0002	
	101719_PM_nist_0_std_0003	
	101719 PM nist 2 std 0001	
	101719_PM_nist_2_std_0002	
	101719_PM_nist_2_std_0003	1
	101719_PM_nist_4_std_0001	1
	101719_PM_nist_4_std_0002	1
	101719_PM_nist_4_std_0003	1
		1
		C
		0
		/ R

Figure 65. Experiment Grouping (Step 5)

Step 2. Group samples

#### 1 Click Add Parameter.

The Edit Parameter Values dialog box appears.

- 2 For the **Parameter name**, type Standard Concentration.
  - You now group the nine samples into sets, each with a different standard. See **Figure 66** on page 69 for the entries given below.
- 3 Select the first three samples with no standards, and click Assign Values.
- 4 Type No standard and click **OK**.
- 5 Select the second set of samples, and click Assign Values.
- 6 Type Standard 2 and click OK.
- 7 Select the third set of samples, and click Assign Values.

#### 8 Type Standard 4 and click OK.

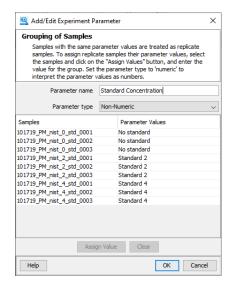


Figure 66. Add/Edit Experiment Parameter dialog box

#### 9 Click **OK** to see a listing of the groups.

MS Experiment Creation Wizard (Step 5 of						
Experiment Grouping						
Experiment parameters define the grouping or button. You may enter as many parameters as parameters can be used in the advanced analy	s you like, but onl	y the first two paramet	ers will be used for an	alysis in the guide		
Displaying 9 sample(	s) with <b>1</b> experim	ent parameter(s). To d	hange, use the buttor	controls below.		
1 in 1 in 1						
Samples			Standa	ard Concentra	tion	
101719_PM_nist_0_std_0001		No stand				
101719_PM_nist_0_std_0002		No stand				
101719_PM_nist_0_std_0003		No stand				
101719_PM_nist_2_std_0001		Standard				
101719_PM_nist_2_std_0002		Standard Standard	-			
101719_PM_nist_2_std_0003 101719_PM_nist_4_std_0001		Standard	-			
101719_PM_nist_4_std_0001		Standard				
101719_PM_mist_4_std_0003		Standard	4			
101/19_PM_INST_4_STG_0003		Standard	4			
101719_PM_nist_4_std_0003	d Parameter	Standard	4 Delete Parameter			

Figure 67. Experiment Grouping after adding groups

## Step 3. Filter data

#### 1 Click Next.

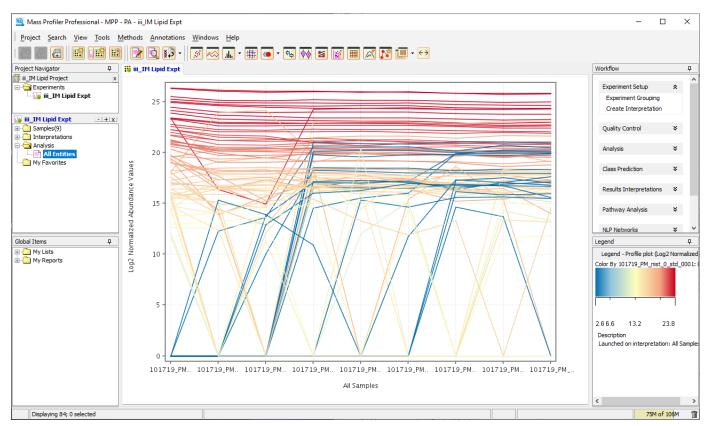
The Filtering step appears. Make the changes below. See Figure 68.

- 2 Clear the Minimum absolute abundance check box.
- **3** Change the **Minimum number of ions** to 1.

MS Experiment Creation Wizard (Step 6 of 11)		×
Filtering Filtering during the data import process may be used to reject lov filtering options that may be applied: Filter by Frequency, Abund is 'Number of Model ions'.		
Abundance	Retention time	
Minimum absolute abundance 5000 counts	🗸 Use all available data	
Minimum relative abundance %	Min RT (1. 1289)	1.1289
Limit to the largest compounds	Max RT (17.9571)	17.9571
Drift time	Mass	
Use All Available Data	🗸 Use all available data	
Min DT (26.1107) 26.1107	Min Mass (343.2707)	343.2707
Max DT (56.1504) 56.1504	Max Mass (914.8304)	914.8304
Number of ions	Charge states	
Single ion compounds only	<ul> <li>All charge states permittee</li> </ul>	đ
	O Multiple charge states requ	uired
Minimum number of ions	O Multiple charge states forb	bidden
Help	<< Back	<u>N</u> ext >> <u>Finish</u> <u>Cancel</u>

Figure 68. Filtering Step after changes

4 Click **Next** four times, then click **Finish**.



The MPP main window appears with an abundance vs sample profile on the display.

Figure 69. MPP main window with log normalized abundance vs samples

For these samples, normalization cannot be set up because internal standards were not included in the study.

You are now ready to move on to the next section, **"Review Lipid Information"** on page 72.

## **Review Lipid Information**

This section shows you how to review the Drift Time (DT), the CCS value and the ion species for each lipid. Once you create interpretations with your experiment groupings, you also learn to use the lipid matrix tool to view the relative abundance of the standards and other lipids in the samples.

# Step 1. View lipid ion mobility data

1 In the top icon toolbar, click the **Spreadsheet** icon,

The spreadsheet with all the sample data appears.

