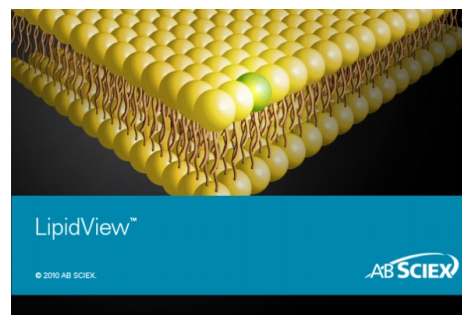


# LipidView™ Software for the Identification and Quantification of Lipids in Complex Biological Extracts

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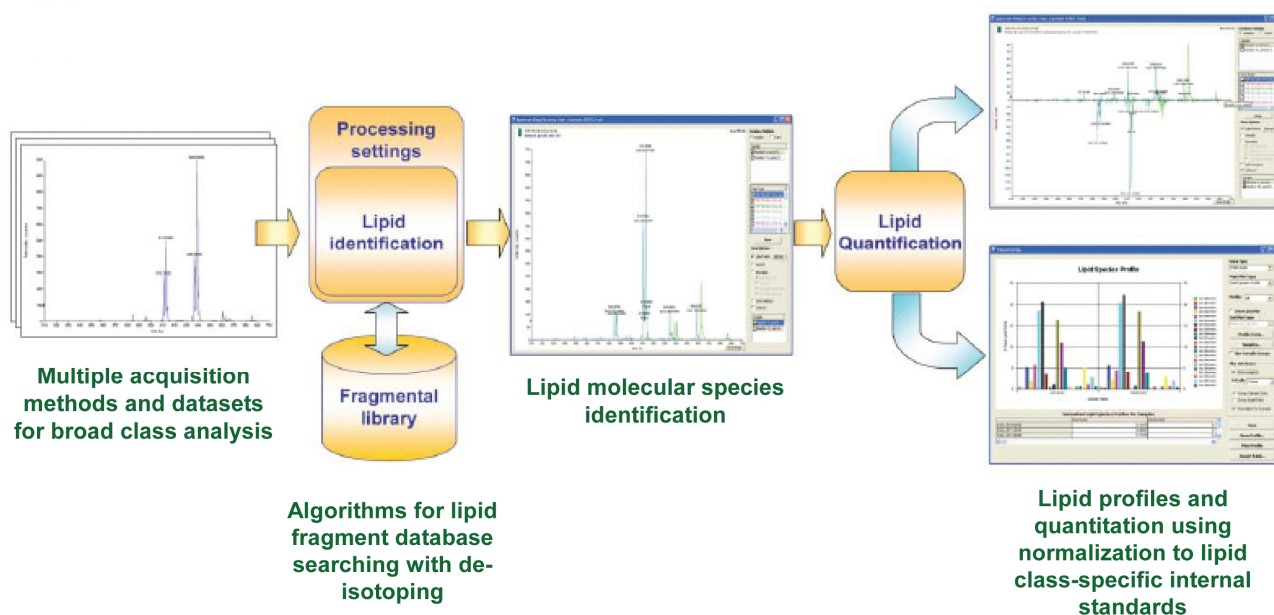
Lipid profiling is performed using electrospray mass spectrometry, where the acquisition of MS and MS/MS data enables for structural characterization of lipid species. These qualitative strategies require lipid fragment and spectral interpretation to identify lipid molecular species by head group, fatty acid, and carbon chain compositions. Lipid profiling can also provide quantitative information when a proper internal standard strategy is implemented. Through the use of complementary scanning techniques, such as multiplexed precursor ion scanning and information dependent MS/MS acquisitions, lipids can be identified and their detection corrected by internal standards to allow for direct comparisons between many samples.

The processing of such complex and complementary datasets is predominantly the bottleneck in lipid profiling experiments of biological lipid extracts and thus only software consisting of sophisticated bioinformatic tools can allow for these analyses in high throughput. LipidView™ Software is a new tool that allows even novice lipid investigators the ability to easily find, identify and interpret lipid species from mass spectrometry data from complex samples.



