Supplementary Information

Accumulation, transformation, and environmental implications of acetaminophen and ibuprofen in sewer systems

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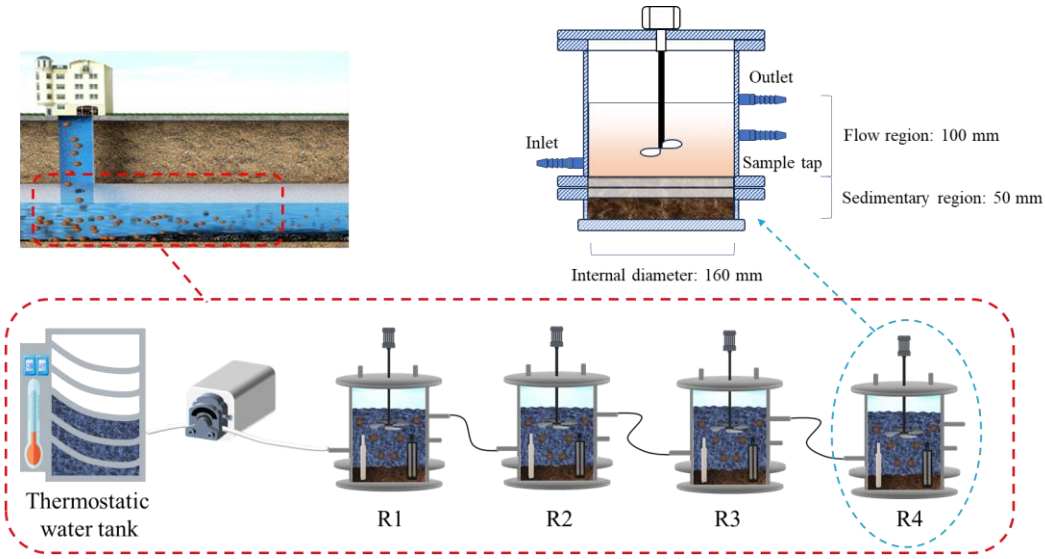

Fig. S1 Sketch of multi-stage, gravity-driven sewer reactor.

Based on previous studies, a multi-stage gravity-driven sewer reactor was designed to simulate a real sewer system. Each reactor had an effective volume of 2L (160mm in diameter, with a water depth of 100mm). Equal amounts of real sewer sediment were carefully weighed and placed at the bottom of each reactor. After one year of stable operation, mature biofilms formed on the reactor walls, with a biofilm area/volume ratio (A/V) of 25m²/m³. The flow rate was adjusted to 16.75 mL/min to achieve a hydraulic retention time (HRT) of 2 hours for each reactor stage.

The water flow velocity was simulated by adjusting the stirring paddle speed to regulate the shear stress of the water flow. The specific calculation is as follows:

Reynolds number and shear stress were calculated using the following equations:

$$Re = \frac{\mathrm{N}d^2}{\gamma}$$

where, *Re* is the dimensionless Reynolds number, N is the stirring speed (rpm), d is the paddle stirrer diameter (m), and γ is the kinematic viscosity of water (m2/s). The kinematic viscosity of water was taken as 9.55×10^{-7} at 22 °C.

$$f = 0.078Re^{-0.25}$$

$$f = \frac{2\tau}{\mu^2 \rho}$$

where, f is the average friction factor, τ is the shear stress (N/m^2), μ is the average velocity (m/s), and ρ is the water density (1000 kg/m^3). With the stirring paddle diameter designed at 80mm, calculations show that at a stirring speed of 50 rpm, the simulated water flow velocity reaches 0.209 m/s. Under a hydraulic retention time of 8 hours, the simulated pipeline length is approximately 6 km.

Table S1 Stirring speed and water flow velocity.