Image: Instructure       Image: Instructure       Image: I	roject Navigator 무	📙 iii_IM Lipid Expt												Workflow	
<b>a</b> IN Lipid Expt <b>a</b> IN Lipid Expt <b>a</b> IN Lipid Expt <b>a</b> IN Lipid Expt <b>a</b> Expension <b>b</b> Expension <b>E</b> Expension </th <th></th> <th>Compound</th> <th></th> <th>11</th> <th></th> <th></th> <th>101710 Linid Class</th> <th>Mass</th> <th>Retention Time</th> <th>DT</th> <th>200</th> <th>Ion Species</th> <th></th> <th></th> <th></th>		Compound		11			101710 Linid Class	Mass	Retention Time	DT	200	Ion Species			
Implementation       TG 16 0, 18 1, 18 2       Implementation       Stander(3)       15, 101       43, 430       320, 49       Mathematic       Experimentation         Implementations       Implementations       TG 16, 0, 16, 0, 18 1, 18       Implementations       24, 437 TG       830, 7256       15, 003       810, 003       18, 100 <td< td=""><td></td><td>•</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>1</td><td>Experiment Setup</td><td>*</td></td<>		•											1	Experiment Setup	*
iiii 111 lipid Expt - i+ixi D surgles(9)       TG 16 0.16 0.20 / 4													^	Experiment Grouping	
III. Includ Expt       - + + x.       TG 14 0, 18 1, 18:1       A       24 4628 TC       830, 7356       15.058       42, 7273       315.79       IN+H4N +         Sample(9)       TG 16 0, 16:0.18:1       24.407 TC       832, 7356       15.058       42, 7273       315.79       IN+H4N +         TG 16 0, 16:0.18:1       24.434 ST G       884, 7819       15.884       43.100       318.90       327.14       IM+H4N +         Analysis       TG 16 0, 16:0.18:1       23.813 TG       684, 7819       15.884       43.100       318.90       237.81       M+H4N +         TG 18 0, 18:0.18:1       23.825 IPC       52.3625       4.074       318.90       237.81       M+H4N +         TG 16:0.16:0.16:1       22.768 TG       860.7819       16.854       43.920       324.06 (M+H4N)+       Participae       Participae       Participae       Participae       11.15 (M+H4N)+       Participae														Create Interpretation	
Damples(P)         To 16 / 0.16 / 16 / 16 / 16 / 16 / 16 / 16 / 16 /	iii TM Linid Evot														
Interpretations         Analysis           Analysis         TG 18:0.18:1.18:1.18:1														Quality Control	¥
Analysis       TG 150.161.182       0.00000000000000000000000000000000000														Quality Control	*
Di All futtues         Di Ococco RT: 4 074         24.355 LPC         22.3 ce         4.074         31.890         227.81 [M+H]+         M+H+           My Pavorites         TG 6.0, 18.0 1.81.           23.238 [G         860.7819         16.854         43.920         324.08 [M+H4N]+         16.854         13.990         237.81 [M+H]+         16.91.61         16.01.61         16.01.61         22.766 [FG         880.7514         14.722         43.863         323.55 [M+H4N]+         Results Interpretations           TG 5.6.0.16.1.16.1           22.766 [FG         804.7200         15.035         42.139         311.27 [M+H4N]+         Results Interpretations           TG 16.0.16.1.16.1            22.064 [FG         804.7200         15.035         42.139         311.27 [M+H4N]+         Results Interpretations           TG 16.0.16.0.16.1            19.965 [LPC         523.3625         3.784         32.029         328.162 [M+H4N]+         Pathway Analysis           TG 16.0.16.0.16.0             13.976 [M+H4N]+         30.68 [M+H4N]+         Pathway Analysis           My Lists         TG 16.0.16.0.16.0			•••• •••• •												
My Favorites       TG 16:0,18:0,18:1       0       23 288 TG       660 7810       16 854       43 920       324.08 [M+HN]+         TG 56:0,18:0,18:1       0       23 043 TG       886 7977       16.816       44.512       328.30 [M+HN]+         TG 56:0,18:0,16:1,16:1       0       22 078 TG       880 7977       16.816       44.512       328.30 [M+HN]+         TG 16:0,16:0,16:1       0       22 776 TG       800 7200       15.035       42.139       311.27 [M+HN]+         TG 16:0,16:1,16:1       0       22 464 TG       802 7045       14.363       41.818       30.802 [M+H+N]+         TG 18:0,16:0,16:0       0       22 044 TG       826 7042       13.916       42.142       311.16 [M+HN]+         PC 18:0,016:0,16:0       0       21.095 TG       826 7358       13.953       43.399       320.214 [M+HN]+         TG 14:0,18:0,18:0       0       21.095 TG       806 7358       15.387       43.213       318.95 [M+HAN]+         My Reports       TG 44:3       0       0.204 710 TG       800 6890       13.859       41.514       30.668 [M+HAN]+         My Reports       TG 14:0,18:0,18:0       0       0.204 81 TG       834 7664       16.912       43.239       319.21 [M+HAN]+         TG 14:0,1														Analysis	¥
al Items       q       TG 54:2															
al Items       q       IC 54:5       I       IC 22:768 TG       880.7514       14.722       43.863       323.55 [M+HA]]+         PC 32:1       III.       IIII.       IIII.       IIII.       IIII.       IIII.       IIII.       IIII.       IIIIII.       IIIIIII.       IIIIIIII.       IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	in My Pavontes													Class Prediction	¥
TG 16:0.16:0.16:1															
Image: Constraint of Constr															~
P C 32:1														Results Interpretations	×
TG 16:1,16:2,18:1															
IPC 18: 0/00         RT:3.784         I         19: 956 (IPC         523: 3625         3.784         32. 029         238: 85 (IH+H)+           al Items         TG         16: 2.18: 2.18: 2.18: 2         IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII											281.42	(M+HJ+		Pathway Analysis	¥
TC 18:2_18:2_18:2       N.       21900TG       878,7350       13.953       43.399       320.14 [M+HA]]+         al Items       TG 16:0_16:0       N.       21.085 TG       806,7358       15.925       42.333       312.69 [M+HA]]+         My Lists       TG 48:3       N.       21.019 TG       800,6890       13.953       43.399       320.14 [M+HA]]+         My Lists       TG 48:3       N.       21.019 TG       800,6890       13.859       41.514       300.68 [M+HAN]+         TG 14:0_18:0_19:2       N.       20.831 TG       844.7516       15.387       43.239       319.21 [M+HAN]+       Legend         LPC 0:0/20:4       N.       20.835 TG       844.7516       15.387       43.239       312.51 [M+HAN]+       Legend - Spreadsheet (Log2         TG 16:0_18:3_18:3       N.       20.621 TG       776.6887       14.336       41.205       304.55 [M+HAN]+       Legend - Spreadsheet (Log2         TG 16:0_12:2:2:2:2:1:1:1:1:1:1:1:1:1:1:1:1:1:1:															
al Items       TG 16:0,16:0       16:0,16:0       12:1900[TG       8/8.7330       13:933       43:399       32:0.14[[H+HH]]+         My Lists       TG 46:0,16:0,16:0         21:095TG       806.7358       15:925       42:333       31:2:09[[H+HH]]+         My Lists       TG 46:0,18:0,18:0          20:835TG       844.7516       15:327       43:213       318.95       [M+HAN]+         TG 14:0,18:0,18:0          20:835TG       844.7516       15:387       43:213       318.95       [M+HAN]+         LPC 0:0/20:4            20:835TG       844.7516       15:387       43:213       318.95       [M+HAN]+         TG 16:0,18:3,18:3            20:835TG       850.7045       13:635       42:598       314:39[[M+HAN]+         TG 16:0,18:3,18:3            20:93TG       850.7045       13:635       42:598       21:49[[M+H]+       16       16:19:197       TG 16:0,20:42:04         20:93TG       774:6731       13:835       40:912       30:41[[M+H]+       17														NI D Networks	¥
My Lists My Reports         TG 48:3															Ý
TG 14:0_18:0_18:0														Legend	
My Reports         IG 14:0_18:0_18:0         IIG         20.814   IG         844.7664         16.912         43.239         319.21 [M+H4N]+           IG 14:0_18:0_19:2														Legend - Spreadsheet (Log2	Norr
LPC 0:0/20:4	My Reports														
TG 46:1															
TG 16:0_18:3_18:3															
TG 53:2															
IPE 17:1       RT:2.933       DT:															
TG 16:0_20:4_20:4															
TG 14:1_16:0_16:1															
CE 20:4         RT:15.508         DT         Image: Margin and Margin															
TG 16:0_16:0_19:1           19.821TG         846.7670         16.288         43.480         320.92         [M+H4N]+           TG 16:0_16:0_17:1           19.882TG         818.7360         15.395         42.610         314.66[[M+H4N]+           SM d33:1           20.510SM         688.5515         8.418         37.890         280.79[[M+H]]+           TG 50:5             19.562[TG         824.6893         13.572         41.950         309.76[[M+H]N]+															
TG 16:0_16:0_17:1															
SM d33:1              20.510 SM         688.5515         8.418         37.890         280.79 [M+H]+           TG 50:5             19.562 TG         824.6893         13.572         41.950         309.76 [M+H4N]+															
TG 50:5			··· ··· ·												
PC 33:2			.				20.074 PC	743.5463	9.366	38.084	281.83		¥		