## Key Features of LipidView™ Software

- LipidView Software enables fast lipid characterization and quantification
- Simple 4-step workflow with automated data processing
- Lipid fragment database with more than 40 lipid classes and 23 000 lipid species
- Easy sample comparison in terms of lipid class, fatty acid, or molecular species profiles
- Works with AB SCIEX Triple Quad™ system, QTRAP® System or QSTAR® System mass spectrometers



**Figure 1. LipidView™ Software Workflow.** The workflow driven format performs the identification and characterization of lipid species by database searching of MS and MS/MS fragment ions, and then reports both numerical and graphical information of various lipid classes and individual lipid species within a class.

LipidView™ Software provides a user-friendly workflow driven format for the identification and quantification of lipid species from ESI-MS data (Figure 1). The software enables identification and characterization through database searching of MS/MS fragment ions and reports a numerical and graphical output for profiling various lipid classes by lipid head groups, fatty acid and long chain base characteristic fragments and neutral losses. LipidView™ Software streamlines the automated data processing with method selection, lipid species identification, isotopic correction of peak intensities, multiple internal standards-based quantification, lipid profile visualization, and reporting of the results.

## LipidView™ Software Utilities for Browsing the Lipid Fragments Database

### Lipid Catalogue Utility

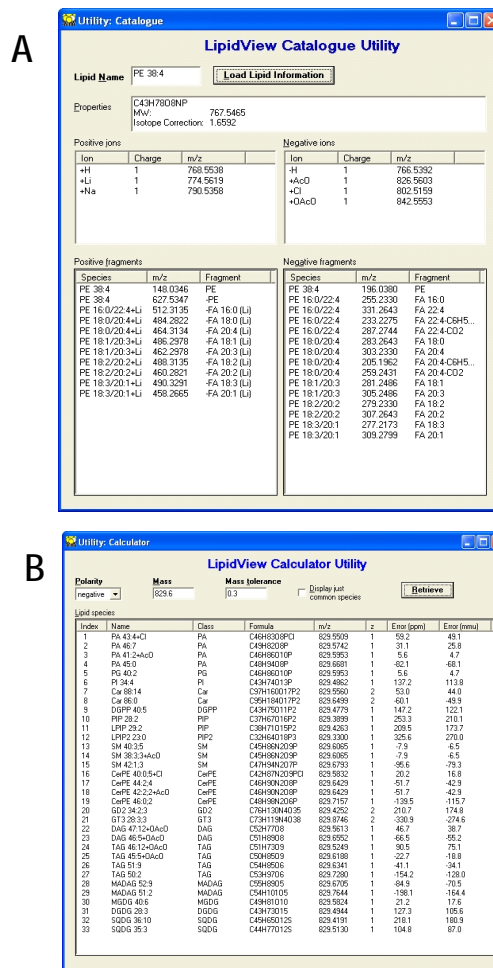
The Lipid Catalogue Utility provides access to the entire database of lipid classification covered by over 23 000 lipid species, retrieving molecular formula, monoisotopic mass, m/z of common adducts, fatty acids, and characteristic fragments listed in either positive or negative polarity. As shown in Figure 2A, the Lipid Catalogue Utility demonstrates the results retrieved from the input of PE 38:4, following the nomenclature: <headgroup> <sum of carbon atoms>:<sum of double bonds> to retrieve the database information for the desired lipid species, including the isotope correction factor implemented when processing data.

### Lipid Calculator Utility

An alternative to retrieving lipid information by lipid name, the user can search lipid species by a given m/z value in either positive or negative polarity with a designated mass tolerance. Figure 2B shows the output of querying potential lipids by parent mass in positive mode listing all lipid species whose monoisotopic mass matches within 0.3 amu. The retrieved list can also be filtered to include only entries containing common fatty acid chain compositions.