Stirring speed	**Reynolds number**	**Average water velocity**	**Shear stress**
rpm		m/s	N/m^2
50	5584	0.209	17.6

T**able S2** Synthetic wastewater composition

Reagent	Concentration (mg/L)	Reagent	Concentration (mg/L)
$C_6H_{12}O_6$	200	$FeSO_4·7H_2O$	2
NH_4Cl	60	Yeast	30
$Na_2HPO_4·12H_2O$	25	$CaCl_2$	3
$NaH_2PO_4·12H_2O$	25	CH_4N_2O	30
$KHCO_3$	50	Peptone	20
$NaHCO_3$	130	Soy peptone	20
$MgSO_4$	50	Tryptone	20
$MnSO_4·H_2O$	2	Casein peptone	20

Table S3 Water quality indicators of the synthetic water

Parameters	Concentration (mg/L)
COD	360±16
TN	46.3±3.6
TP	7.1±0.3
NH_4^+-N	20.4±1.8
NO_3^-N	4.0±0.5
SO_4^{2-}S	14.1±0.7
pH	7.5±0.3

Text S1 The methods for the Ultimate 3000 UHPLC-Q Exactive mass spectrometry.

Acetaminophen (APAP): mobile phase A consisted of 0.1% formic acid in 5 mM ammonium formate solution, and mobile phase B was acetonitrile. The flow rate was set at 0.4 mL/min. The gradient elution program was as follows: 0 min, 95% A; 10 min, 5% A; 25 min, 5% A; 27 min, 95% A; 30 min, 95% A. Mass spectrometry detection utilized a high-efficiency electrospray ionization source (HESI), with sheath gas and auxiliary gas flow rates set at 40 arb and 10 arb, respectively. The spray voltage was set to 3.8 kV in positive ion mode and 3.0 kV in negative ion mode, with the capillary temperature maintained at 320°C. The mass spectrometry detection was performed using full scan mode (range: 100–1500 m/z) and fragment ion scanning (range: ≥50 m/z).

Ibuprofen (IBU): mobile phase A consisted of 0.1% formic acid in 5 mM ammonium formate solution, and mobile phase B was methanol. The flow rate was set at 0.4 mL/min. The gradient elution program was as follows: 0-2 min, 90% A; 13 min, 20% A; 13-20 min, 20% A; 25 min, 90% A. Mass spectrometry detection utilized a high-efficiency electrospray ionization source (HESI), with sheath gas and auxiliary gas flow rates set at 40 arb and 10 arb, respectively. The spray voltage was set to 3.8 kV in positive ion mode and 3.0 kV in negative ion mode, with the capillary temperature maintained at 320°C. The mass spectrometry detection was performed using full scan mode (range: 100–1500 m/z) and fragment ion scanning (range: ≥50 m/z).

Table S4 Metagenomic Quality Summary.

Samples	Clean reads	Clean base (bp)	Percent in raw reads (%)	Percent in raw bases (%)	N50	Average length (bp)
Control-U1	42850020	6456006693	99.04	98.82	771	478.50
Control-U2	42919640	6467454595	99.04	98.83	877	502.19
Control-U3	47371634	7138357701	99.16	98.96	908	504.99
APAP-U1	47245988	7116347761	99.09	98.85	791	482.93
APAP-U2	44569832	6714179734	99.21	98.97	709	464.83
APAP-U3	44343088	6680363196	99.28	99.05	687	456.59
IBU-U1	39619142	5960245341	97.57	97.21	663	474.07
IBU-U2	40118774	6034874971	97.89	97.52	649	469.13
IBU-U3	38896628	5854270307	97.96	97.64	687	472.30
Control-D1	42255066	6363359002	98.53	98.26	929	518.20
Control-D2	41512094	6251680300	98.61	98.35	981	526.06
Control-D3	48524732	7308443595	98.83	98.57	984	524.11
APAP-D1	49872854	7507292895	98.73	98.42	871	508.05
APAP-D2	51085026	7690763754	98.65	98.36	877	510.32
APAP-D3	53756014	8092351932	98.74	98.44	912	515.11
IBU-D1	43509164	6546807334	98.28	97.94	824	506.13
IBU-D2	42709182	6430564624	98.49	98.21	746	488.48
IBU-D3	44504208	6701834302	98.70	98.43	763	487.01