Figure 70. Spreadsheet View of the main MPP window

2 Note the Drift Time (DT), CCS values and ion species for each lipid.

Each ion species listed is the one whose CCS has been calculated for the corresponding lipid.

To add columns to the Spreadsheet View, right-click the **All Entities** item in **Project Navigator** on the left and then select **Inspect List**.

# Step 2. Create interpretations

An interpretation is a grouping of experiment conditions for display and analysis. In this step you create two interpretations -- averaged and non-averaged.

## 1 In the Workflow menu under Experiment Setup, click Create Interpretation.

Step 1 of the Create Interpretation wizard appears.

Create Interpretation (Step 1 of 4)				>
Select parameters An Interpretation specifies how samples will be grou analysis. Select the parameter(s) to group samples grouped into an experimental condition.				
elect experiment parameters				
elect experiment parameters Standard concentration				
✓Standard concentration				
Select all				
/ Select all				
Help	<< Back	Next >>	Finish	Cancel

Figure 71. Step 1 of the Create Interpretation wizard - Select parameters

2 Click **Next** until you reach the next to last page (Step 3).

Create Interpretation (Step 3 of 4)	$\times$
Select conditions Select the conditions defined by the selected parameter(s) to include in the interpretation. Samples within a condition are considered as replicates. If Averaged, for each entity, the average intensity value across replicates will be used for visualization and analysis. For experiments with flags, only measurements with permitted flags will be used for visualization and analysis.	
Unselect conditions to exclude	
∑[Std 0] ∑[Std 2] ∑[Std 4]	
Select all Average over replicates in conditions	
Averaged	
Non-Averaged © Both	
Use Measurements flagged ☐Present ☐Marginal ☐Absent	
Help <<< Back Next >> Einish Cancel	

Figure 72. Step 3 of the Create Interpretation wizard - Select conditions

Notice that both Averaged and Non-Averaged options have been selected. This selection of default settings creates two separate interpretations.

**3** Click **Next** to reach the last page (Step 4).

Create Interpretation (Step 4 of 4)			×		
Save Interpretation This page displays the details of the	e interpretatior	n created.			
Objects		N	ame	Standard concentra	tion
Standard concentration Standard concentration (Non-averaged)		N	otes		
		Creation	late	Mon Mar 30 20:42:2	10 PDT 2020
	L	ast modified (	late	Mon Mar 30 20:42:2	10 PDT 2020
		Ov	vner	gxuser	
	Average ove	r replicates in	со	Yes	
	Parameters	Conditions	Use N	Aeasurements Flagge	d
	Paramet	er Name	Pa	arameter Type	Display Mode
	Standard c	oncentra	Non	Numeric	Categorical
Help		<< B	ack	<u>N</u> ext >>	<u>Finish</u>

Figure 73. Last Step of Create Interpretation wizard - Save Interpretation

4 Click Finish.

Step 2. Create interpretations

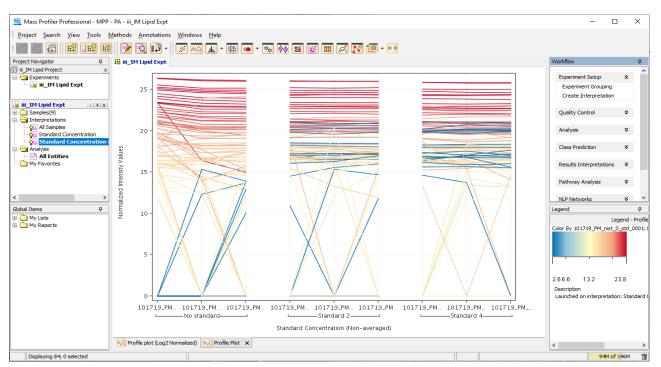




Figure 74. MPP main window - non-averaged interpretation, Standard concentration (Non-averaged)

# Step 3. Create a lipid matrix

To view the abundance of different lipids, you use the lipid matrix tool.

