Supplementary information:

Screening and Validation for Plasma Biomarkers of Nephrotoxicity Based on Metabolomics in Male Rats

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Figure and table captions：

Figure S1 Histopathological examination in the validation stage by H&E staining (100× magnification).

Figure S2 BPI chromatograms of the plasma QC samples in positive ion mode based on UPLC-Q-TOF/MS.

Figure S3 PCA and PLS-DA score plots of the three nephrotoxic drugs (GM, ETI and AMB) at different timepoints after drug administration.

Figure S4 Venn diagrams of the three nephrotoxic drugs (GM, ETI and AMB) at different times for the integration analysis. A total of 117, 255, and 88 potential nephrotoxicity metabolites were obtained in GM, ETI and AMB groups, respectively.

Figure S5 Mass spectrum of LysoPC(22:5).

Table S1 Relative standard deviation of the QC samples in the methodology experiments.

Table S2 The parameters of each PLS-DA model.

The figures and tables are listed according to their order in the manuscript:

1. The histopathological examination in the validation stage

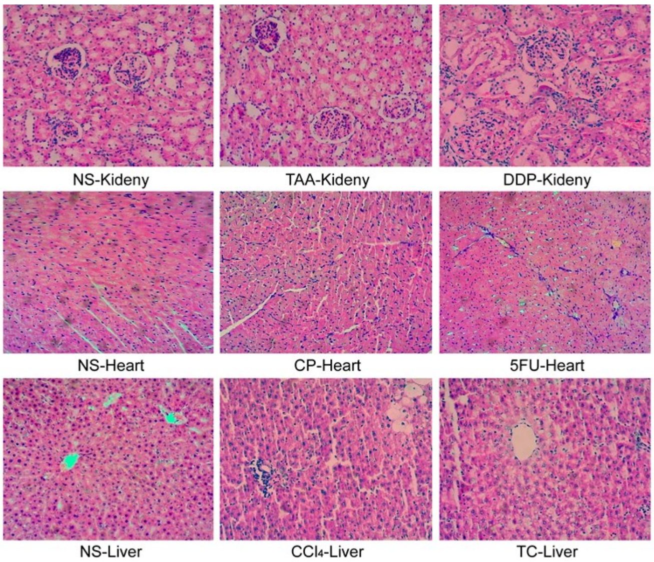

Figure S1 Histopathological examination in the validation stage by H&E staining (100× magnification).

2. The results of methodology experiments

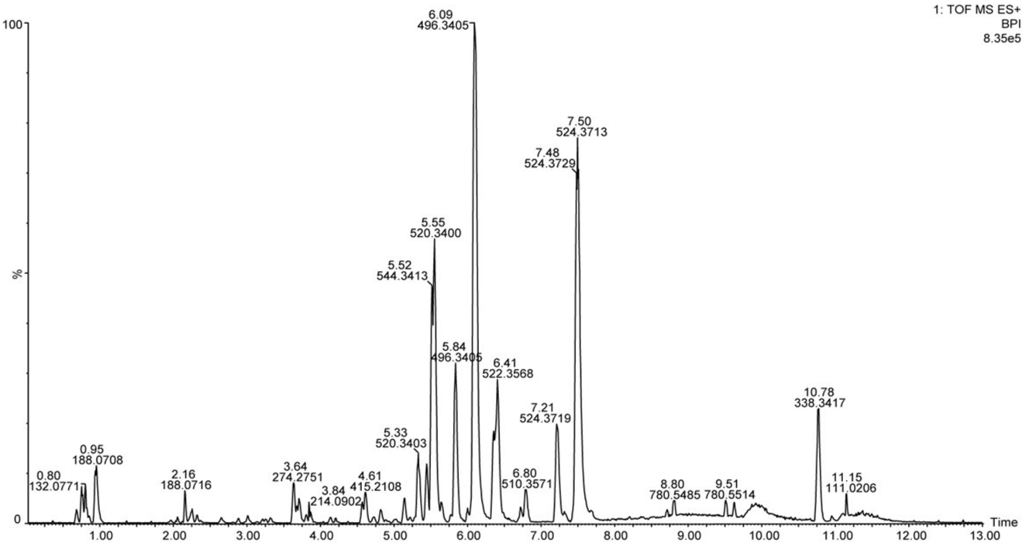

Figure S2 BPI chromatograms of the plasma QC samples in positive ion mode based on UPLC-Q-TOF/MS.

Table S1 Relative standard deviation of the QC samples in the methodology experiments.

	t_R(%)	**Peak area (%)**
Instrument precision	<0.87	<7.33
Method repeatability	<0.83	<9.32
Sample stability	<0.91	<9.95

3. The multivariate statistical analysis using PCA and PLS-DA

We obtained the PCA and PLS-DA score plots using SIMCA-P+11.5 software (Umetrics, Sweden), and the plots are shown in Figure S3. We used R^2X, R^2Y and Q^2 to evaluate the quality of the PLS-DA model. A R^2Y/Q^2 ratio closer to 1 indicates a more stable and reliable model. The R^2X, R^2Y and Q^2 values for each PLS-DA model are listed in Table S2.