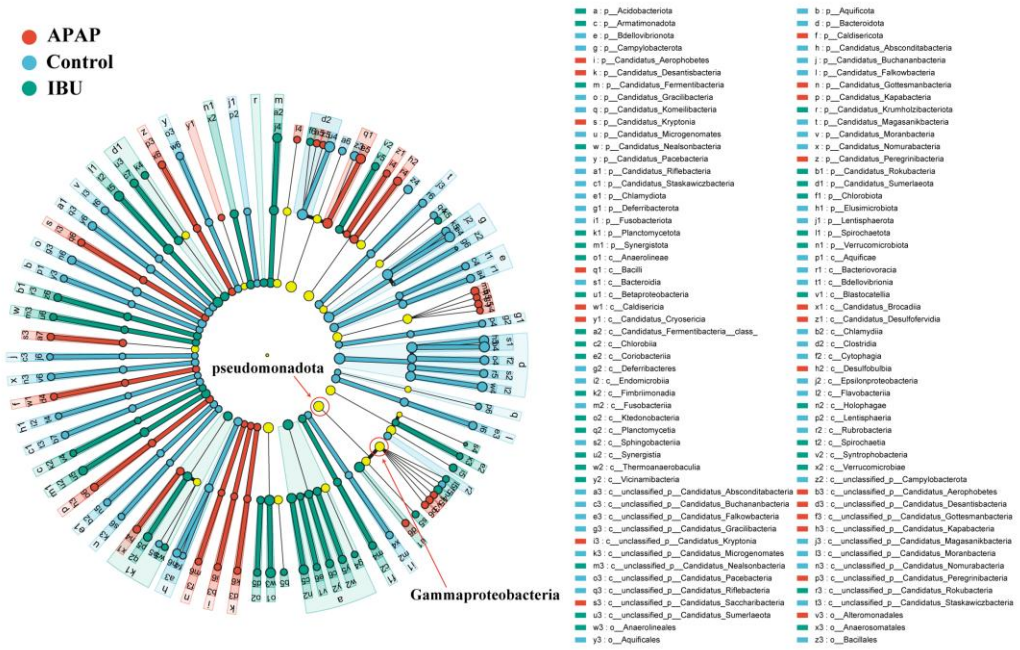

Fig. S2 The LEfSe hierarchical diagram (from Phylum to Order) of the communities in sewer systems.

Pseudomonadota was significantly enriched in all three groups (control, APAP, and IBU). Given that both *Escherichia coli* and *Pseudomonas aeruginosa* belong to the *Gammaproteobacteria* class of the *Pseudomonadota* phylum, their protein profiles are highly relevant in modeling pharmaceutical-protein interactions under these conditions. Thus, this taxonomic advantage supports the rationale for using the proteins (Wza and PslG) of these two well-characterized model strains as representative receptor candidates in docking simulations. In addition, these strains are known for their metabolic versatility and are frequently detected in sewage, which provides ecological and functional relevance to the simulation results.