## Defining Lipid Internal Standards in LipidView™ Software

The use of lipid class-specific internal standards is critical to making quantitative measurements or generating normalized comparisons between samples analyzed by any mass spectrometry experiment. LipidView™ Software offers a module dedicated to the implementation and management of internal standards, as shown in Figure 3. The application contains a pre-loaded list of non-natural, internal standards (consisting of odd chained fatty acid moieties) commonly used for application against glycerophospholipids, glycerolipids, sphingolipids, and



**Figure 2. Lipid Catalogue (A) and Lipid Calculator (B) Utilities.** These utilities enable browsing of the lipid fragments database by way of monoisotopic mass, molecular ion, and fragment ions in either polarity.

sterol lipid classes consisting of odd chained fatty acid moieties. In addition, users can also enter custom internal standards by filling in the required information, such as molecular formula, molecular ion and specific scan information (Figure 3, inset)..

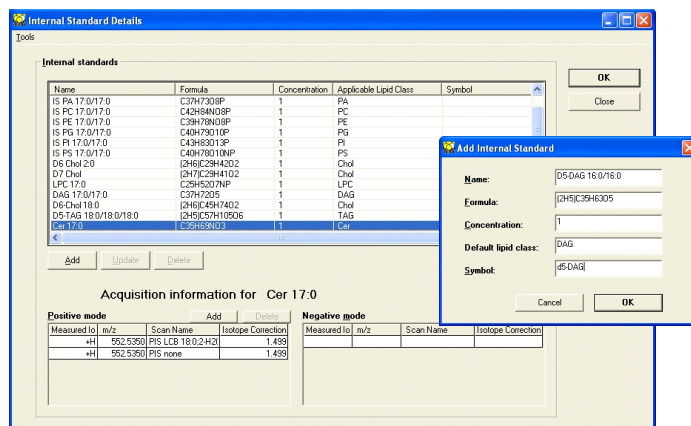
Once internal standards have been added and the lipid database, users can then select specific internal standards within a processing method to apply when processing lipid mass spectrometry data.

## Developing Processing Methods for Lipid Identification and Quantitation

LipidView™ Software enables batch processing of ESI lipid data by applying processing methods to samples individually, or to a group of samples. The processing method set-up module is shown in Figure 4. Here a user defines the parameters for extracting the raw data and spectral peak preprocessing, then selects the relevant lipid classes for searching the data against the database. Processing methods are set-up by 5 primary steps, as outlined in the Figure 4 below.

1. Selecting the acquisition type and polarity.
2. Defining spectrum pre-processing.
3. Providing the sample introduction details: liquid chromatography (LC), flow injection analysis (FIA) or direct infusion.
4. Selecting the candidate lipid classes for identification
5. Applying internal standards, confirmatory molecular ions (adducts), and chain/double bond limits for each selected lipid category.

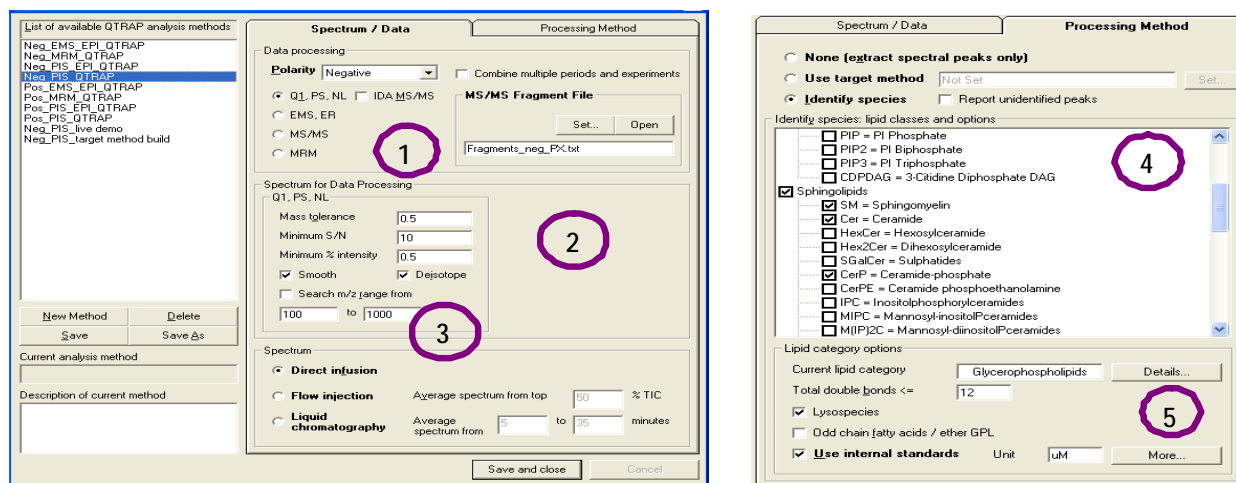
Processing methods can be saved and are added to the list for future use. A set of pre-installed processing methods is available in the software; these methods serve as templates to derive user specific methods.



