Validation of untargeted metabolomics method

A pooled QC representing "mean" sample containing all analytes was used to monitor the stability and repeatability of the analytical methods. The QC sample was run three times at the beginning of analysis to ensure instrument equilibration, and then one every ten tested samples. The robustness test was conducted using the relative peak intensity (RPI) and relative retention time (RRT) to evaluate the anti-interference performance of the analytic method [1]. As shown in Table S1 and Table S2, eight representative peaks in the entire ion chromatogram were selected to calculate the RSD values of the RPI and RRT. These results revealed that the RSD values were all less than 3.0 %, indicating that the established LC-MS method was stable enough to perform the analysis.

Additionally, the QC datasets were also analyzed by PCA to assess the system stability and repeatability. The QC sample sets were found to be clustered in the center and segregated in a tight cluster in the PCA score plots in either positive or negative ionization mode (Supplementary Figure S1 and Figure S2). These data indicated that the developed LC-MS method was robust with excellent repeatability and stability, and could be utilized in metabolomics study.

Table S1 Reproducibility of retention time in QC sample in the metabolomics study

No.	Retention time (min)							
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8

No.								
1	1.79	3.21	4.85	7.26	9.00	10.01	12.75	15.08
2	1.79	1.79	4.85	7.26	8.99	10.01	12.75	15.08
3	1.79	1.79	4.85	7.26	8.99	10.01	12.75	15.08
4	1.79	1.79	4.85	7.26	8.99	10.01	12.75	15.08
5	1.79	1.79	4.85	7.26	8.99	10.01	12.75	15.08
6	1.79	1.79	4.85	7.26	8.99	10.01	12.75	15.08
RSD (%)	0	0	0	0	0.05	0	0	0

Table S2Reproducibility of area in QC sample in the metabolomics study

No.	Retention time (min)							
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8
1	53272	59205	380331	127880	226907	367832	169828	16882
2	51507	56820	368869	122688	226926	368099	168832	16294
3	51529	57642	371919	129263	222728	365857	168350	16614
4	50614	56113	369324	121524	220153	363364	160017	16785
5	51756	57229	364161	125141	223075	364146	165580	16780
6	52821	59923	388707	129262	228828	369339	169339	16861
RSD (%)	1.87	2.52	2.42	2.68	1.47	0.69	2.26	1.33

Table S3 Calibration curve in the targeted metabolomics study

Compound	unit	LOD	S/N	range
Uric acid	ug/mL	0.5	10	0.5~50
pc16	ug/mL	0.5	10	0.5~50
Eicosapentaenoic acid	ug/mL	0.2	10	0.2~20
Prostaglandin E2	ug/mL	0.1	10	0.1~10
Arachidonic acid	ug/mL	0.5	10	0.5~50
Palmitic acid	ug/mL	0.5	10	0.5~50
RvE1	ng/mL	0.1	3	0.1~10
5-HETE	ng/mL	0.1	3	0.1~10

[1] Y. Peng, F. Zhang, H. Tao, W. Wang, L. Sun, W. Chen, C. Wang, Simultaneous determination of multiple platycosides with a single reference standard in Platycodi Radix by high-performance liquid chromatography coupled with evaporative light scattering detection, J Sep Sci, 38 (2015) 3712-3719.
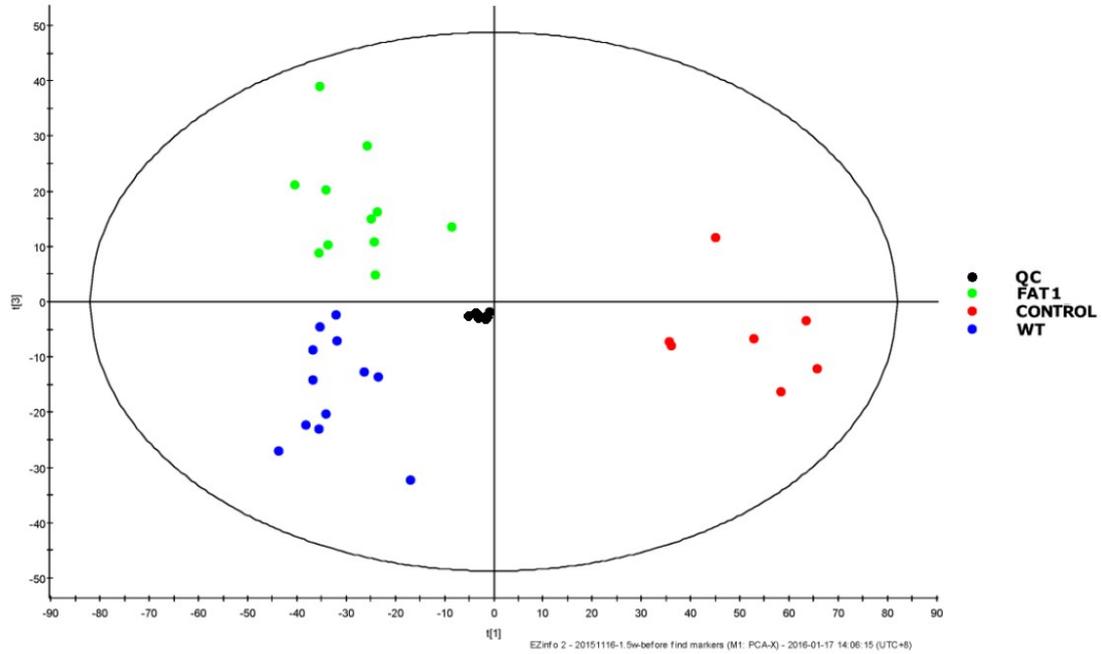
Supplementary figure legends