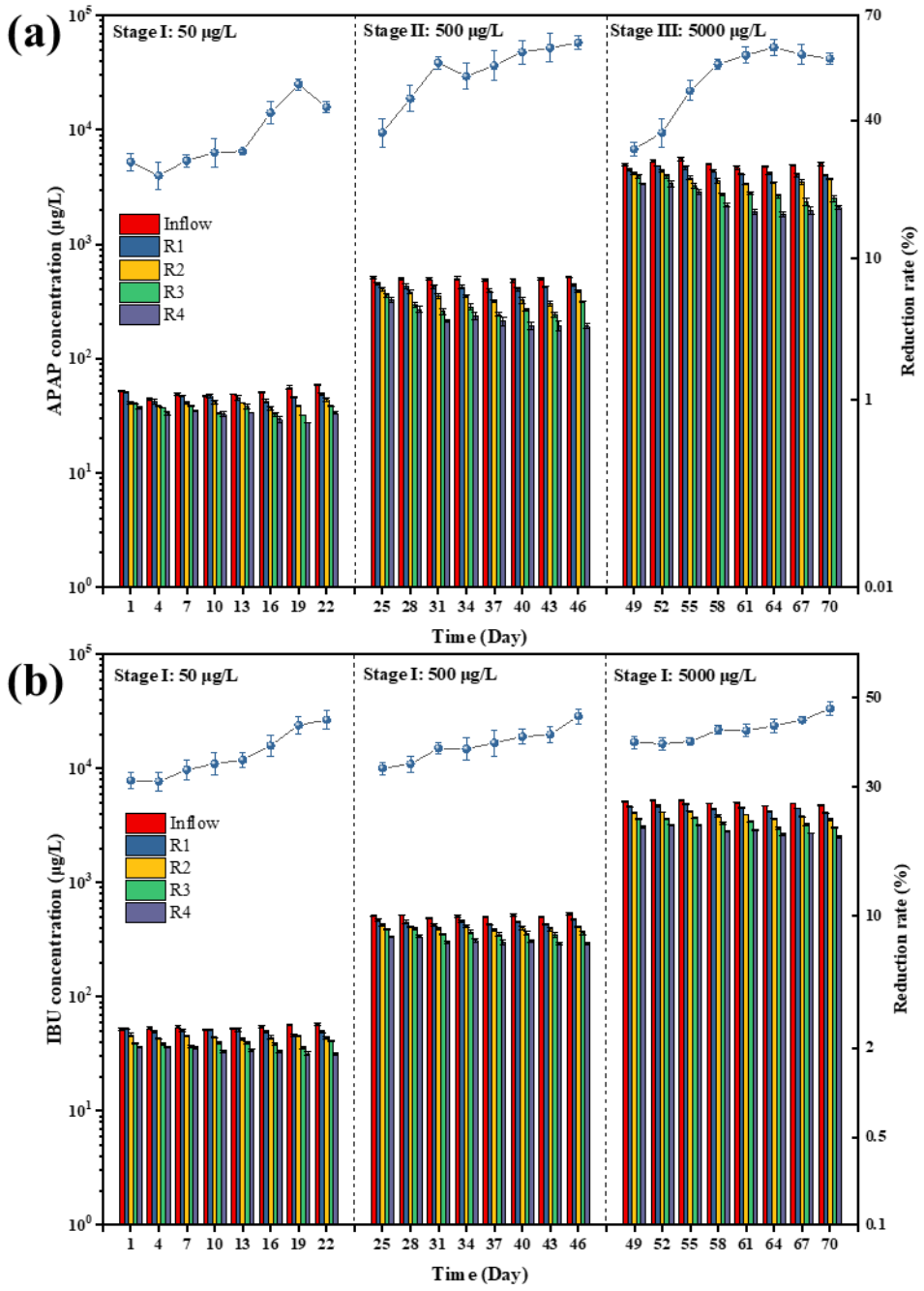

Fig. S3 Concentration distribution and attenuation rates of APAP (a) and IBU (b) in the laboratory gravity sewer system during the experiment.

Table S5 Pseudo-first-order kinetic model parameters for each stage.

Stage	*b*		*k*		*P*-value	Pearson	R^2	Adj-R^2
	95% confidence interval		95% confidence interval					
	lower limit	upper limit	Lower limit	upper limit				
Acetaminophen (APAP)								
Equation: $Ln(Ct) = b + kt$								
I	3.932 ± 0.021		-0.057 ± 0.004		< 0.001	-0.904	0.818	0.813
	3.889	3.975	-0.065	-0.048				
II	6.236 ± 0.031		-0.100 ± 0.006		< 0.001	-0.932	0.869	0.865
	6.174	6.299	-0.113	-0.087				
III	8.552 ± 0.041		-0.093 ± 0.008		< 0.001	-0.873	0.762	0.756
	8.468	8.636	-0.111	-0.076				
Ibuprofen (IBU)								
Equation: $Ln(Ct) = b + kt$								
I	4.008 ± 0.012		-0.059 ± 0.002		< 0.001	-0.969	0.939	0.937
	3.984	4.033	-0.064	-0.054				
II	6.234 ± 0.012		-0.060 ± 0.002		< 0.001	-0.969	0.940	0.938
	6.209	6.258	-0.065	-0.055				
III	8.533 ± 0.019		-0.070 ± 0.004		< 0.001	-0.949	0.901	0.898
	8.496	8.571	-0.078	-0.063				