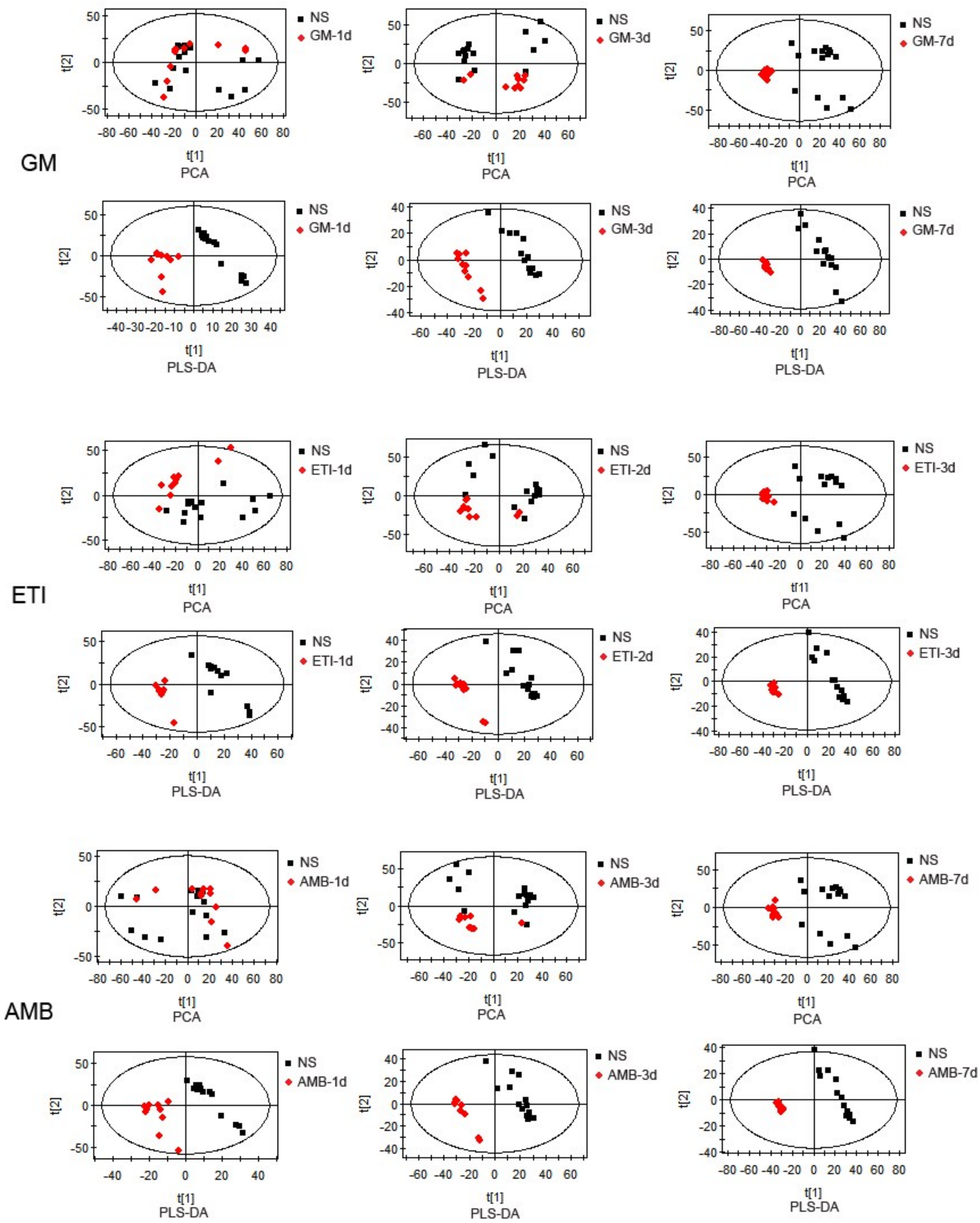

Figure S3 PCA and PLS-DA score plots of the three nephrotoxic drugs (GM, ETI and AMB) at different timepoints after drug administration.

Table S2 The parameters of each PLS-DA model.

