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Data Article

Data processing on a comparative evaluation of the extraction and analysis procedures for urinary phospholipid and lysophospholipid using MALDI-TOF/MS

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ABSTRACT

In this dataset we provide MALDI-TOF/MS spectra for the testing and application of a quantitative method using external ionization standards (ionization STDs) for peak-intensity normalization. The presented data is related to our recent article entitled "a comparative evaluation of the extraction and analysis procedures for urinary phospholipid and lysophospholipid using MALDI-TOF/MS". Gradient dilutions of mixture containing thirteen phospho- and lysophospho-lipid species (internal STDs) were mixed with constant concentration of the ionization STDs and analyzed together. Peak intensities of the internal and ionization STDs were picked by a homemade workflow based on OpenMS (steps including noise filtration, baseline subtraction and peak-picking). The peak-intensity ratios between the internal and ionization STDs were linearly correlated with their concentration ratios. Using this method, the evaluation of efficiencies of six different lipid extraction methods was performed in urine samples. In summary, a free and easy-to-use method for phospholipid and

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lysophospholipid quantitative analysis based on MALDI-TOF/MS is provided in this article.

Specifications table

Subject area	*Chemistry*
More specific subject area	*Analytical Chemistry, Mass Spectrometry, Clinical Biochemistry and Clinical Chemistry.*
Type of data	*Raw MS spectra, processed and converted spectra data in excel files*
How data was acquired	*MALDI-TOF/MS (Axima Performance, Shimadzu Kratos Analytical, Manchester, UK); Launchpad (Ver. 2.8.4); OpenMS (Ver. 2.3.0).*
Data format	*mzXML, xlsx*
Experimental factors	*Data processing of MALDI-TOF/MS spectra; evaluation of six different lipid extraction methods in urine samples.*
Experimental features	*Internal STDs mixture containing 13 lipid species (before and after extractions) were analyzed together with constant concentration of ionization STDs. Ten mM of 9-AA was used as matrix. LPC, PC and SM species were analyzed in positive mode while LPA, PA, LPS, PS, LPE, PE, LPG, PG, LPI and PI species were analyzed in negative mode. Peak intensities were normalized by ionization STDs.*
Data source location	*Department of Urology, Graduate School of Medicine, Kyoto University, Kyoto, Japan.*
Data accessibility	*Data is with this article with additional data available at* https://doi.org/10.17632/ds3wbvyhpg.1

Value of the data
- A normalization approach of MALDI-TOF/MS peak intensities using external ionization standards is provided.
- A method for quantitative MALDI-TOF/MS is provided and tested.
- The data can be used for evaluation of the analytical conditions of urinary phospholipids and lysophospholipids using MALDI-TOF/MS.

1. Data

This data article contains the phospholipids (PLs) and lysophospholipids (LPLs) spectra obtained by MALDI-TOF/MS. A quantitative analysis of PLs and LPLs were performed using the ionization STDs for normalization. The data processing steps were performed by OpenMS software and parameter settings shown in the Fig. 1. In order to evaluate this method, totally 13 lipid species (compositions were shown in Table 1) were spiked into 1 mL of urine sample before extracted by 6 different methods. The lipid extracts were analyzed by AXIMA Performance (Shimadzu Kratos Analytical, Manchester, UK) using 9-aminoacridine (9-AA) as a matrix. The spectra were measured in *m/z* range from 450 to 1,000 and from 400 to 1,000 in the positive and negative mode, respectively. Raw mass spectra in mzXML format and calculated data in xlsx format are available at the Mendeley dataset.

