Electronic Supplementary Materials

1. Detailed parameters for MS and MS/MS detection

The mass spectrometry analysis was performed on a Thermo Fisher Scientific Q Exactive Focus instrument equipped with an ESI ion source. The system operated at resolutions of 70000 FWHM (full width at half maximum) for full MS scans and 35000 FWHM for MS/MS scans. Automatic maximum injection time was enabled to dynamically optimize signal acquisition. Automatic gain control (AGC) targets were set at 1×10^6 ions for MS and 5×10^4 ions for MS/MS detection. Precursor ion isolation was achieved using isolation window as 3 m/z to ensure optimal mass selection. For MS/MS, a stepped normalized collision energy (NCE) protocol was implemented with three energy levels (30, 40, and 45 eV) to maximize fragment ion coverage while maintaining structural information. These parameters ensure high mass accuracy (<5 ppm) in MS detection while maintaining sufficient scan speed.

2. MS and MS/MS spectra of melatonin

The MS spectra of melatonin in both positive and negative ion modes, as well as the MS/MS spectra of collision induced dissociation of quasi molecular ions, are shown in Fig. S1. Although the ion response intensity of melatonin was similar in both positive and negative ion modes, the ion peak was relatively pure in negative ion mode. From the spectra, it can be seen that in the positive ion mode, the MS spectrum not only shows hydrogenation peak ($[M+H]^+$) and adduct ion peaks ($[M+K]^+$ and $[M+Na]^+$), but also a small mount of fragment ion peak, indicating that melatonin is more prone to cleavage in positive ion mode. In negative ion mode, both MS and MS/MS spectra exhibit strong and stable ion response intensity, which is more conducive to the structural analysis of compounds.

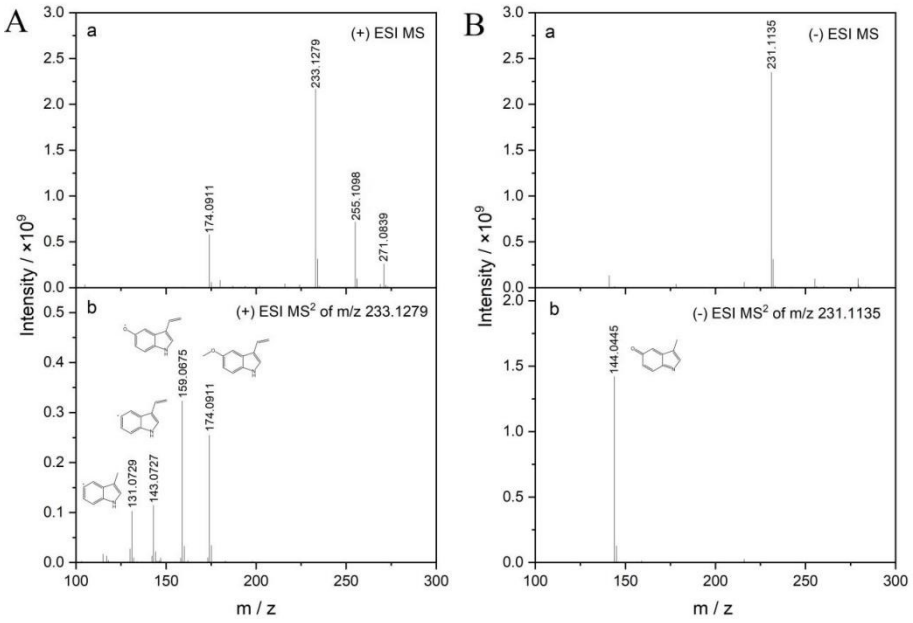

Fig. S1 (a) MS and (b) MS/MS of melatonin in (A) positive ion mode and (B) negative ion mode.

3. Extracted ion chromatograms (EICs) of m/z 463.2335, 231.1125, 263.1388 and 249.1230 in electrochemically simulated metabolite

The effects of applied potential on the electrochemical oxidation and simulated metabolism of melatonin as the target compound was investigated with the result shown in Fig. S2. By comparing the extracted ion chromatograms (EICs) of characteristic metabolites (m/z 463.2335, 231.1125, 263.1388 and 249.1230) in positive ion mode under open-circuit potential versus graded potentials (0.4 V, 0.8 V, and 1.0 V), the relative abundance of the products increased significantly with higher applied potentials. Additionally, elevated potentials promoted the formation of polymeric products. Corresponding EICs of these compounds in negative ion mode were also detected (Fig. 3A-D in the manuscript).

