Systematic optimization of mass-spectrometric analysis of complex lipids and primary metabolites from human blood plasma

Do Yup Lee[1], Tobias Kind[2], Young-Ran Yoon[3], Oliver Fiehn[‡], Kwang-Hyeon Liu[4]

[1]The Department of Bio and Fermentation Convergence Technology, Kookmin University, Seoul, Korea
Email: rome73@kookmin.ac.kr
[2] UC Davis Genome Center, Metabolomics, UC Davis, Davis, USA
[3]Department of Biomedical Science and Clinical Trial Center, Kyungpook National University, Daegu 700-734, Korea
[4]College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 702-701, Korea

Introduction

For comprehensive and simultaneous analysis of complex lipids and primary metabolites, five different extraction methods were tested, which can be categorized into two classes, liquid-liquid extraction and protein precipitation methods. Extraction efficiency was evaluated for the analysis of blood plasma lipids and primary metabolites by using chip-based direct infusion nano-electrospray tandem mass spectrometry and gas chromatography coupled to time-of-flight mass spectrometry, respectively. Extraction strategies were compared by the criteria such as the number of identified metabolites, extraction efficiency and compound diversity, reproducibility, and convenience for high-throughput sample preparations. Results presents two liquid-liquid extraction methods (the Folch and Matyash methods) were equally valid and robust for lipidomic assessments while primary metabolites were better assessed by the protein precipitation methods with organic solvent mixture.

Study Design

	Method name	Extraction Solvent	Fractionation	MS
Protein Precipitation	LAB I	CH_3CN:iPrOH:H_2O (3:3:2)	M + L → M; L (insoluble)	M → GC-MS; L → Direct infusion -MS
	LAB II	Acetone:MeOH (3:7)		
Liquid-Liquid Extraction	Folch	MeOH, $CHCl_3$ (1, 2)	M; insoluble; L	
	Bligh	$CHCl_3$: MeOH (1:2)		
	Matyash	MeOH, MTBE (1, 3.3)	L; M; insoluble	