**Figure 3. Defining and Updating Internal Standards.** Internal standards can be added or updated by indicating the formula and measured ion in each polarity. The software will automatically calculate the isotope correction factor.

## Spectrum Pre-processing

In the Spectrum Tab of the process method set-up module, the user can select whether the data should be smoothed (recommended) and isotopic contribution from lower mass species be removed before lipid identification is carried out. This feature is essential when processing data from complex samples where lipid species are not resolved in time to ensure accurate lipid identification from isobaric peaks especially lipids differing by one double bond.



**Figure 4. Processing Method Set-up for QTRAP® System Acquisitions.** Batch processing methods are developed using this set-up module with five easy steps.

## Applying Internal Standards for Lipid Quantification

Internal standard reference peaks can be used for correcting the peak areas or peak intensities of identified lipid species. By default internal standards are assigned to lipids based on the Default Lipid Class specified on internal standard information entry. To customize how the internal standards are to be applied, the user will open Profile Tests to view the complete list of identified lipids and review the automatic assignment of each internal standard to the applicable lipid species (Profile Tests Editor shown in Figure 5). Once all lipids have the appropriate internal standard assigned, the numerical data is normalized and quantitative lipid profiles can be created to compare lipid abundances between samples.

## Creating a Method with a Target Lipid List

Once a data file has been processed by LipidView™ Software and internal standards have been designated to correct a particular lipid-class or specific lipid species, the user can create a Target Lipid List consisting of a set of specific lipid targets and internal standard reference peaks to apply against the processing of new datasets. Target Lipid Lists can be saved and then loaded into the application when the user develops a processing method, as shown in upper view of the Processing Method dialog shown in Figure 4. Processing datasets using a Target Lipid List relies purely on the information pertaining to the targeted lipids and provides a defined search within the lipid fragments database. Such an approach is advantageous for the batch processing of multiple samples in lipid class-specific screening experiments, since it is fast, data can be aligned easily and species which are not described in the lipid database can be a part of the target lipid list.

## Interpreting Data in the LipidView™ Software Results Workspace

Data files acquired from AB SCIEX Triple Quad™ system, QTRAP® System or QSTAR® System mass spectrometers can be processed by LipidView™ Software to identify lipid species through extraction of spectral peaks acquired via specific survey or MS/MS scans. Spectra from survey scans are processed independently from spectra in dependant MS/MS scans (MS triggered MS/MS). However, survey scan and MS/MS lipid identification results are coupled within the results workspace. In addition, if user selected to store information on spectral peaks that could not be assigned to a lipid species, such results are available in parallel as well.