1 In the Interpretation list, double-click the averaged interpretation, **Standard concentration**.

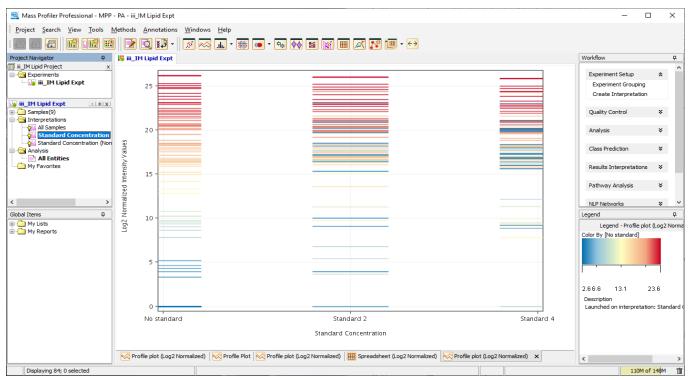
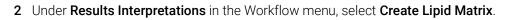


Figure 75. MPP main window - averaged interpretation, Standard concentration



Step 3. Create a lipid matrix

nput Parameters			
Select entity list and interpretation.			
Entity List	All Entities		Choose
Interpretation	Standard concentration		Choose
Help		<back next="">&gt;</back>	Einish Cancel

Step 1 of the Create Lipid Matrix wizard appears.

Figure 76. Create Lipid Matrix wizard - Input Parameters

The interpretation you see is the interpretation highlighted in the Project Navigator, which in this case is the averaged Standard concentration.

## 3 Click Next.

Step 2 of the wizard appears, which displays 3 graphics showing relative abundance in the sample sets. The first that appears is the Sum Composition.

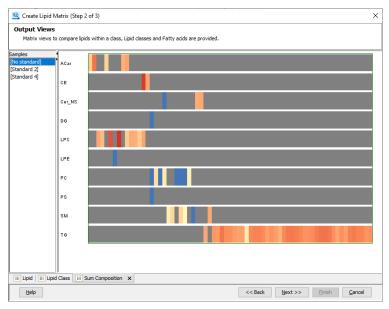


Figure 77. Create Lipid Matrix wizard - Output Views - Sum Composition

To see these graphics more clearly, you will view them in the main window.

4 Click Next.

The Save Lipid Matrix dialog box appears.

🚇 Create Lipid Matrix (Step 3 of 3)	×
Save Lipid Matrix This window displays the results for the Lipid Matrix views or	eated.
Name	Lipid Matrix on Standard concentration
Notes	Created from Entity List: Al Entities Interpretation: Standard concentration
Creation date	Tue Mar 31 09:17:04 PDT 2020
Last modified date	Tue Mar 31 09: 17:04 PDT 2020
Owner	gxuser
Technology	Lipidomics_MassHunterQual.IDENTIFIED_UNIDENTIFIED_COMPOUNDS.eme_IM lipid expt_2020_1
Help	< <back next="">&gt; Einish Cancel</back>

Figure 78. Save Lipid Matrix dialog box

5 Click Finish.

The main window appears with the Lipid display.

Step 3. Create a lipid matrix



## 6 From the Lipid Classes list, select PC.

Figure 79. MPP main window - Lipid display for averaged concentration interpretation of the PC lipid class

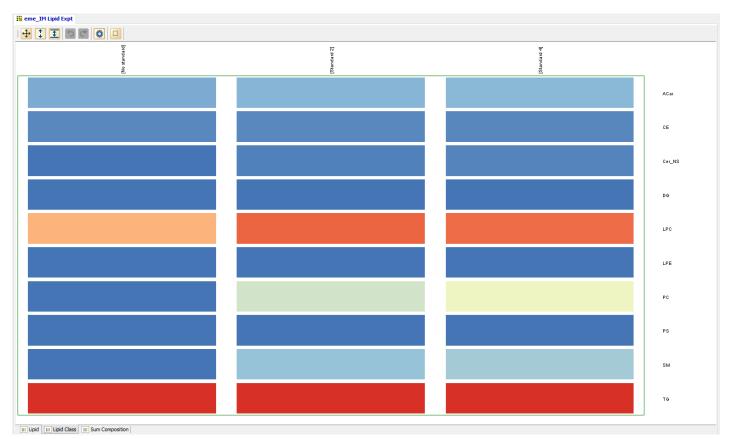
This graphic display shows the different levels of abundance in the standardspiked samples for each of the lipids in the PC class. Blue means less abundance. Red means strong abundance, and the colors between blue and red mean increasing degrees of abundance, from yellow to orange to red. See the Legend to the right of the matrix.

7 To see the full name of a lipid in the matrix and its abundance, pass the mouse over the rectangle of interest.

Notice that for the standard lipid PC 14:1\_14:1, the abundance increases from no standard > standard 2 > standard 4.

8 Click the Lipid Class tab.

Step 3. Create a lipid matrix



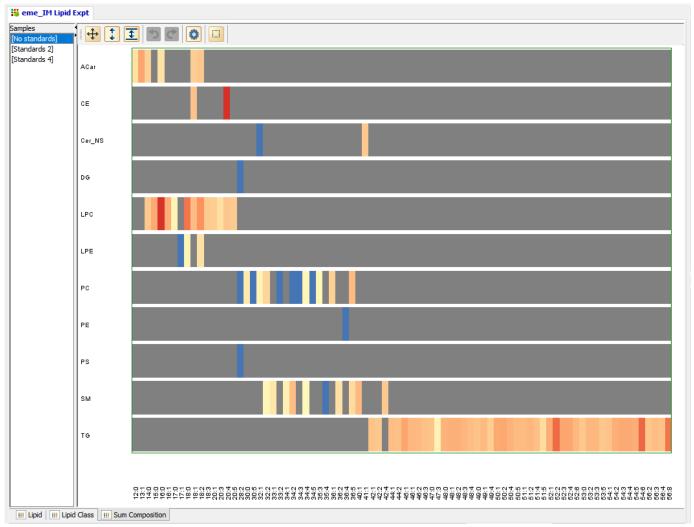
A different graphical display appears.

Figure 80. MPP main window - Lipid class display for averaged concentration interpretation

Even though there is one lipid standard spiked and found for the PC and SM classes, the results are muted because in this graphic you are viewing the results for all the lipids together in the class. Blue means less abundance, yellow or orange means some abundance, and red means strong abundance. See the Legend to the right of the matrix.

9 Click the Sum Composition tab.

Step 3. Create a lipid matrix



Yet another graphical display appears.

Figure 81. MPP main window - Sum Composition display for an averaged concentration interpretation

The sum composition shows the abundance (log of normalized abundance) of each lipid in a class for each sample set. Again, see the Legend to the right of the matrix for the color meanings.