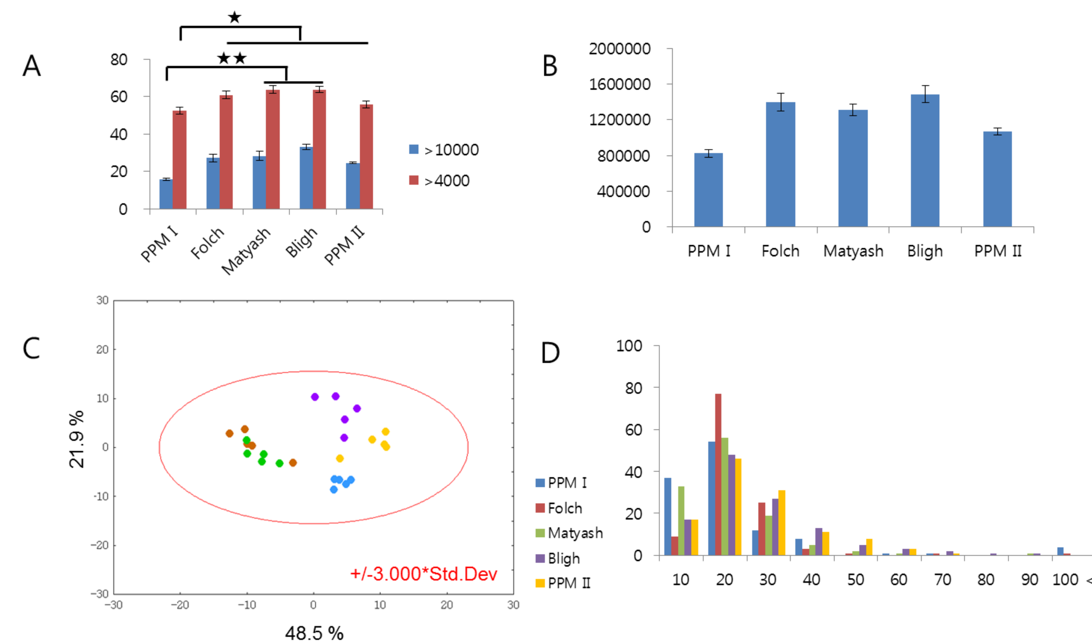
Methods

Lipid profiling*:* Experimental spectra were obtained on nano-electrospray infusion MS (LTQ XL linear ion trap mass spectrometer). The data collection method performed a full scan with an infusion time of 30 s, and a data dependent tandem mass-spectrometry (MS/MS) scan of the most abundant ions with 35V collision-induced dissociation (CID) to obtain specific MS/MS fragmentations. All spectra were recorded using the Thermo Xcalibur software.

Primary metabolite profiling*:* Metabolite analysis was performed on a with Leco GC-TOF mass spectrometer at 10 spectra/s from m/z 85 to 500 using automatic liner exchange, 50°C injection and a GC program ramping from 50-330°C by 20°C/min on a 30m rtx-5SilMS column. Data were processed by the Fiehnlab SetupX and BinBase databases.

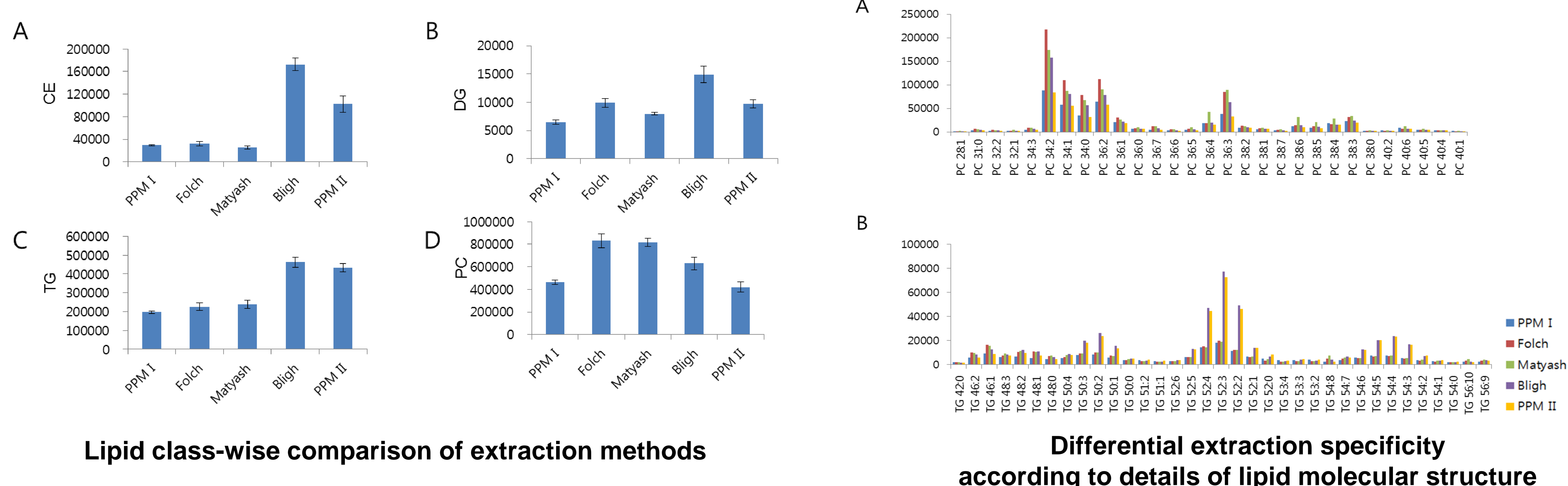
Statistics: Statistical analyses were performed on all continuous variables using the Statistica software vs. 7.1 (StatSoft, Tulsa OK). Univariate statistics for multiple study design classes was performed by breakdown and one-way ANOVA. F-statistics and p-values were generated for all metabolites. Multivariate statistics was performed by unsupervised principal component analysis (PCA) to obtain a general overview of variance of metabolic phenotypes in the study, by entering metabolite values without study class assignments.

The comparison of extraction methods for lipids collected by positive ionization mode.