Internal Standards						Target Lipids					
Index	Scan Name	Scan m/z	m/z	Name	Use	# Nam	Polarity	Lipid Class	Lipid Name	Correction Fac	IS Symbol
312	FA 18:0/PA 22:6	283.2	480.3	LPE 18:0	<input checked="" type="checkbox"/>	1	Negative	PE	LPE 18:0	1.323	PE 34:0
313	FA 18:0/PA 22:6	283.2	511.3	LPG 18:0	<input checked="" type="checkbox"/>	1	Negative	PG	LPG 18:0	1.339	PG 34:0
314	FA 18:0/PA 22:6	283.2	582.4	LPC 18:0+AcO	<input checked="" type="checkbox"/>	1	Negative	PC	LPC 18:0+AcO	1.360	PC 34:0
315	FA 18:0/PA 22:6	283.2	599.4	PA 30:10	<input checked="" type="checkbox"/>	2	Negative	PA	PA 30:10	1.475	PA 34:0
316	FA 18:0/PA 22:6	283.2	605.4	PA 30:7	<input checked="" type="checkbox"/>	1	Negative	PA	PA 30:7	1.476	PA 34:0
317	FA 18:0/PA 22:6	283.2	609.4	PA 30:5	<input checked="" type="checkbox"/>	1	Negative	PA	PA 30:5	1.477	PA 34:0
318	FA 18:0/PA 22:6	283.2	611.4	PA 30:4	<input checked="" type="checkbox"/>	1	Negative	PA	PA 30:4	1.477	PA 34:0
319	FA 18:0/PA 22:6	283.2	613.4	PA 30:3	<input checked="" type="checkbox"/>	1	Negative	PA	PA 30:3	1.479	PA 34:0
320	FA 18:0/PA 22:6	283.2	620.4	PE 28:7	<input checked="" type="checkbox"/>	1	Negative	PE	PE 28:7	1.482	PE 34:0
321	FA 18:0/PA 22:6	283.2	624.4	PE 28:5	<input checked="" type="checkbox"/>	1	Negative	PE	PE 28:5	1.482	PE 34:0
322	FA 18:0/PA 22:6	283.2	627.4	PA 32:10	<input checked="" type="checkbox"/>	1	Negative	PA	PA 32:10	1.509	PA 34:0
323	FA 18:0/PA 22:6	283.2	643.4	PA 32:2	<input checked="" type="checkbox"/>	1	Negative	PA	PA 32:2	1.511	PA 34:0
324	FA 18:0/PA 22:6	283.2	652.4	PE 30:5	<input checked="" type="checkbox"/>	1	Negative	PE	PE 30:5	1.516	PE 34:0
325	FA 18:0/PA 22:6	283.2	665.5	PA 34:5	<input checked="" type="checkbox"/>	1	Negative	PA	PA 34:5	1.545	PA 34:0
326	FA 18:0/PA 22:6	283.2	669.5	PS 28:5	<input checked="" type="checkbox"/>	1	Negative	PS	PS 28:5	1.506	PS 34:0
327	FA 18:0/PA 22:6	283.2	669.5	PA 34:3	<input checked="" type="checkbox"/>	1	Negative	PA	PA 34:3	1.546	PA 34:0
328	FA 18:0/PA 22:6	283.2	701.5	PS 32:10	<input checked="" type="checkbox"/>	1	Negative	PS	PS 32:10	1.560	PS 34:0
329	FA 18:0/PA 22:6	283.2	707.5	PA 38:12	<input checked="" type="checkbox"/>	2	Negative	PA	PA 38:12	1.613	PA 34:0
330	FA 18:0/PA 22:6	283.2	718.5	PE 34:0	<input checked="" type="checkbox"/>	1	Negative	PE	PE 34:0	1.589	PE 34:0
331	FA 18:0/PA 22:6	283.2	728.5	PC 28:4+AcO	<input checked="" type="checkbox"/>	3	Negative	PC	PC 28:4+AcO	1.533	PC 34:0
332	FA 18:0/PA 22:6	283.2	744.5	PE 36:1	<input checked="" type="checkbox"/>	2	Negative	PE	PE 36:1	1.623	PE 34:0
333	FA 18:0/PA 22:6	283.2	746.6	PC 30:9+AcO	<input checked="" type="checkbox"/>	3	Negative	PC	PC 30:9+AcO	1.567	PC 34:0
334	FA 18:0/PA 22:6	283.2	761.6	PG 36:8	<input checked="" type="checkbox"/>	2	Negative	PG	PG 36:8	1.641	PG 34:0
335	FA 18:0/PA 22:6	283.2	762.6	PE 38:6	<input checked="" type="checkbox"/>	2	Negative	PE	PE 38:6	1.652	PE 34:0

