

Lipidomics supplement

Internal and external standards for lipidomic analysis

The internal standard mixture contained the following lipid compounds (mg/ml) with heptadecanoic acid (C17:0) as the esterified fatty acid: *D-erythro*-Sphingosine-1-Phosphate (9.3 mg/ml; C17 Base, Avanti Polar Lipids), GPCho(17:0/0:0) (8.8 mg/ml; Avanti Polar Lipids), MG(17:0/0:0/0:0)[rac] (9.3 mg/ml; Larodan Fine Chemicals), GPGro(17:0/17:0)[rac] (9.6 mg/ml; Avanti Polar Lipids), Cer(d18:1/17:0) (9.2 mg/ml; Avanti Polar Lipids), GPSer(17:0/17:0) (8.6 mg/ml; Avanti Polar Lipids), GPCho(17:0/17:0) (9.9 mg/ml; Avanti Polar Lipids), GPA(17:0/17:0) (8.5 mg/ml; Avanti Polar Lipids), GPEth(17:0/17:0) (8.9 mg/ml; Avanti Polar Lipids), DG(17:0/17:0/0:0)[rac] (10.2 mg/ml; Larodan Fine Chemicals), and TG(17:0/17:0/17:0) (10.4mg/ml; Larodan Fine Chemicals). The labeled standard mixture consisted of GPCho(16:0/0:0-D3) (9.3 mg/ml; Larodan Fine Chemicals), GPCho(16:0/16:0-D6) (11.7 mg/ml; Larodan Fine Chemicals) and TG(16:0/16:0/16:0-¹³C3) (10.0 mg/ml; Larodan Fine Chemicals).

PLS/DA model

Partial least squares discriminant analysis (PLS/DA) (1; 2) was utilized as a supervised modeling method. Contiguous block cross-validation method (3) and Q^2 scores were used to optimize the model. Top loadings for latent variables associated with drug specific effects were reported. The VIP (variable importance in the projection) values (4) were calculated to identify the most important molecular species for the clustering of specific groups. Multivariate analyses were performed using Matlab version 7.2 (Mathworks, Inc.) and the PLS Toolbox version 4.0 Matlab package (Eigenvector Research, Inc.).

References

1. Geladi P, Kowalski BR: Partial least-squares regression: a tutorial. *Anal Chim Acta* 185:1-17, 1986
2. Barker M, Rayens W: Partial least squares for discrimination. *J Chemometrics* 17:166-173, 2003
3. Wise BM, Gallagher NB, Bro R, Shaver JM, Windig W, Koch JS: *PLS Toolbox 3.5 for use with Matlab*. Manson WA, Eigenvector Research Inc., 2005
4. Wold S, Esbensen K, Geladi P: Principal component analysis. *Chemometr Intell Lab Syst* 2, 1987

Lipidomics supplement figures

- Figure Lipidomics S1. Concentration levels of selected phospholipids. (A) Ether linked phosphatidylcholines and phosphatidylethanolamines. (B) Ester linked phosphatidylcholines. * $p < 0.05$, ** $p < 0.01$.
- Figure Lipidomics S2. PLS/DA model of lipidomics profile data. (A) Model scores for the first two latent variables. (B) Corresponding model loadings.

Figure Lipidomics S1