Figure S1. Score plots in PCA statistic of method validation with QC in the positive electrospray ionization mode
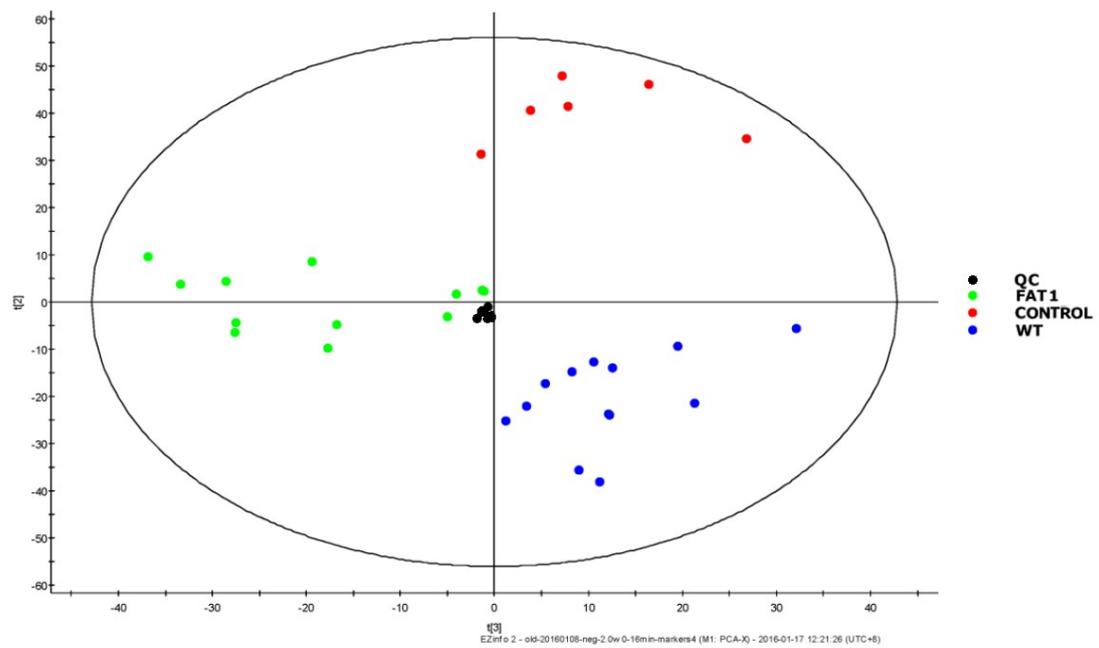

Figure S2. Score plots in PCA statistic of method validation with QC in the negative electrospray ionization mode
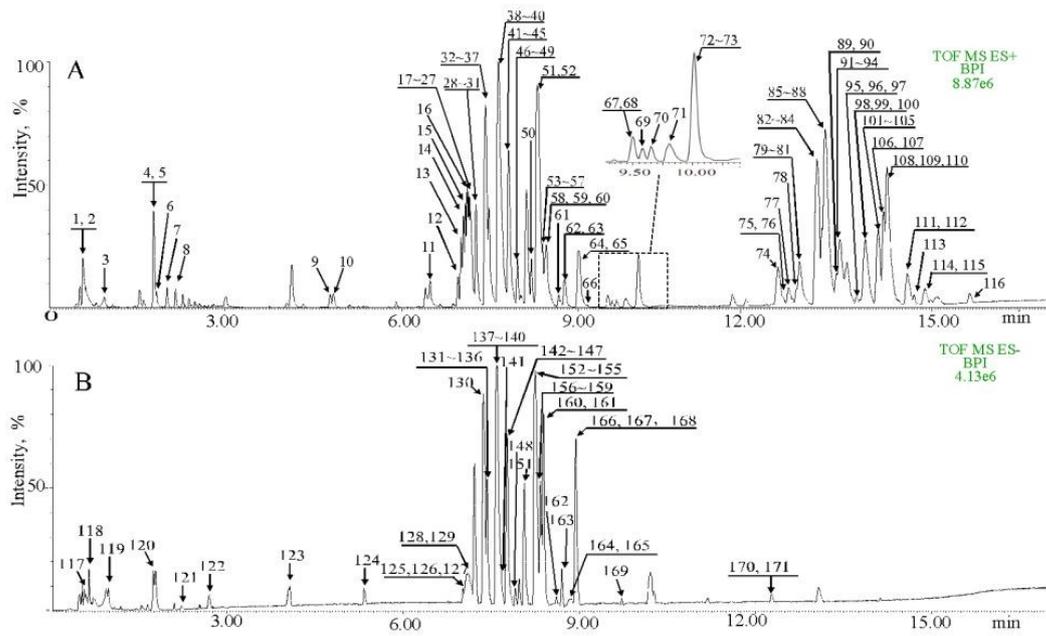
Figure S3. The Chromatogram of QC samples in the ESI positive mode and negative mode. The features with VIP more than one in Chromatogram were numbered and marked.