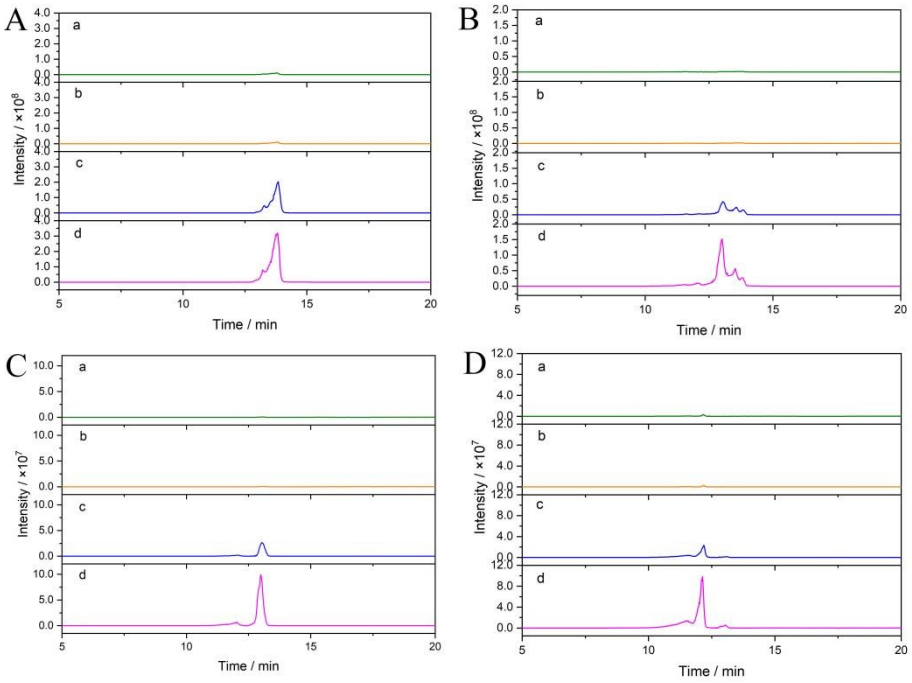

Fig. S2 EICs of melatonin simulated metabolites of m/z (A) 463.2335, (B) 231.1125, (C) 263.1388 and (D) 249.1230 in positive ion mode at (a) no voltage applied, (b) 0.4 V, (c) 0.8 V and (d) 1.0 V by EC-HPLC-MS.

4. EIC of m/z 229.0977 for simulating metabolites of rat liver microsomes

In the simulated metabolites of rat liver microsomes, the EIC of m/z 229.0977 in negative ion mode showed two other peaks in addition to the peak that appeared at about 13.6 min, which is different from the electrochemical products (Fig. S3). This may be attributed to the selectivity and specificity of oxidation sites in electrochemical oxidation.

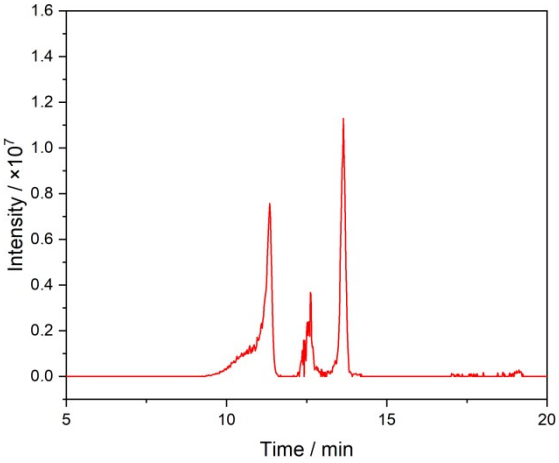

Fig. S3 EIC of m/z 229.0977 in negative ion mode for simulating metabolites of rat liver

microsomes.

5. EIC of m/z 536.1816 for phase II metabolites simulated by rat liver microsomes

In the phase II metabolites incubated with rat liver microsomes, remarkable conjugation product of melatonin and GSH was found (m/z 536.1816 in negative ion mode, Fig. S4), while other phase II metabolites were not detected. However, this product was not found in the phase II metabolites of electrochemical simulation.

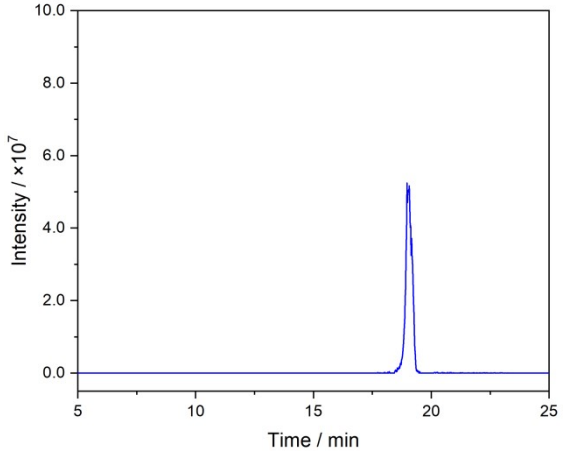

Fig. S4 EIC of m/z 536.1816 in negative ion mode for phase II metabolites incubated with rat liver microsomes *in vitro*.

6. Comparison of response signals of major metabolites obtained through electrochemical simulation and *in vitro* liver microsomes incubation

To further validate the feasibility and detection sensitivity of electrochemical simulation for melatonin metabolism under the experimental conditions of this study, the mass spectrometry response signals (peak areas of EICs for corresponding product ions) for identical metabolites obtained from these two experimental approaches were compared, as detailed in the following Table S1. As evidenced by the table, the EIC peak area ratio (S_E/S_L) for corresponding product ions consistently exceeds unity (>1), indicating enhanced detection sensitivity.

Table S1 Response signals in negative ion mode of major metabolites obtained through electrochemical simulation and *in vitro* liver microsomes incubation.

Symbol	Molecular formula	Electrochemical simulation (S_E)	Liver microsomes incubation (S_L)	S_E/S_L
P1	$C_{13}H_{14}O_2N_2$	5.55×10^9	1.20×10^8	46.25
P2	$C_{14}H_{18}O_3N_2$	2.70×10^9	4.19×10^7	64.44
P3	$C_{13}H_{16}O_3N_2$	1.17×10^9	5.89×10^7	19.86
P7	$C_{13}H_{16}O_4N_2$	1.48×10^8	7.65×10^7	1.93
P8	$C_{12}H_{12}O_3N_2$	1.95×10^7	1.41×10^7	1.38