	R^2X	R^2Y	Q^2
GM-1d	0.271	0.990	0.711
GM-3d	0.176	0.984	0.685
GM-7d	0.262	0.982	0.820
ETI-1d	0.291	0.997	0.769
ETI-2d	0.299	0.997	0.878
ETI-3d	0.231	0.988	0.780
AMB-1d	0.271	0.992	0.723
AMB-3d	0.311	0.996	0.888
AMB-7d	0.232	0.992	0.800

4. Venn diagrams of the three nephrotoxic drugs for the potential nephrotoxicity-associated metabolites

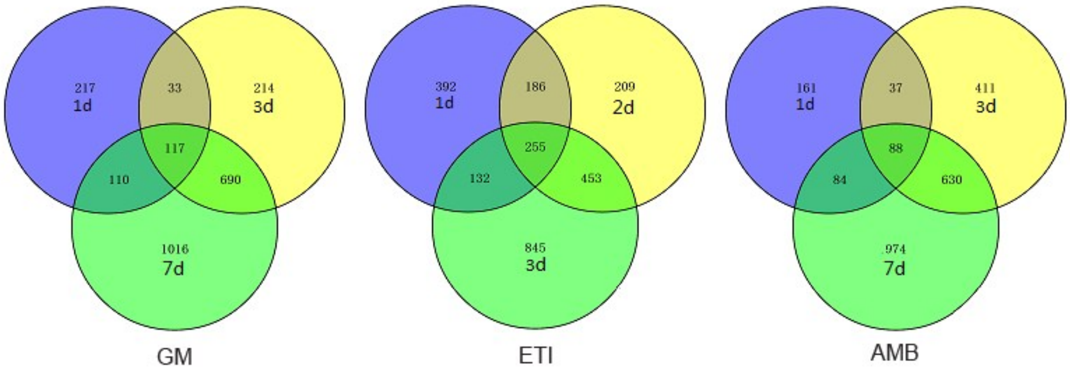

Figure S4 Venn diagrams of the three nephrotoxic drugs (GM, ETI and AMB) at different times for the integration analysis. A total of 117, 255, and 88 potential nephrotoxicity metabolites were obtained in GM, ETI and AMB groups, respectively.

5. The substance identification process:

The metabolites were identified based on MS/MS information. The ion at m/z 570.3549 is used as an example to explain the identification process; this ion had a relative retention time of 6.1319 min. First, the MarkerLynx V4.1 forecasted a molecular formula ($C_{30}H_{52}NO_7P$). The main fragment ions in the positive MS/MS spectrum were found at m/z 552.3, 184.0, 125.0 and 104.1, which could be ions formed by the $[M+H]^+$ after losing $-H_2O$, $-C_{25}H_{38}O_3$, $-C_{28}H_{47}NO_3$, and $-C_{25}H_{38}O_6P$, respectively. According to the ChemSpider and HMDB databases, we preliminary concluded that the metabolite was LysoPC(22:5). The mass spectrum of LysoPC(22:5) is shown in Figure S5.

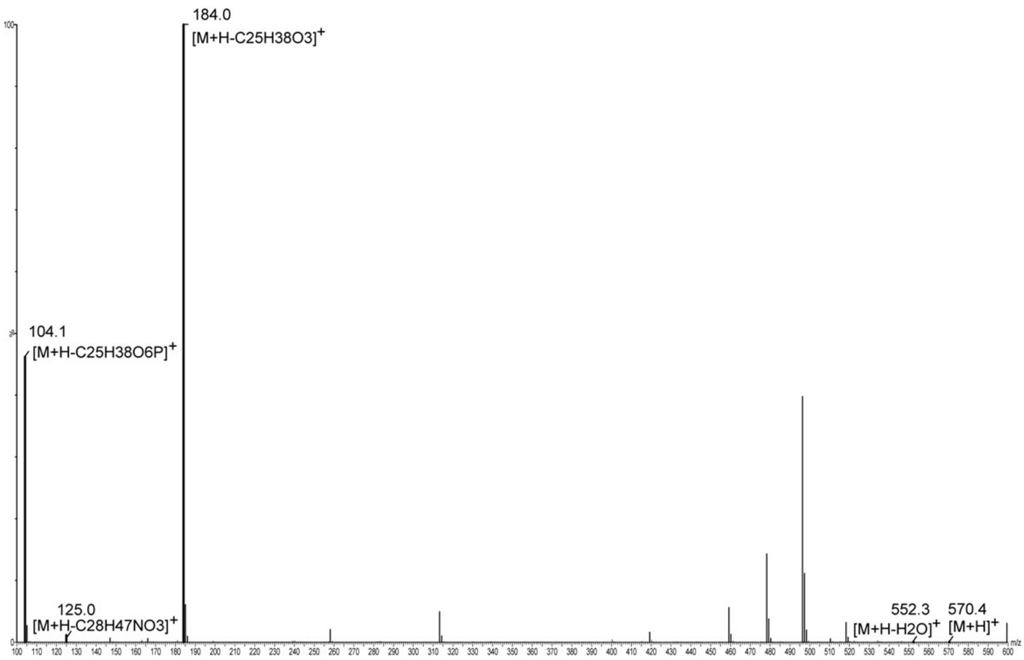

Figure S5 Mass spectrum of LysoPC(22:5).