Electronic Supplementary Material (ESI) for Toxicology Research.

Appendix

Metabolites analysis was carried out by the instrument of HPLC-MS/MS consisting symbiosis liquid chromatography system and an API 5500 triple quadruple mass spectrometer. After reaction of CYP-catalyzed NNK metabolism in aqueous solution, the incubation was terminated by pre-cold acetonitrile with a dilution factor of 10:1 (acetonitrile account for 90%), facilitating good peak shape of chromatograph and high sensitivity of instrument. As shown in Fig. 1, the chromatographic separation of metabolites was successfully achieved on a Waters Atlantis HILIC silica column (2.1×150 mm i.d. 5.0 μm) at 40℃ and equilibrated with 5% solvent A (10 mM ammonium acetate in water) and 95% solvent B (acetonitrile). The gradient program was (time, % A): 0–2 min, 5–15; 2–6 min, 15–25; 6–6.1 min, 25–5; 6.1–9 min, 95. The flow rate was kept at 0.5 mL/min throughout the run, and the sample volume injected was 10 μL.

ESI was performed in the positive ion mode (ionspray voltage 5000 V) with nitrogen as nebulizing (gas 1), heater (gas 2), curtain, and collision gas. Gas flow parameters were optimized (nebulizer 65, heater 60 and curtain gas 35 psi) by making successive flow injections while introducing mobile phase into the ionization source at 200 μL/min. The turbo ion spray temperature was set at 600℃. The dwell time was set at 100 ms.

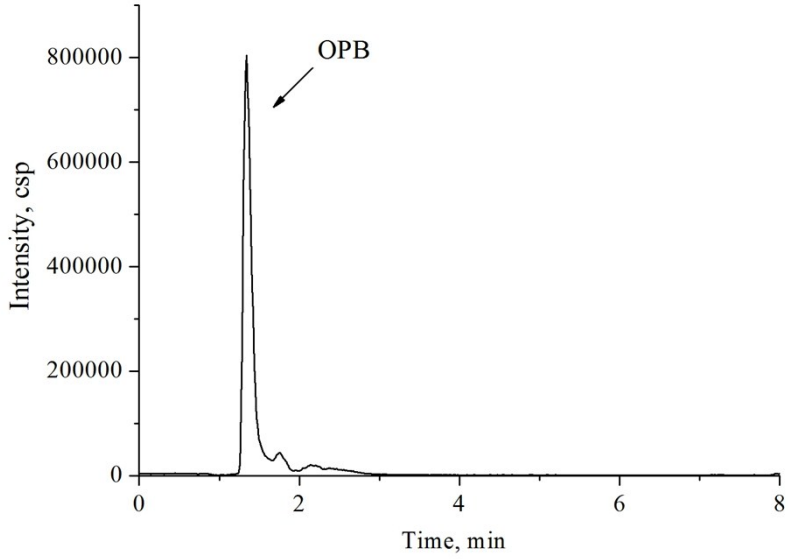
Mass spectral data on precursor and product ions were collected in multiple reaction monitoring (MRM) mode. The declustering potential, collision energy, and cell exit potential were optimized for each analyte (Table 1).

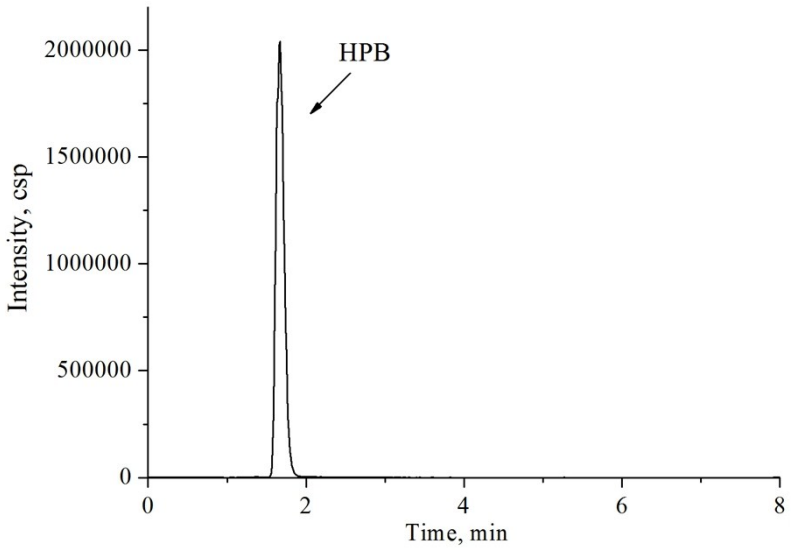
Table 1 Multiple reaction monitoring analysis of three metabolites and their respective internal standards

Analyte	Precursor ion (m/z)	Product ion (m/z)	Declustering potential (V)	Collision energy (V)	Cell exit potential (V)
OPB	164.0[a]	78.9	160	31	11
	164.0[b]	134.0	160	31	11
HPB	166.1[a]	106.0	140	23	13
	166.1[b]	80.0	140	23	13

OPBA	180.1[a]	134.1	140	28	12
	180.1[b]	80.0	140	28	12
d_4-HPB	170.1	106.0	140	23	13
d_4-OPBA	184.1	138.1	140	28	12

[a] Quantitation ion. [b] Confirmation ion.





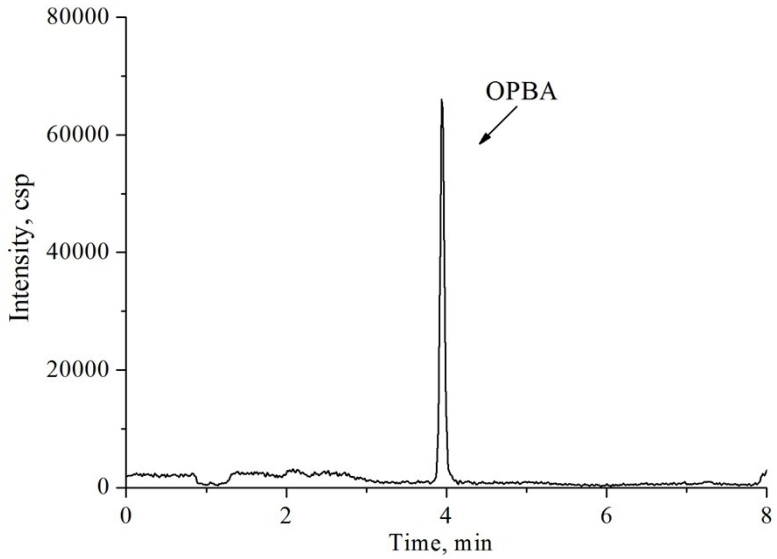


Fig. 1 Chromatograms of OPB, HPB and OPBA in the background of acetonitrile/water solution contained NNK, CYP2A13, NADPH-generating system, $MgCl_2$ and Tris buffer.