(A) The number of peaks above 4000 and 10000 intensities. ★ indicates significantly different groups with 10000 intensity while ★★ presents significantly different groups with 4000 intensity (B) The sum of intensities of identified lipids. (C) Score scatter plots of principal component analysis. (D) %CV.

The extraction efficiency for lipids collected by positive ionization mode.



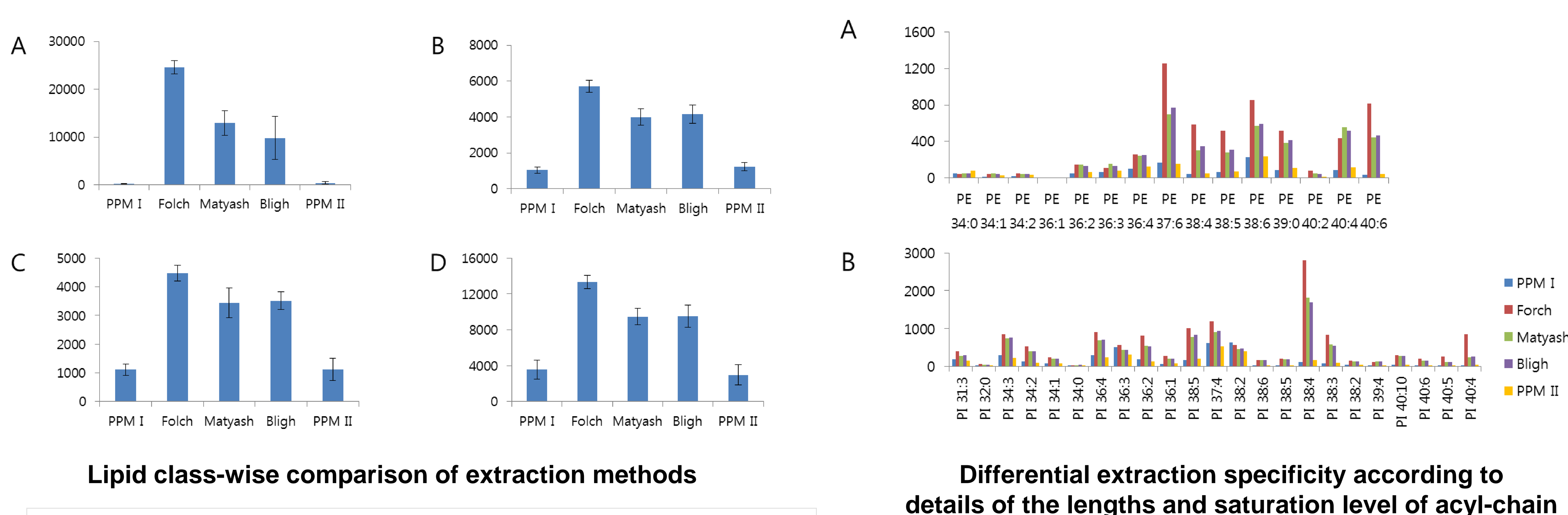
Lipid class-wise comparison of extraction methods

The sum of peak intensities of each lipid class.(A) CE: cholesteryl ester, (B) DG: diacylglycerol, (C) TG: triacylglycerol, (D) PC: phosphatidylcholine, and (E) LPC: lyso-phosphatidylcholine in positive ionization mode.

Differential extraction specificity according to details of lipid molecular structure

The intensities of each lipid of (A) PCs, and (B) TGs in positive ionization mode. Each lipid is arranged in X axis according to the molecular weight and the number of carbon. Y axis presents the intensity as a.u. (arbitrary unit).

The differential extractability for lipids collected by negative ionization mode.