The x-axis shows the number of carbons in the acyl tail groups for each lipid in a class. For example, the lipids in the ACar class have fewer carbons in their tails than the lipids in the other classes, and the lipids in the TG class have more carbons than the others, primarily because they have 3 tails and the ACar lipids have only one.

Also notice that PC 28:2 increases its abundance from No standard > Standard 2 > Standard 4.

## Step 4. Plot CCS vs Mass

In addition to viewing lipid and lipid class relationships in MPP, you can generate plots with ion mobility data.

In this step you learn how to generate a CCS vs Mass plot.

1 In the MPP main window, click the **Mass vs RT Plot** icon,



The plot display appears in the MPP window.

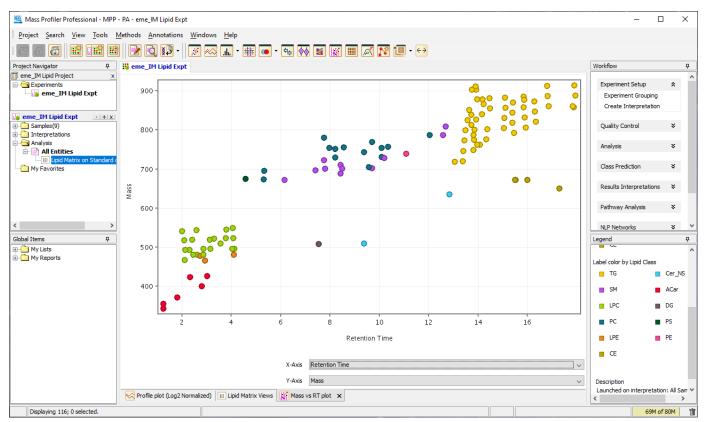
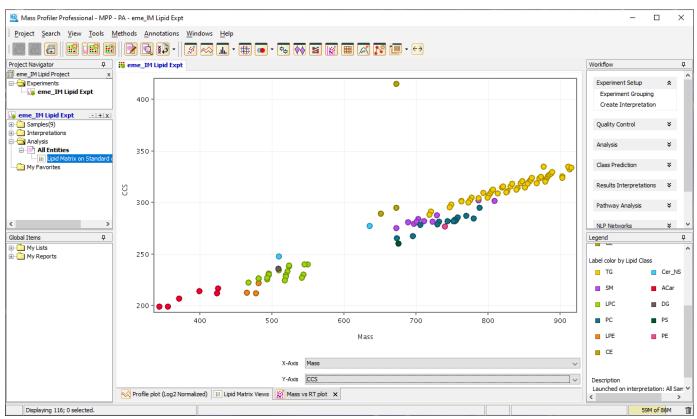


Figure 82. Mass vs RT plot in MPP window

- 2 From the X-Axis list, select Mass.
- 3 From the Y-Axis list, select CCS.



You now see the CCS vs Mass plot.

Figure 83. CCS vs Mass plot in MPP main window

ΝΟΤΕ

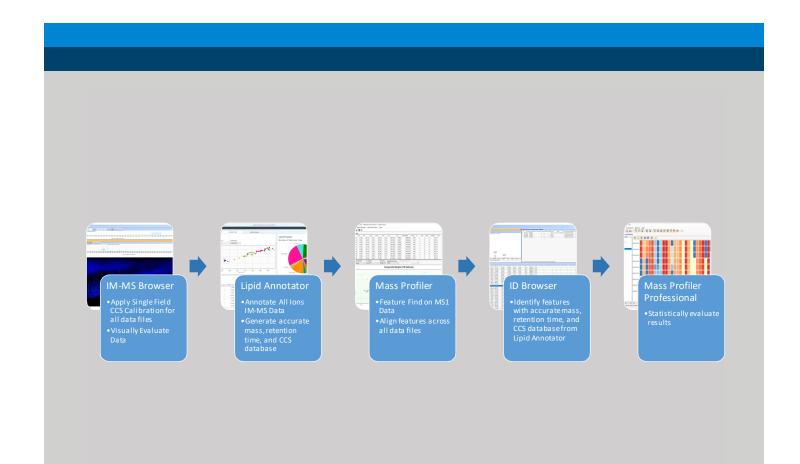
Each lipid class is color-coordinated according to the legend on the right. Note how the lipids are grouped together according to class and structure (CCS values).

This page intentionally left blank.

## 5 Reference Information

This chapter consists of definitions of ion mobility, lipidomics and software terms, as well as references to Agilent publications that can help you use Agilent products to perform lipidomics analyses.

Definitions	86
Resource Apps	94
Agilent Web Site	94
Agilent Community	94



Definitions	Review of the terms and definitions presented in this section helps you understand the Agilent software wizards and the lipidomics workflow. Because this guide does not show you MPP statistical tools, refer to the <i>Agilent Metabolomics Workflow</i> <i>Guides</i> mentioned in <b>"Resource Apps"</b> on page 94 for these definitions.
Abundance	When mentioned in this guide, it usually refers to the log normalized abundance, or concentration, of the identified lipids in the sample set.
Alignment	Adjustment of the chromatographic retention time of eluting components to improve the data correlation among data sets, based on the elution of specific component(s) that are (1) naturally present in each sample or (2) deliberately added to the sample through spiking the sample with a known compound or set of compounds that do not interfere with the sample.
All lons data	Ion mobility data files containing both MS1 and fragmentation data, an option specified for acquisition. All Ions data is used in Lipid Annotator because the fragmentation data is necessary for lipid annotation. See <b>"MS1 data"</b> on page 91.
Beta and TFix values	Constants similar to the slope and intercept of a straight line, determined with tune- mix data and used to determine CCS values for either All lons data or MS1 data.
Biomarker	An organic compound that is an indication of a biological state and which by analytical measurement of its presence and concentration in a biological sample indicates a normal or altered function of higher level biological activity.
Calibration	Using a standard with known accuracy to determine the values of other compounds present in the sample.
CCS	Collision cross section (CCS) is the rotationally averaged surface area of the ion. The measured drift time of an ion from an ion mobility experiment is used along with other experimental parameters (temperature, pressure, drift tube length and voltage), information about the ion (mass and charge) and buffer gas (mass and number density) to calculate the CCS value. CCS values serve as additional descriptors in analytical workflows, adding not only more specificity, but also unique structural information about the ion that is not reflected in retention time or mass from traditional LC-MS experiments.
CEF file	A binary file format called a Compound Exchange Format (CEF) that is used to exchange data between Agilent software programs and between Agilent programs and third-party programs. In the lipidomics workflow, CEF files are used to share molecular features between Mass Profiler and Mass Profiler Professional.
Charge carriers	Specific ions, either positive or negative, such as +NH <sub>4</sub> or -Cl.
Charge state	Actual charge, either +N or -N, where N is the number of the charge.
Composite sample	In Mass Profiler, features from all the samples plotted together on the X-Y plot (i.e., m/z vs RT) are called the "composite sample".