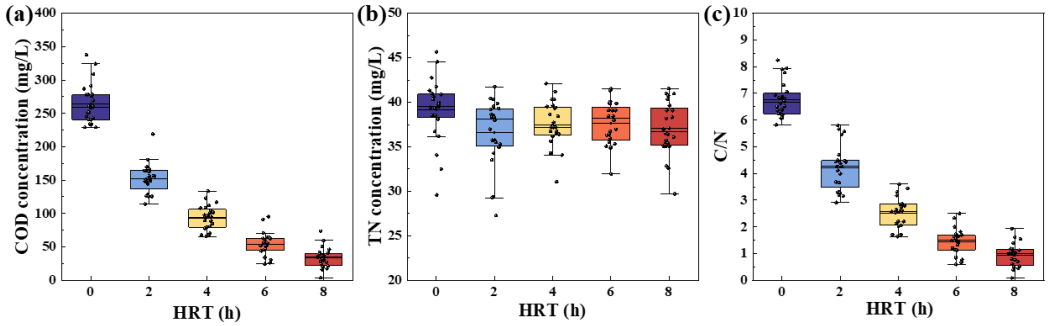

Fig. S4 Chemical oxygen demand (COD) (a), total nitrogen (TN) (b), and carbon-to-nitrogen ratio (C/N) (c) in the reactors under different HRT.

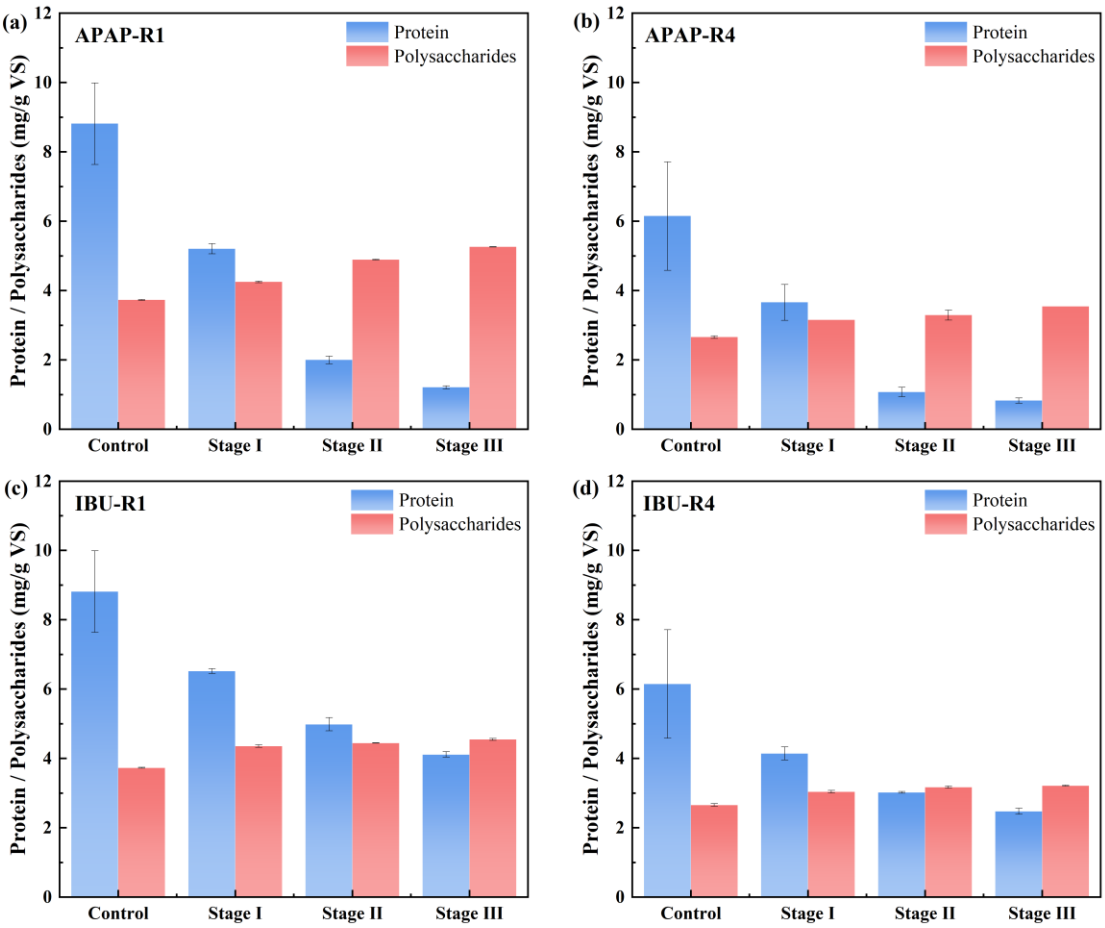

Fig. S5 Protein and polysaccharide content in the extracellular polymeric substances (EPS) of sediments at different stages, (a) APAP-R1; (b) APAP-R4; (c) IBU-R1; and (d) IBU-R4.