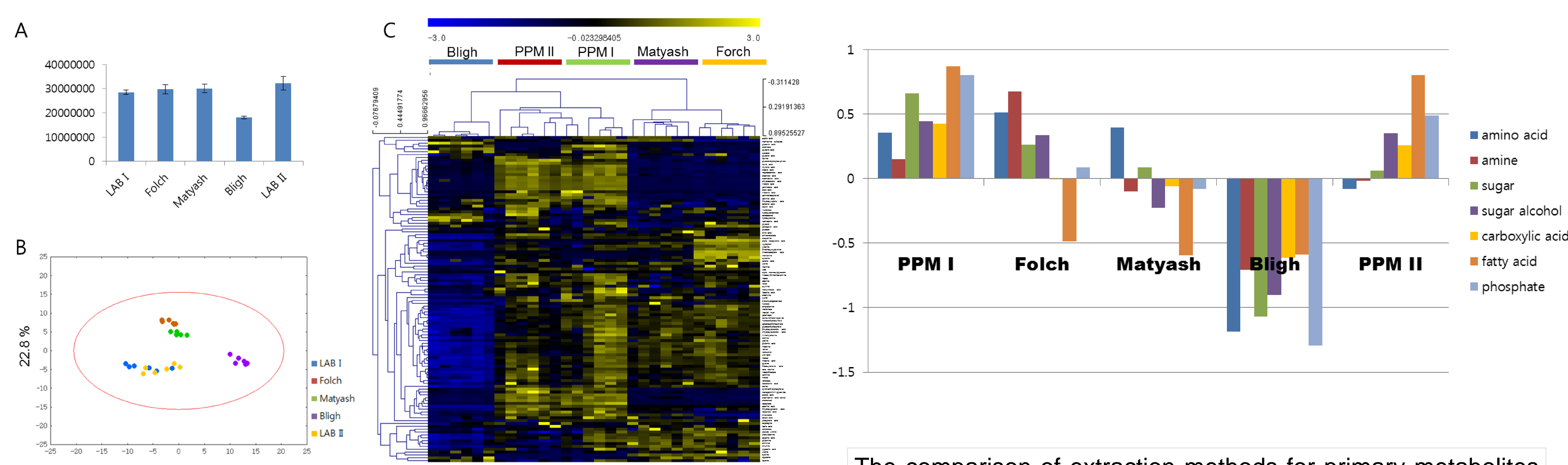
Lipid class-wise comparison of extraction methods

The sum of peak intensities of (A) lyso forms of phospholipids (LPE: lyso-phosphatidylethanolamine, LPG: lyso-phosphatidylglycerol, and LPI: lyso-phosphatidylinositol), (B) PE: phosphatidylethanolamine, (C) PG: phosphatidylglycerol, and (D) PI: phosphatidylinositol in negative ionization mode.

Differential extraction specificity according to details of the lengths and saturation level of acyl-chain

The intensities of each lipid of (A) PEs, and (B) PIs in negative ionization mode. Each lipid is arranged in X axis according to the molecular weight and the number of carbon. Y axis presents the intensity as a.u. (arbitrary unit).

The quantitative assessment of extraction methods for blood primary metabolites



The comparison of extraction methods for primary metabolites detected by GC-MS. (A) The sum of peak intensities (B) score scatterplot by PCA, and (C) Hierarchical Clustering Analysis using Spearman rank correlation and average linkage methods

The comparison of extraction methods for primary metabolites detected by GC-MS. Comparison of peak intensities of structurally classified metabolites between the extraction methods, PPM I, PPM II, Bligh, Forch, and Matyash methods. The relative peak intensity was computed by sums of peak intensities normalized by unit variance scaling.

Conclusions

		Precipitation method		Liquid-liquid extraction		
Method		PPM I	PPM II	Folch	Matyash	Bligh
Lipid profiling	Positive	★	★	★★★	★★★	★★
	Negative	★	★	★★★	★★★	★★
GC-MS profiling		★★★	★★★	★★	★★	★
Convenience		★★	★★	★★	★★★	★★

In overall, the Folch and Matyash methods were comparably effective and reproducible for lipidomic assessments The primary metabolite profiles were better assessed by the protein precipitation methods with organic solvent mixture. ★ fair ★★good ★★★ excellent

Acknowledgement

This study was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2013M3A9B6046416) and the Korea Healthcare Technology R&D Project, Ministry of Health & Welfare (grant No.: HN13C0076), Republic of Korea. The authors are grateful to Gert Wohlgemuth at UC Davis Genome Center for establishing the primary metabolite database.