## 5 Reference Information Definitions

Compound	A lipid that may be individually referred to as a compound, feature, element, or entity during the various steps of the lipidomics data analysis.
Condition	Another term for one of several values within a parameter for which exist correlating samples. Condition may also be referred to as a parameter value during the various steps of the lipidomics data analysis.
Database	A saved set of information that includes all of the important qualities of a feature used to identify a feature as a particular compound. One of the lipidomics workflow steps is to create a custom lipid database for identifying unknown lipids.
Demultiplexed data	As you set up to acquire ion mobility data, you can select either standard IM mode or multiplexed IM mode. To take multiplexed data through the lipidomics workflow you must first demultiplex the data. This process combines the signals from the multiple ion pulses, which increases the sensitivity of the measurement. Also see <b>"Multiplexed data"</b> on page 91 and <b>"Standard-mode data"</b> on page 93.
Dependent variable	An element in a data set that can only be observed as a result of the influence from the variation of an independent variable. For example, in a typical discovery experiment the lipid profiles of a set of human serum samples make up the dependent variables of a study whose independent variable is the disease state.
Element	A lipid that may be individually referred to as a compound, feature, element, or entity during the various steps of the lipidomics data analysis.
Entity	A lipid that may be individually referred to as a compound, feature, element, or entity during the various steps of the lipidomics data analysis.
Experiment	Data acquired in an attempt to understand causality, where tests or analyses are defined and performed on an organism to discover something that is not yet known, to demonstrate proof of something that is known, or to find out whether something is effective.
Experiment grouping	Grouping samples into sets with the same qualities, conditions or treatments.
Externality	A quality, attribute, or state that originates and/or is established independently from the specimen under evaluation.
Extraction	The process of retrieving a deliberate subset of data from a larger data set whereby the subset of the data preserves the meaningful information as opposed to the redundant and less meaningful information. Also known as data extraction.
Feature	Independent, distinct characteristics of phenomena and data under observation. Features are an important part of the identification of patterns within the data, whether processed by human intelligence, or artificial intelligence programs such as Agilent MassHunter Mass Profiler and Agilent Mass Profiler Professional. In lipidomics analysis a feature is a lipid and may be individually organized (extracted) by mass, abundance and CCS values before it is identified.

Feature extraction	The reduction of data size and complexity through the removal of redundant and non-specific data by using the important variables (features) associated with the data. Careful feature extraction yields a smaller data set that is more easily processed without any compromise in the information quality. This is part of the filtering and statistical analysis processes employed by Agilent Mass Profiler for the lipidomics workflow.
Frame	In ion mobility mass spectrometry, data acquisition is the ultra-high-speed signal digitization in the detector, which acquires arrival-time spectra, called transients, at a rate of one per 120 usec. To capture the speed of peaks being separated by the ion mobility drift tube, it is necessary to record each transient individually. A full drift spectrum of 60 msec is the result of 500 TOF transients, often referred to as the Frame transient. The acquisition system then sums Frame transients until the period specified by the Frame data rate for saving the file is reached. The mass and drift time data within this period is known as the Frame. See the <i>Agilent 6560 Ion Mobility LC/QTOF Fundamentals Guide</i> for a visual explanation of a Frame.
Frame extraction	The process of summing the signals within a selected region of the Frame as implemented in the IM-MS Browser.
Filtering	The process of establishing criteria by which entities are removed (filtered) from further analysis during the lipidomics workflow.
ID Browser	Agilent software that annotates the feature lists by assigning compound names based on database searching and spectral matching. It can be launched from either Mass Profiler or Mass Profiler Professional to assign compound names to features.
Identified compound	Chromatographic components that have an assigned identity, such as compound name and molecular formula, based on prior assessment or comparison with a database. See also <b>"Unidentified compound"</b> on page 93.
IM-MS Browser	Agilent software that displays and lets you analyze ion mobility data.
IM-MS Reprocessor	Agilent software designed to help you correct mass calibration in ion mobility data.
Independent variable	An essential element, constituent, attribute, or quality in a data set that is deliberately controlled in an experiment. For example, in a typical discovery experiment the lipid profiles of a set of human serum samples make up the dependent variables of a study whose independent variable is the disease state. An independent variable may be referred to as a parameter and is assigned a parameter value during the various steps of the lipidomics data analysis.
Interpretation	Groupings of samples based on the experimental parameters. By default in MPP, when you open an experiment, the "All Samples" interpretation is active. You can click another interpretation to activate it. To learn how to create an interpretation, see <b>"Step 2. Create interpretations"</b> on page 73.

## **Reference Information** Definitions

Ion mobility	An analytical technique that separates ions based on their mobility through a drift gas. For drift tube IM, the velocity of the ion is the result of counteracting forces. The driving force is an electric field established along the length of the drift tube. The decelerating force arises from collisions with the drift gas within the tube, affected by the ion's shape and size, as well as by the number density of gas molecules in the drift tube. Ions that manifest a higher velocity have higher ion mobility.
Isotope model	The kind of isotopic envelope pattern a given data set may contain. The isotope model controls how the assignment algorithm uses peak abundances in addition to peak spacing when it tries to group peaks into isotope clusters (from which it can then determine the charge state). For example, an isotope model could be "Common organic compounds" or "Common organic (No Halogens)". See " <b>Method</b> " on page 91.
Lipid Annotator	Agilent software designed to annotate lipids based on matching fragmentation data with theoretical fragmentation spectra. Lipid Annotator provides a pie chart summary of lipid classes present in the sample and creates a custom lipid database, or PCDL.
Lipids	Lipids are a very broad, diversified group of hydrophobic or amphipathic (both hydrophobic and hydrophilic) small molecules that are critically involved in maintaining structural integrity of cellular membranes, serve as cellular energy stores and are also key intermediates of several signal transduction pathways. The amphipathic nature of some lipids lets them form structures such as vesicles, liposomes, or membranes in an aqueous environment. These lipids are <b>named</b> according to their "head group" component and their acyl tails.
	<b>Lipid class</b> Categories of lipids organized by their component compounds. Hydrophobic lipids, such as Cholesterol, belong in their own class. Amphipathic lipids contain the same backbone or "head group" component, such as Carnitine, Glycerol or Sphingosine, and 1-3 long-chain acyl groups from fatty acids attached to an alcohol group or an amine on the "head group". Some examples of lipid classes for these three "head groups" are Acylcarnitine (ACar), Triacylglycerol (TG) Ceramide Non-hydroxy Fatty Acid Sphingosine (CerNS).
	<b>Lipid constituent</b> A lipid constituent of a lipid class is a lipid whose name includes its class abbreviation and the individual number of acyl chains, carbons and double bonds. For example, the name PC 16:0_18:2, indicates this constituent is a phosphatidylcholine with two attached acyl tails, one of 16 carbons and no double bonds and one of 18 carbons and 2 double bonds. TG 16:0_16:1_18:1 is a triacyl glycerol with 3 acyl tails, one with 16 carbons and no double bonds. and one double bond and one with 18 carbons and one double bond.
	<b>Lipid sum composition</b> A lipid or set of lipids can also be identified by their class and the total number of carbons and double bonds in the acyl chain or chains their sum composition. For example, PC 34:2 is a sum composition of phosphatidyl- choline with a total of 34 carbons and two double bonds in the attached acyl groups. Another example of a sum composition is TG 50:2, which has a total of 50 carbons and 2 double bonds in the attached acyl groups.