2. Experimental design, materials, and methods

2.1. Experimental design

For comparison of the target concentrations between different MALDI-plate wells, the ionization standards with a fixed concentration in the sample-matrix mixture were used for the normalization of
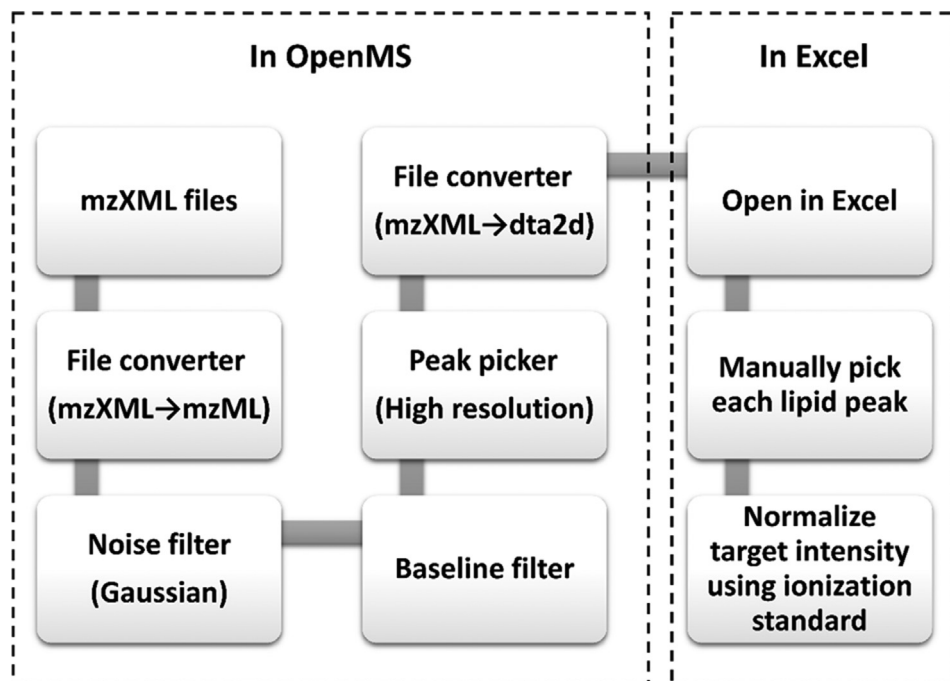
Fig. 1. Flow chart of the data processing. OpenMS was used for converting peaking processing (noise and baseline filtering, and peak picking). In the noise filter, the gaussian filter width was set as 1 and tolerance was set as 10 ppm; in baseline filter, length of the structuring element was set as 3; in the peak picker, the minimal signal-to-noise ratio for a peak to be picked was set as 0.1. The finally converted dat2d files were open using Microsoft Excel and stored as xlsx format. The *m/z* values and intensities of target and ionization standard peaks were manually picked in the excel files. The intensities of target peaks were normalized by ionization standard in each analysis.

intensity values. For evaluation of the extraction and analysis procedures for urinary PLs and LPLs using MALDI-TOF/MS before the clinical application, commercially available PLs and LPLs were mixed and spiked into urine samples before extraction. The relative recovery rate of each lipid was calculated by comparison of the normalized intensities between the urinary extraction group and the 100% recovery group (PLs and LPLs mixture without any extraction steps).

2.2. Test of the normalization approach using ionization STDs

To test if the ionization STDs could be used for the normalization of peak intensities among different MALDI-TOF/MS analysis, the standard calibration curves were prepared by serial dilution of the internal STDs. The internal STD was firstly diluted for four times using $CHCl_3$:MeOH (1:1, v/v), this was set as the 100% group. The 100% group was further gradient diluted to 75%, 50% and 25% groups using $CHCl_3$:MeOH (1:1, v/v). $CHCl_3$:MeOH (1:1, v/v) without the internal STD was used as the 0% group. These solutions were equally mixed with 9-AA matrix solution containing fixed concentrations of the ionization STDs [PC (14:0/14:0) for positive mode and PG (17:0/14:1) for negative mode, respectively] and analyzed by MALDI-TOF/MS in triplicate on three different days. Lysophosphatidylcholine (LPC), sphingomyelin (SM) and phosphatidylcholine (PC) species were analyzed in a reflectron positive mode and normalized by PC (14:0/14:0) peaks. Lysophosphatidic acid (LPA), lysophosphatidylserine (LPS), lysophosphatidylethanolamine (LPE), lysophosphatidylglycerol (LPG), lysophosphatidylinositol (LPI), phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and phosphatidylinositol (PI) were detected in a reflectron negative mode and normalized by PG

Table 1
Lipid standard mixtures [1].