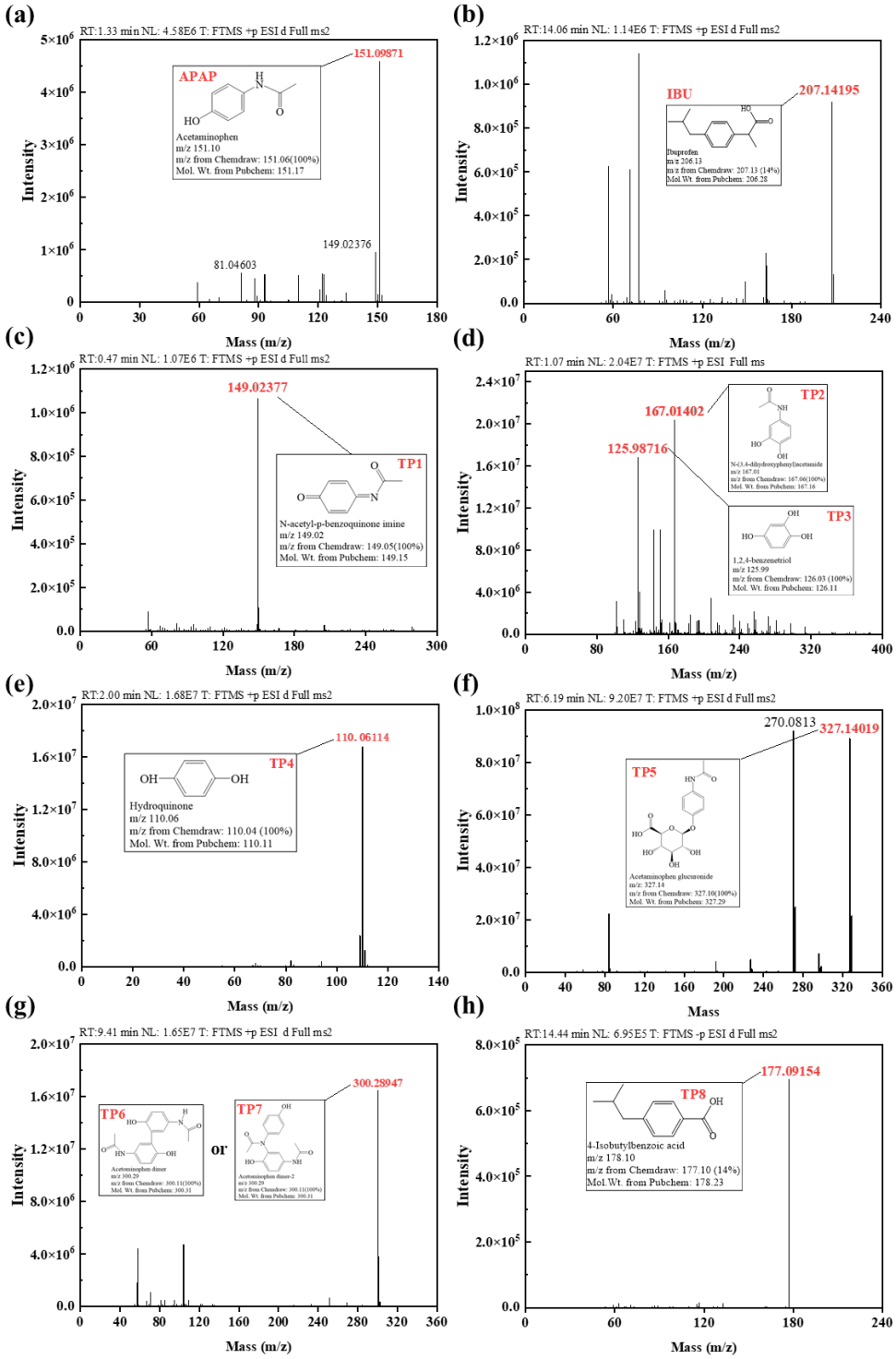

Fig. S6 Mass spectra of APAP and its intermediates, (a) Acetaminophen; (b) Ibuprofen; (c) Hydroquinone; (d) 1,2,4-benzenetriol and N-(3,4-dihydroxyphenyl)-acetamide; (e) N-acetyl-p-benzoquinone imine (NAPQI); (f) Acetaminophen glucuronide; (g) Acetaminophen dimers; (h) 4-Isobutylbenzoic acid.