	For a given sum composition one or multiple lipid constituents are possible based on evidence in the fragmentation spectra. These are listed in the bottom table on the Match Details view of Lipid Annotator. If only one lipid constituent is listed, evidence for only those specific fragment ions was found in the fragment spectra (e.g., for PC 34:2 if only PC 16:0_18:2 is listed, then only fragments for the acyl groups 16:0 and 18:2 were found). If multiple constituents are listed, then multiple fragment ions were detected in the fragment spectra for all the acyl groups (e.g., for TG 50:2 if TG 16:0_16:1_18:1, TG 15:0_17:1_18:1 and TG 16:0_16:0_18:2 are listed, then fragments were detected for the 15:0, 16:0, 16:1, 17:1, 18:1, and 18:2 acyl groups).
	The percentages in the table represent the comparative abundance of each of the unique fragment ions, and the dominant constituent is the one present in the greatest abundance.
	<b>Lipidome</b> The complete set of lipids found within a biological sample.
	<b>Lipidomics</b> Identification and quantification of cellular lipids from an organism in a specified biological situation. The study of lipids is a subset of metabolomics.
Mass Profiler	Agilent software for extracting features from data files. This software serves as the batch processing software tool for ion mobility data. Once extracted, the features can be identified using the embedded ID Browser software tool. Mass Profiler also supports differential analysis for two sample groups and exports CEF files for more statistical analysis in MPP.
Mass Profiler Professional (MPP)	Agilent software with many statistical and display tools for analyzing identified and unidentified compounds. Its lipid matrix tool is especially useful for comparing abundances of compounds within and between lipid sample sets of differing qualities, conditions or treatments.
Mass calibration	In mass spectrometry, tuning compounds are used to adjust the mass scale and relative intensities of the mass spectral peaks.
Mass variation	The mass to charge (m/z) resolution of the mass spectral data enables the identification of compounds with nearly identical, or identical, chromatographic behavior by adjusting the m/z range for extracting ion chromatograms.
Mean	The numerical result of dividing the sum of the data values by the number of individual data observations.
Metabolism	The chemical reactions and physical processes whereby living organisms convert input compounds into living compounds, structures, energy and waste.
Metabolites	Small organic molecules that are intermediate compounds and products produced as part of metabolism. Metabolites are important modulators, substrates, by- products and building blocks of many different biological processes. They are typically in the range of 50 to 600 Da.

**Metabolome** The complete set of small-molecule metabolites that may be found within a biological sample.

	<b>Metabolomics</b> The process of identification and quantification of all metabolites of an organism in a specified biological situation. The study of the metabolites of an organism presents a chemical "fingerprint" of the organism under the specific situation. See " <b>Metabonomics</b> " for the study of the change in the metabolites in response to externalities.
	<b>Metabonomics</b> The metabolic response to externalities such as drugs, environmental factors, and disease. The study of metabonomics by the medical community may lead to more efficient drug discovery and to individualized patient treatment. Meaningful information learned from the metabolite response can be used for clinical diagnostics or for understanding the onset and progression of human diseases. See <b>"Metabolomics"</b> for the identification and quantitation of metabolites.
Method	A procedure using specified, but modifiable, values of known parameters to process data.
MS1 data	Data file containing only MS level data (i.e., no fragmentation data).
Multiplexed data	IM data can be acquired in multiplexed mode, which is specified during data acquisition. In multiplexed mode multiple packets of ions are sent into the drift tube for each frame vs. a single packet during standard mode. Both 3-bit and 4-bit multiplexing are available options that result in either 4 or 8 packets of ions per frame. (See <b>"Frame"</b> on page 88.) Trap fill time must be reduced to 3-4 ms to fit the multiple packets within a typical maximum drift time frame of 60 ms. Also see <b>"Demultiplexed data"</b> on page 87 and <b>"Standard-mode data"</b> on page 93.
Multi-omics	Biological analysis approach in which the data sets are multiple "omes", such as the genome, proteome, metabolome or lipidome. In other words, it is the use of multiple "omics" technologies to study life in a concerted way. By combining these "omes", researchers analyze complex biological big data to find novel associations between biological entities, pinpoint relevant biomarkers and build elaborate markers of disease and physiology. Ion mobility is an excellent tool for multi-omics studies because it provides an additional dimension of separation for these complex samples. Additionally, each "omics" class of compounds contains distinct structural characteristics that manifest in class separation on the plot of CCS values vs. mass.
Parameter	Another term for an independent variable. Referred to as a parameter or parameter name and is assigned a parameter value during the various steps of the lipidomics data analysis.
Parameter value	Another term for one of several values within a parameter for which exist correlating samples. Parameter value may also be referred to as a condition during the various steps of the lipidomics data analysis. See also <b>"Condition"</b> on page 87.