1-1. Internal lipid standards (internal STDs)		
Standards	Concentration (μM)	Detected *m/z*
LPC (17:1)[a]	0.34	508.3[c]/530.3[d]
SM (d18:1/17:0)[a]	2.44	717.6[c]/739.6[d]
PC (17:0/17:0)[a]	1.03	762.6[c]/784.6[d]
LPA (17:1)[b]	1.80	421.2
LPS (13:0)[b]	8.80	454.2
LPE (17:1)[b]	4.23	464.3
LPG (17:1)[b]	1.77	495.3
LPI (17:1)[b]	1.35	583.3
PA (16:0/18:1)[b]	6.31	673.5
PS (14:0/14:0)[b]	8.74	591.5[e]/678.5
PE (17:0/20:4)[b]	3.95	752.5
PG (17:0/20:4)[b]	1.35	783.5
PI (21:0/22:6)[b]	1.11	951.6
1−2. External ionization standards (ionization STDs)		
Standards	Concentration (μM)	Detected *m/z*
PC (14:0/14:0)[a]	1.48	678.5[c]/700.6[d]
PG (17:0/14:1)[b]	1.39	705.5

[a] Detected in positive mode.
[b] Detected in negative mode.
[c] H$^+$ adduct.
[d] Na$^+$ adduct.
[e] Loss of serine head group from PS.

(17:0/14:1) peak. Standard curves for each lipid were constructed by plotting lipid concentrations against the normalized lipid peak intensities. The coefficient of determination (R^2) of each curve was calculated to evaluate linearity and the reliability of MALDI-TOF/MS for quantitative analysis.

2.3. Urine sample preparation

This study was approved by the Ethics Committee of the Kyoto University Graduate School of Medicine and Kyoto University Hospital (Approval No. G52). Informed consent was obtained from each of the participants prior to sample collection. Urine samples were collected and centrifuged at 2,000 × g for 10 min at 20 °C. The supernatant was filtered by 100 μm nylon cell strainers (BD Falcon, San Jose, CA, USA) to exclude urinary debris. One mL of the filtered supernatants was divided into a 1.5 mL Flex-Tube® 3810X (Eppendorf, Hamburg, Germany) and stored at −80 °C until analyzed.

2.4. Lipid extractions from urine samples

After thawing at room temperature, 1 mL urine in the 1.5 mL microtube was centrifuged at 2,000 × g for 5 min at room temperature. The urinary supernatant was picked up and was transferred into a 10 mL glass centrifugal tube to remove a little insoluble particle. Fifty μL of the lipid standard (contents shown in Table 1) was spiked into the urine sample and mixed well. The mixture was extracted by Folch [2], Bligh and Dyer [3], Acidified Bligh and Dyer [4,5], MTBE [6], Improved BUME [7] and HIP [8] method which were detailly described in our research article.

2.5. MALDI-TOF/MS analysis of lipid extraction

Ten mM 9-AA dissolved in 60/40 (v/v) isopropanol/ACN was used as the matrix. The lipid extractions containing 1.48 μM of PC (14:0/14:0) and 1.39 μM of PG (17:0/14:1) as external ionization STDs were mixed with equal volume of 9-AA solution and the 1 μL of the mixture was spotted onto the

μFocus MALDI plate 700-μm (384 circles, Hudson Surface Technology, Inc., Fort Lee, NJ, USA) and left to dry at room temperature. Mass spectra were acquired in *m/z* range from 450 to 1,000 and from 400 to 1,000 in the positive and negative mode, respectively. LPC, SM and PC species were analyzed in the reflectron positive mode. LPA, LPS, LPE, LPG, LPI, PA, PS, PE, PG and PI were detected in the reflectron negative mode. The nitrogen UV laser (337 nm) power was adjusted to a point just above the ionization threshold of the sample. The laser rate was set at 10 Hz with 100 laser shots per profile and 253 profiles were averaged for each individual sample.