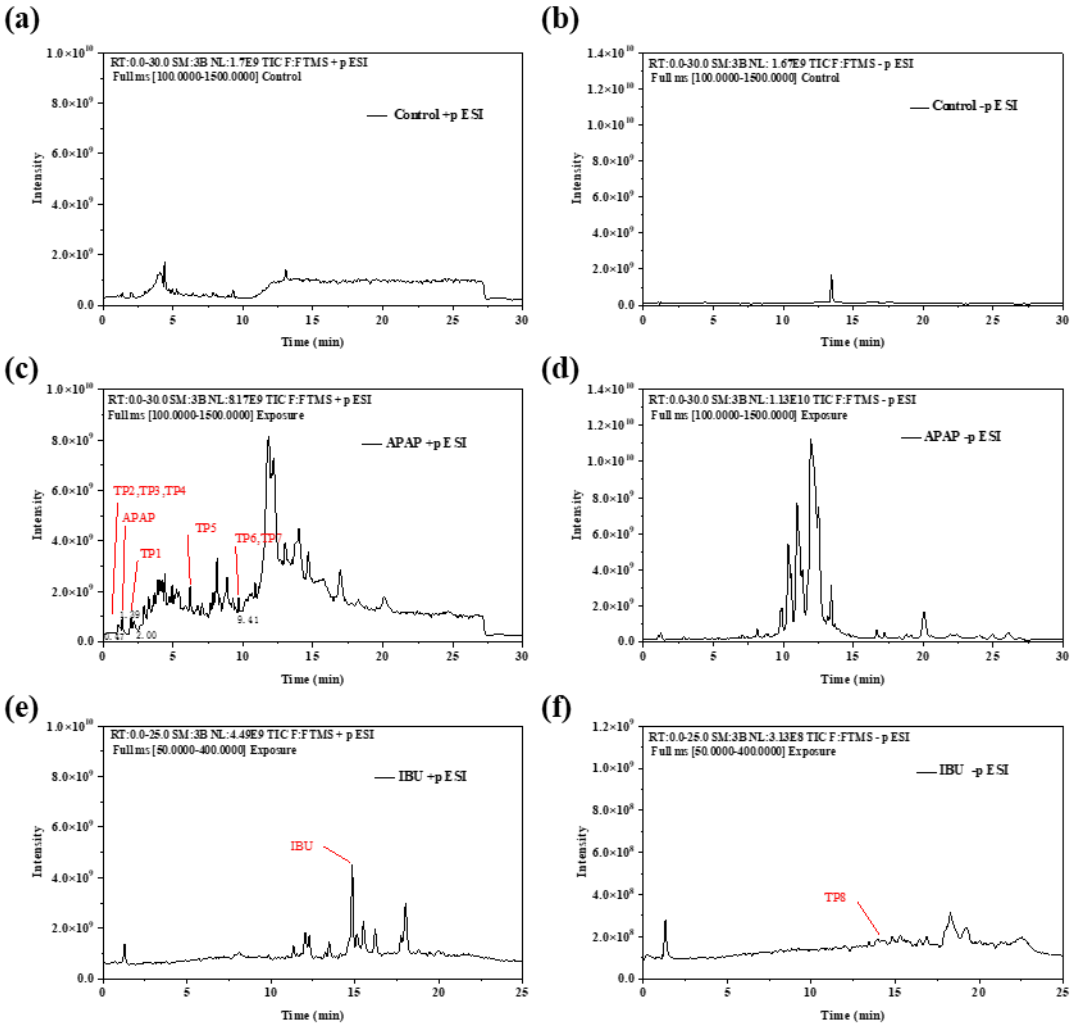

Fig. S7 Total ion chromatograms (TIC), (a) + p ESI control group; (b) -p ESI control group; (c) + p ESI APAP exposure group; (d) -p ESI APAP exposure group; (e) + p ESI IBU exposure group; (f) -p ESI IBU exposure group.