## 5 Reference Information Definitions

PCDL	PCDL stands for Personal Compound Database and Library. Agilent has designed a number of programs for creating and managing PCDL's, including Pathways to PCDL, PCDL Manager and Lipid Annotator. PCDL's are then used for database searching and spectral library matching with the Agilent ID Browser software.
PNNL Preprocessor	A software program designed by the Pacific Northwest National Laboratory (PNNL) in conjunction with Agilent for the purposes of preprocessing IM data prior to data analysis. Smoothing, saturation repair, and demultiplexing are a few of the techniques available in the PNNL Preprocessor.
Polarity	Positive or negative mode used for mass spectral data acquisition.
Quality	A feature, attribute, and/or characteristic element of a sample. The quality's analytical result shows the sample is representative of the larger specimen to a high degree of certainty.
Recalibration	If an application requires the best possible mass accuracy, a recalibration may need to be applied to the data file. To perform a recalibration, reference mass ions must be present throughout the entire analytical run. An IRM (internal reference mass) recalibration can then be carried out with the IM-MS Reprocessor utility, which stores the new mass calibration coefficients in the data file for use in other software applications.
Reduction	The process whereby the number of variables in a data set is decreased to improve computation time and information quality, e.g., an extracted ion chromatogram obtained from GC/MS and LC/MS data files. Reduction provides smaller, viewable and interpretable data sets by employing feature selection and feature extraction. Also known as dimension reduction and data reduction. This is part of the filtering and statistical analysis processes employed by Agilent Mass Profiler and by Agilent Mass Profiler Professional.
Replicate	Collecting multiple identical samples from a population to obtain a measured value that more closely approximates the true value.
Sample	A part, piece, or item that is taken from a specimen and understood as being representative of the larger specimen (e.g., blood sample, cell culture, body fluid, aliquot) or population. An analysis may be derived from samples taken at a particular geographical location, taken at a specific period of time during an experiment, and taken before or after a specific treatment. A small number of specimens used to represent a whole class or group.
Single-field CCS calibration	A method for calculating reliable CCS values. A data file is acquired for tune-mix ions that utilizes the exact same IM-MS settings used for the analytical run. This tune-mix data file is processed in IM-MS Browser to determine the Beta and Tfix single-field CCS calibration coefficients. These values are then applied to the analytical run so each feature's drift time, mass, and charge can be easily converted into a CCS value during feature finding.

## 5 Reference Information Definitions

Specimen	An individual organism, e.g., a person, animal, plant, or other organism, of a class or group that is used as a representative of a whole class or group.
Spike	The specific and quantitative addition of one or more standard compounds to a sample.
Standard	A chemical or mixture of chemicals selected as a basis of comparing the quality of analytical results or of measuring and compensating for the precise offset or drift incurred over a set of analyses.
Standard deviation	A measure of variability among a set of data that is equal to the square root of the arithmetic average of the squares of the deviations from the mean. A low standard deviation value indicates that the individual data tend to be very close to the mean, whereas a high standard deviation indicates that the data is spread out over a larger range of values from the mean.
Standard-mode data	This term refers to IM data that is acquired without multiplexing. See <b>"Multiplexed</b> data" on page 91.
State	A set of circumstances or attributes characterizing a biological organism at a given time. A few sample attributes may include temperature, time, pH, nutrition, geography, stress, disease, and controlled exposure.
TIC	Total Ion Current
Tune mix	A solution made up of compounds of known masses; used to optimize conditions for producing the best resolution and highest signal in a mass spectrometer. Used with IM-MS Reprocessor to correct the mass calibration of raw IM data. Used for single-field CCS calibration to calculate the Beta and TFix constants.
Unidentified compound	Chromatographic components that are only uniquely denoted by their mass, abundance and retention times, or in the case of ion mobility data, their CCS values, and that have not been assigned an identity, such as compound name and molecular formula. Unidentified compounds are typically produced by feature finding and deconvolution algorithms. See also <b>"ID Browser"</b> on page 88.
Variable	An element in a data set that assumes changing values, i.e., values that are not constant over the entire data set. The two types of variables are independent and dependent.
Wizard	A sequence of dialog boxes presented by Agilent software programs that guide you through well-defined steps to enter information, organize data, and perform analyses.

Resource Apps	MassHunter software includes a Resource App from where you can access relevant resource material. Some of these guides can also be found on <b>www.agilent.com</b> .
	These guides, tutorials and videos help you use Agilent products for lipidomics analyses. For citations to Agilent application notes, presentations, product brochures, technical overviews, and software written especially for metabolomics analyses but also useful for lipidomics analyses, see the <i>Agilent Metabolomics</i> <i>Discovery Workflow Guide</i> .
MassHunter Mass Profiler Professional Resources	<ul> <li>Agilent G3835AA MassHunter Mass Profiler Professional Software Quick Start Guide</li> </ul>
	<ul> <li>Agilent G3835AA MassHunter Mass Profiler Professional Software Familiarization Guide</li> </ul>
	<ul> <li>Agilent G3835AA MassHunter Mass Profiler Professional Software Application Guide</li> </ul>
	Agilent Mass Profiler Professional - User Manual
	Agilent Getting Started with MPP
	Agilent MPP Statistics and Machine Learning
	Agilent MPP Training Videos
	Agilent Metabolomics Discovery Workflow Overview
	Agilent Metabolomics Discovery Workflow Guide
TOF and Q-TOF LC/MS Resource App	Agilent Lipid Annotator eFam
Agilent Web Site	This guide is available from <b>www.agilent.com.</b>
-	<ul> <li>Metabolomics: Approaches Using Mass Spectrometry (Agilent publication 5990-4314EN, October 27, 2009)</li> </ul>

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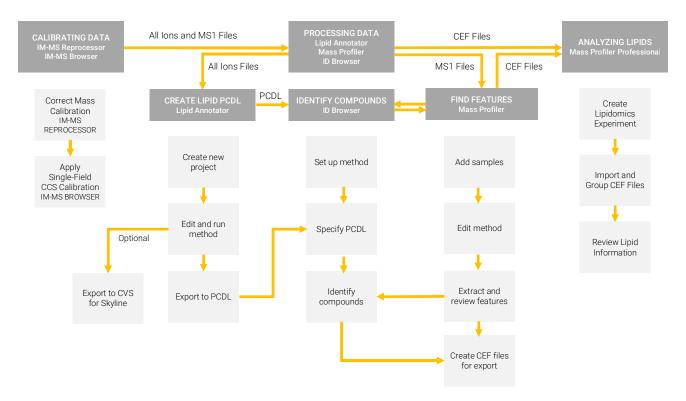
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#### In This Guide

This guide describes the Lipidomics workflow using the Agilent 6560 Ion Mobility Mass Spectrometer and MassHunter software.



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