Supplementary Materials: Nonylphenol Toxicity Evaluation and Discovery of Biomarkers in Rat Urine by a Metabolomics Strategy through HPLC-QTOFMS

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2. Materials and Methods

2.3. Metabolomics Analysis in Urine with HPLC-QTOF-MS

2.3.2. HPLC-QTOF-MS Data Acquisition

The HPLC system was equipped with a Waters XBridge™ C18 column (2.1 × 150 mm, 5 μm), and the column temperature was set to 25 °C. The mobile phases for metabolic fingerprinting consisted of 0.1% formic acid in Milli-Q water and 5 mM ammonium acetate in Milli-Q water (solvent A, positive electrospray ionization (ESI+) and negative electrospray ionization (ESI–), respectively), acetonitrile (solvent B), and methanol (solvent C) in both (ESI+) and (ESI−) analyses. The following multi-step elution gradient was used: 0–2 min, 90% solvent A; 2–40 min, 90%–5% solvent A, which was kept for 10 min; 50–51 min, 5%–90% solvent A, which was kept for 10 min and then changed back to the initial mobile phase rate; 40–50 min, 30% solvent B; 0% solvent B in other periods. The flow rate of the mobile phases was 0.3 mL/min. The sample injection volume was 5 μL for all experiments.

The ion source was a separated ESI ion source in TurboSpray™. In ESI+ mode, the initial parameters for metabolomics were as follows: ion spray voltage, 5500 V; nebulizing gas pressure (GS1), 60 psi; drying gas pressure (GS2), 50 psi; ion source temperature, 500 °C; focusing potential, 265 V; curtain gas pressure, 25 psi; declustering potential, 80 V. In ESI-mode, the ion spray voltage was −4200 V; the declustering potential was −60 V; the focusing potential was −265 V; the other parameter settings were the same with ESI+. At the same time, the TOF-MS and information-dependent acquisition (IDA) methods were used to collect MS and MS/MS spectra. The methods involved a TOF-MS experiment with spectra ranging from m/z 50 to 1200 for metabolomic analysis. Dynamic background ions were subtracted to acquire MS spectra, which were recorded with automatic collision energy. In this way, low- and high-energy fragment ions were both present in a single spectrum.

2.6. HPLC-MS/MS-Based Validation Test

Table S1. The conditions of HPLC-MS/MS to validate potential biomarkers.

Q1 Mass (Da)	Q3 Mass (Da)	DP (V)	CE (V)	Ion Mode
194.1	125/68.5	80	25/40	+
73.2	52.1/30.3	60	20/30	+
177.1	130/85.2	65	25/40	+
76.0	45.9/29.2	50	25/30	+
205.1	145.1/103.8	60	30/35	+
258.2	180.2/95.4	60	30/40	+
160	108/75.2	70	15/25	+
285.4	139/104.1	60	20/25	+
141.1	85.2/67	50	20/25	+
246.4	156/88.3	60	30/35	+
247.5	156.2/102	70	30/40	+
432.2	312/204.8	80	30/35	+
296.9	157.3/89	70	25/40	+
283.2	122.3/90.3	–65	–35/–30	−

445.2	203.8/134.7	–70	–25/–30	–
119.1	76/43.2	–70	–22/–28	–
268.7	172.2/126	–60	–30/–40	–
111.0	66/45.2	–65	–35/–40	–
216.9	156.3/89	–70	–35/–30	–
498.3	232.8/165.9	–60	–30/–40	–

3. Results

3.4. Multivariate Data Analysis of HPLC-TOF-MS Spectra

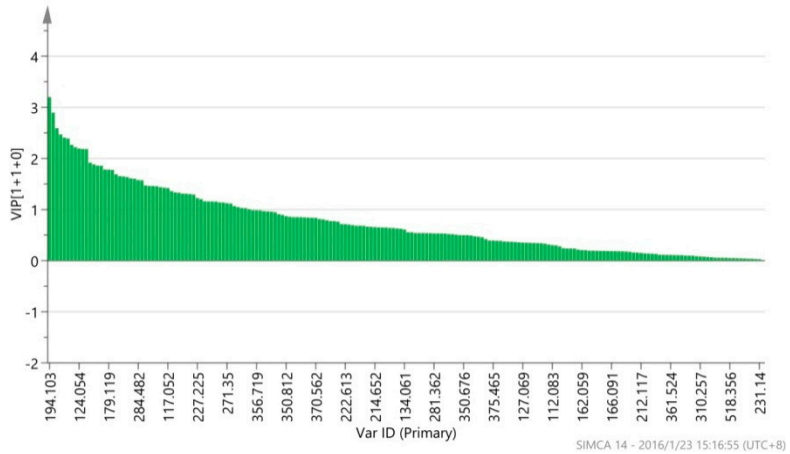
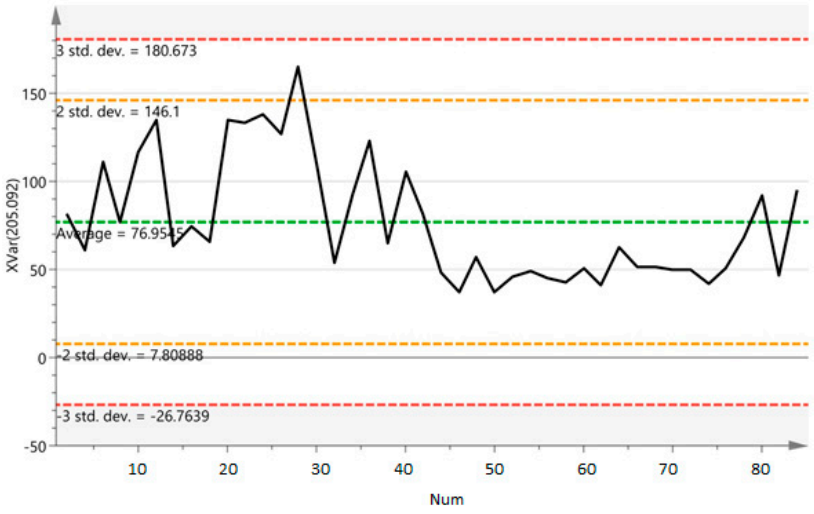


