Carl Fruehan, David Johnson, and Ruth Welti

LipidomeDB Data Calculation Environment Has Been Updated to Process Direct-Infusion MRM Data

Addresses:

C. Fruehan
110 Chandler Drive
Mundelein, IL 60060

D. Johnson
Molecular Graphics and Modeling Laboratory
Computational Chemical Biology Core
University of Kansas
2034 Becker Drive
Lawrence KS 66045

R. Welti
Kansas Lipidomics Research Center
Division of Biology
Kansas State University
1717 Claflin Road
Manhattan, KS 66506

Running head:

LipidomeDB Data Calculation Environment Updates

Corresponding author information:

Ruth Welti
welti@ksu.edu
(785) 532-6241
Fax: (785) 532-6653

Key words:

Mass spectrometry (MS); Lipid analysis; Lipid chemistry; Phospholipid analysis

Abstract

LipidomeDB Data Calculation Environment (DCE), a web application for processing data from direct-infusion tandem mass spectrometer data on lipids, was described by Zhou et al. (2011, Lipids, 46, 879-884). The original version processed multiple precursor and/or neutral loss scans on multiple samples. A recent update to LipidomeDB DCE, http://lipidome.bcf.ku.edu:8080/Lipidomics/, extends LipidomeDB DCE's functionality to process data acquired in multiple reaction monitoring (MRM) mode by direct-infusion mass spectrometry. Both the precursor-neutral loss workflow and the multiple reaction monitoring workflow remove signals due to isotopic overlap of lipid analytes and calculate of the amount of each target lipid in comparison with internal standards.

Introduction

LipidomeDB Data Calculation Environment was created to provide an easy-to-use method to process lipidomics data obtained by direct infusion using precursor or neutral loss scanning (Zhou et al., 2011). The original online processing system found the peaks of interest in spectral lists, performed isotopic deconvolution of the data, and calculated amounts of lipid in samples compared to internal standards.

The work described here extends LipidomeDB DCE functionality to quantify data from multiple reaction monitoring (MRM) on a triple quadrupole mass spectrometer in direct-infusion mode or with differential ion mobility spectrometry at the front end of the mass spectrometer. This new workflow in LipidomeDB DCE is freely available and should facilitate data processing for direct-infusion MRM lipidomics data, where isotopologue corrections are required, but are not typically included in mass spectrometer manufacturers' workflows.

Materials and Methods

LipidomeDB DCE is written in JSP with Javascript for client-side calculations. It is available online at http://lipidome.bcf.ku.edu:8080/Lipidomics/. More technical details are provided in the "tutorial" at the site.

Results and Discussion

A major update to LipidomeDB DCE has added a processing strategy for MRM data. The use of this and other features of LipidomeDB DCE are described in detail in an updated "tutorial" available from the front page of the site. The tutorial also describes the acquisition of data for lipidomics using a triple quadrupole mass spectrometer. Example data for both Precursor/Neutral Loss processing and MRM data processing are provided to demonstrate the function of the calculation programs.

MRM data are uploaded in an Excel file, which must be formatted as described in the "tutorial" and as shown in the example upload file. Each row contains data for one lipid analyte or internal standard, including its name, its chemical formula, the adduct used in the analysis, and the formula of the charged MRM fragment. The user also designates whether or not isotopic deconvolution should be used, and, if so, which lipids form each group that should be considered for deconvolution together. For example, if differential mobility spectrometry is used to separate ions in the front end of the mass spectrometer (Lintonen et al., 2014), then unseparated (and separated) groups of lipids can be designated. If no physical separation device is used, lipids analyzed in negative and positive modes can (and should) be distinguished. Additionally, for each analyte, the user designates which 1 to 3 compound(s) will serve as internal standards, and whether the quantification should be performed by averaging the intensity/amount for each standard for comparison to the lipid intensity or by using a plot of standard intensity/amount vs. *m/z* for comparison to the lipid intensity. The user also provides the *m/z*s of the MRM transitions and the intensity data from each transition for each sample.

After the user uploads the Excel file, results are provided online, and an Excel file containing the input data plus the results can be downloaded. The output includes the data isotopically deconvoluted, as specified by the user, and normalized to the internal standards. Additionally, the file includes calculated MRM transition *m/z*s based on the provided lipid chemical formula, adduct, and fragment formula information. Any difference between the calculated and user-

provided *m/z*s will be flagged to indicate a problem with either the formula information in the upload file or the acquisition *m/z*s.

The updates to LipidomeDB DCE also include a user-editable database of lipids and their formulas. Several hundred commonly analyzed lipids have been entered into the database, and each user can add additional lipids. If the lipids are in the database, the user can enter a lipid name without a formula in their MRM data upload file, and the formula will be retrieved from the database.

Additional changes to LipidomeDB Data Calculation Environment for lipid analysis by Precursor or Neutral Loss scanning include addition of an editable fragment database and a mechanism to edit user-saved target lists.

Acknowledgements

The authors would like to thank Yu Song for preparing the document, linked to the LipidomeDB DCE tutorial, describing how to export MRM data using a Sciex 6500+ triple quadrupole mass spectrometer. We also would like to thank Mary Roth for her comments on the function of LipidomeDB DCE. We would like to acknowledge support from a Core Facility award from Kansas INBRE, which operates under an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health with NIH grant number P20GM103418 and the Chemical Biology of Infectious Disease Centers of Biomedical Research Excellence (COBRE) with NIH grant number P20GM113117. Contribution 19-104-J from the Kansas Agricultural Experiment Station.

Conflict of Interest

The authors have no conflict of interest.

References

Lintonen, T. P. I., Baker, P. R. S., Suoniemi, M., Ubhi, B. K., Koistinen, K. M., Duchoslav, E., Campbell, J. L. and Ekroos, K. (2014) Differential mobility spectrometry-driven shotgun lipidomics. Anal. Chem. 86, 9662-9669.

Zhou, Z., Marepally, S. R., Nune, D. S., Pallakollu, P., Ragan, G., Roth, M. R., Wang, L., Lushington, G. H., Visvanathan, M., and Welti, R. (2011) LipidomeDB Data Calculation Environment: Online processing of direct-infusion mass spectral data for lipid profiles. Lipids 46, 879-884.