**Figure 5. Profile Test Editor.** The Profile Tests Editor is used to assign internal standards (IS) (right most column), to imply correction to the identified lipid species (left columns). This editor then saves the identified lipids and IS into a Targeted Method

## Survey Scan and MS/MS Data Review

Precursor Ion, Neutral Loss, Q1, Enhanced MS or MRM survey scans undergo peak extraction and direct searching of precursor masses against the lipid fragments database to obtain lipid identifications and spectral data shown in the results workspace (Figure 6A). Identified lipids can be presented in three categories: All, Confirmed, and Confirmed & Common.

- Confirmed lipids refer to identifications where multiple complementary spectral peak measurements point to a single lipid species.
- Confirmed and Common lipids describe lipids whose evidence confirms a head-group or a fatty acids with common chain compositions.
- All can be selected to view lipids that have been identified based on evidence from only one scan where confirmatory data is absent (i.e. lacking fatty acid data) or propose species with uncommon chain arrangement.

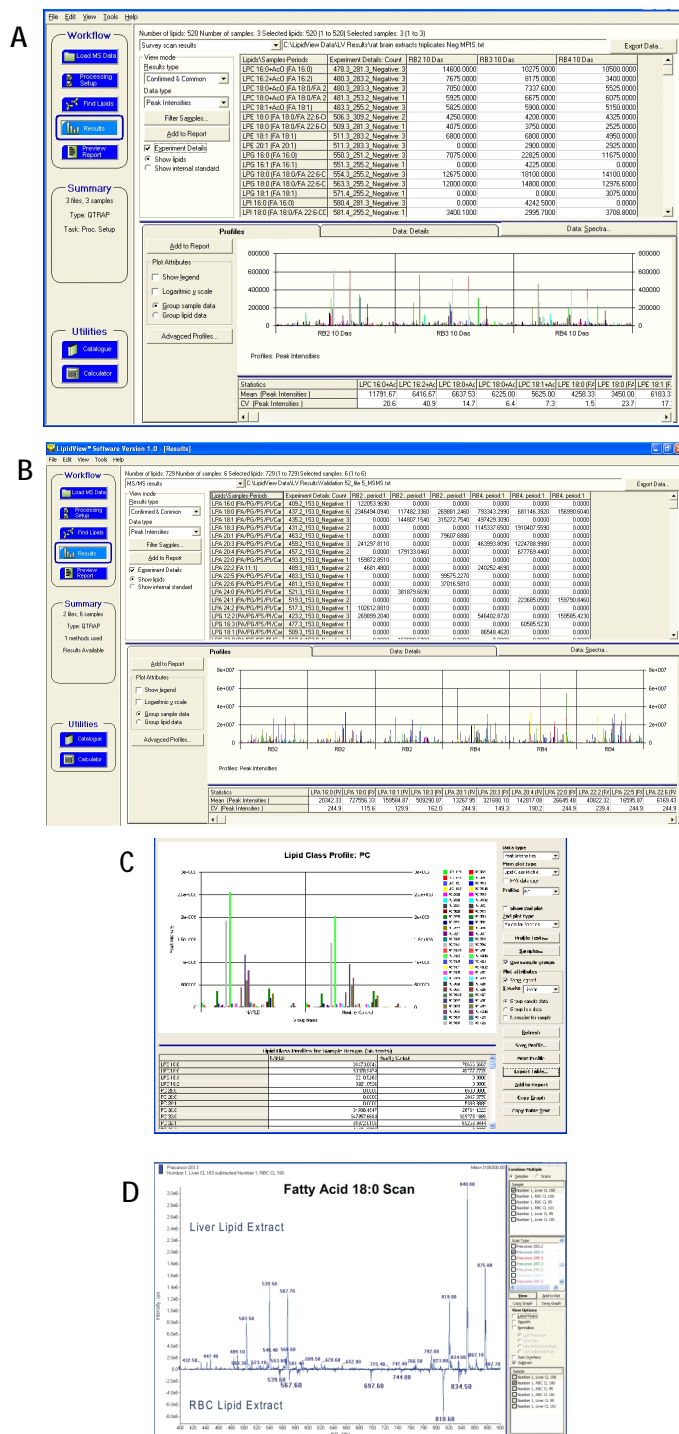
Numerical data is aligned per lipid identification in the form of peak intensities, peak areas, % intensities, or representative values corrected by internal standards. MS/MS data collected by information dependant acquisition (IDA) is processed separately and is coupled to the results workspace yet viewed independently (Figure 6B). As MS/MS data is more likely to contain several confirmatory ions, the lipids identified by positive and negative MS/MS spectra are often well validated and can be reviewed and exported separately. Samples can be filtered and grouped where lipid profile plots are created using averaged data between sample groups. Moreover, there is a direct link between the sorted numerical data and the lipid profiles quick view within the results workspace.