Figure S1. VIP distribution in the OPLS-DA model.

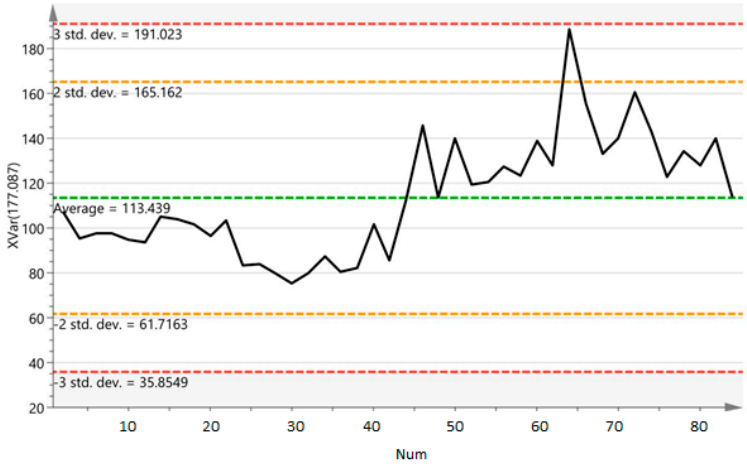
Table S2. The VIP, *p*-value, and trends of different ions found by OPLS-DA.

Varable ID (Primary)	M2 VIP (1 + 0 + 0)	*p*-Value	Trends
194.103	3.20173	0.032	upward
73.0634	2.89664	0.046	upward
177.087	2.59281	0.021	upward
309.232	2.47136	0.148	downward
76.0335	2.40796	0.022	upward
205.092	2.39113	0.046	downward
258.103	2.2673	0.027	upward
355.839	2.22234	0.147	downward
124.054	2.19655	0.074	upward
351.047	2.187	0.059	upward
160.041	2.18602	0.038	downward
144.038	1.91818	0.064	upward
288.473	1.88484	0.086	downward
236.605	1.86005	0.541	downward
130.113	1.85904	0.356	not obvious
796.756	1.78462	0.086	not obvious
179.119	1.78306	0.126	upward
266.679	1.77678	0.054	upward
285.377	1.69169	0.026	not obvious
141.13	1.65387	0.017	upward
246.426	1.64913	0.039	not obvious
360.631	1.63763	0.076	upward
247.535	1.61167	0.025	upward

301.742	1.6044	0.082	upward
284.482	1.57816	0.137	not obvious
125.099	1.57493	0.167	upward
399.577	1.47335	0.095	upward
432.229	1.4631	0.022	not obvious
306.731	1.46207	0.052	not obvious
211.972	1.42949	0.564	not obvious
117.052	1.42164	0.078	upward
296.961	1.36267	0.016	upward
283.192	1.33699	0.046	not obvious
445.242	1.33155	0.037	upward
119.081	1.31319	0.009	not obvious
268.686	1.31028	0.019	upward
284.302	1.30409	0.057	not obvious
122.07	1.29508	0.127	not obvious
227.225	1.22513	0.146	not obvious
116.087	1.20611	0.166	upward
111.078	1.16245	0.039	upward
314.246	1.1605	0.057	not obvious
216.917	1.15817	0.044	not obvious
366.265	1.15671	0.176	not obvious
240.168	1.11843	0.178	not obvious
498.326	1.06967	0.038	not obvious



(**A**)



(**B**)

Figure S2. (**A**) The m/z value 205.092 (upward for the 50 units group compared with the 0 unit) and (**B**) the m/z value 177.087 (downward for the 50 units group compared with the 0 unit).