2.6. Data processing

The spectra in mzXML format were processed using OpenMS software [9]. Our workflow is shown in Fig. 1. The parameters for peak processing were set as follows: the gaussian filter width was set as 1 and tolerance was set as 10 ppm in 'Noise filter' part; length of the structuring element was set as 3 in 'Baseline filter' part; the minimal signal-to-noise ratio for a peak to be picked was set as 0.1 in 'Peak picker' part. In our peak processing, we set 0.1 as the minimal signal to noise ratio for picking peaks since very low concentration of lipids were used in our analysis and we could calculate each peak intensity in our method. After peak processing, the finally converted dat2d files were open in Excel® (Microsoft Corp., Redmond, WA) and stored as xlsx format for the next calculation steps. The *m/z* values (shown in Table 1) and intensities of targets and the ionization standard peaks were manually picked in the excel files. The intensity of each target peak was normalized by the ionization standard [PC (14:0/ 14:0) for positive mode and PG (17:0/14:1) for negative mode, respectively)]. (All files are available on-line: https://doi.org/10.17632/ds3wbvyhpg1). The normalized intensities were used for calculation of the recovery rates by comparing with the 100% recovery group.

Conflict of interest

This study was supported in part by a Research Grant for Collaboration Research Projects from the Shimadzu Corporation. KN is the principal investigator in the Support Center for Precision Medicine, Shimadzu Techno-Research, Inc. and is also a program-specific associate professor in the Department of Urology in the Graduate School of Medicine at Kyoto University. The authors declare no competing interests.

References

[1] X. Li, K. Nakayama, T. Goto, S. Akamatsu, K. Shimizu, O. Ogawa, T. Inoue, Comparative evaluation of the extraction and analysis of urinary phospholipids and lysophospholipids using MALDI-TOF/MS, Chem. Phys. Lipids. 223 (2019) 104787, https://doi.org/10.1016/j.chemphyslip.2019.104787.
[2] J. Folch, M. Lees, G.H. Sloane Stanley, A simple method for the isolation and purification of total lipides from animal tissues, J. Biol. Chem. 226 (1957) 497–509. http://www.ncbi.nlm.nih.gov/pubmed/13428781.
[3] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917, https://doi.org/10.1139/o59-099.
[4] K. Retra, O.B. Bleijerveld, R.A. Van Gestel, A.G.M. Tielens, J.J. Van Hellemond, J.F. Brouwers, A simple and universal method for the separation and identification of phospholipid molecular species, Rapid Commun. Mass Spectrom. 22 (2008) 1853–1862, https://doi.org/10.1002/rcm.3562.
[5] A. Reis, A. Rudnitskaya, G.J. Blackburn, N.M. Fauzi, A.R. Pitt, C.M. Spickett, A comparison of five lipid extraction solvent systems for lipidomic studies of human LDL, J. Lipid Res. 54 (2013) 1812–1824, https://doi.org/10.1194/jlr.M034330.

[6] V. Matyash, G. Liebisch, T.V. Kurzchalia, A. Shevchenko, D. Schwudke, Lipid extraction by methyl- *tert* -butyl ether for high-throughput lipidomics, J. Lipid Res. 49 (2008) 1137−1146, https://doi.org/10.1194/jlr.D700041-JLR200.

[7] M. Cruz, M. Wang, J. Frisch-Daiello, X. Han, Improved butanol−methanol (BUME) method by replacing acetic acid for lipid extraction of biological samples, Lipids 51 (2016) 887−896, https://doi.org/10.1007/s11745-016-4164-7.

[8] A. Hara, N.S. Radin, Lipid extraction of tissues with a low toxicity solvent, Anal. Biochem. 90 (1978) 420−426, https://doi.org/10.1016/0003-2697(78)90046-5.

[9] H.L. Röst, T. Sachsenberg, S. Aiche, C. Bielow, H. Weisser, F. Aicheler, S. Andreotti, H. Ehrlich, P. Gutenbrunner, E. Kenar, X. Liang, S. Nahnsen, L. Nilse, J. Pfeuffer, G. Rosenberger, M. Rurik, U. Schmitt, J. Veit, M. Walzer, D. Wojnar, W.E. Wolski, O. Schilling, J.S. Choudhary, OpenMS: a flexible open-source software platform for mass spectrometry data analysis 13 (2016) 741−748, https://doi.org/10.1038/nmeth.3959.