## Advanced Lipid Profiles View

Lipid profiles can also be created in the Advanced Profiles View enabling the plotting of large numbers of lipids by species, by class, or by fatty acids in an expanded view. As shown in Figure 6C, the average corrected peak intensities are plotted for two sample groups for direct comparisons. Advanced Profiles View also allows the user to create secondary plots. For example, in Figure 6C, the total PE lipid class is plotted in the major view while the lower plot displays the fatty acid composition of the PE 40:4 from the complementary fatty acid data. The data plotted in each profile is displayed below each graph for easy export of such plots.

## Spectral View

To view the raw spectra, a Spectral View module allows for the recall of specific scans or averaged spectra from processed data files. Figure 6D is a spectrum corresponding to the PIS of 283.3 m/z in negative mode for a liver tissue extract displayed subtracted from an identical spectrum from red blood cell (RBC) lipid extract. Spectral peaks loaded into the spectral viewer can also be labeled with the corresponding lipids identified.



**Figure 6. LipidView Software Results Workspace.** Data Details listing confirmed lipid molecular compositions are provided in the results workspace, for MS (A) and MS/MS data (B). Advanced Profile View enables plotting of lipid classes and fatty acids (C). Spectral View is used to view specified scans from raw data (D).

## Exporting Data from LipidView™ Software

Corrected peak intensity, peak area data displayed in the upper grid of the results workspace, Advanced Profiles graphical values, or the lipids identified from the Profile Tests can be exported to a text file (\*.txt). Exporting of data maintains the sample grouping or filters created within the LipidView™ Software Session. Also, samples processed maintain all experimental details, such as parent mass and fragment mass(es) aligned for each lipid identified.

## Creating a Report

LipidView™ Software enables users to create a report directly to MS Word format. Samples and processing methods are automatically printed on the report template and users can create numeric tables automatically from the results workspace. Additionally, lipid profile plots and labeled spectral views can be sent to the report directly from the results workspace, Advanced Profiles View, or Spectral View. Reports generated are automatically saved with a given LipidView™ Software session and can be easily re-accessed when reviewing archived lipid result.

## Conclusions

LipidView™ Software enables processing and review of MS and MS/MS data from infusion and LC ESI experiments from a variety of different acquisition types – supporting Analyst® Software raw data files.

- Lipid identifications are obtained by searching of lipid fragments and lipid classification in an internal database.
- Processing methods can be derived and modified by the user from a suite of pre-designed template methods for lipid identification and quantification in both polarities and further customized for specified acquisition strategies.
- Supports batch file processing with multiple methods and full traceability of the processing parameters.
- Flexible data review workspace with linked numerical and graphical results workspace incorporating advance profiles view, spectral view, and data exporting features.
- Custom reporting of data into MS Word format

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