Supporting Information

Spatial and Temporal Variability of Micropollutants Within a Wastewater Catchment System

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Supplemental Experimental

Sample Collection

Briefly, samples were collected as twenty-four-hour time-weighted composites at the wastewater treatment plant influent, effluent, and seven sub-catchment locations before the treatment plant.

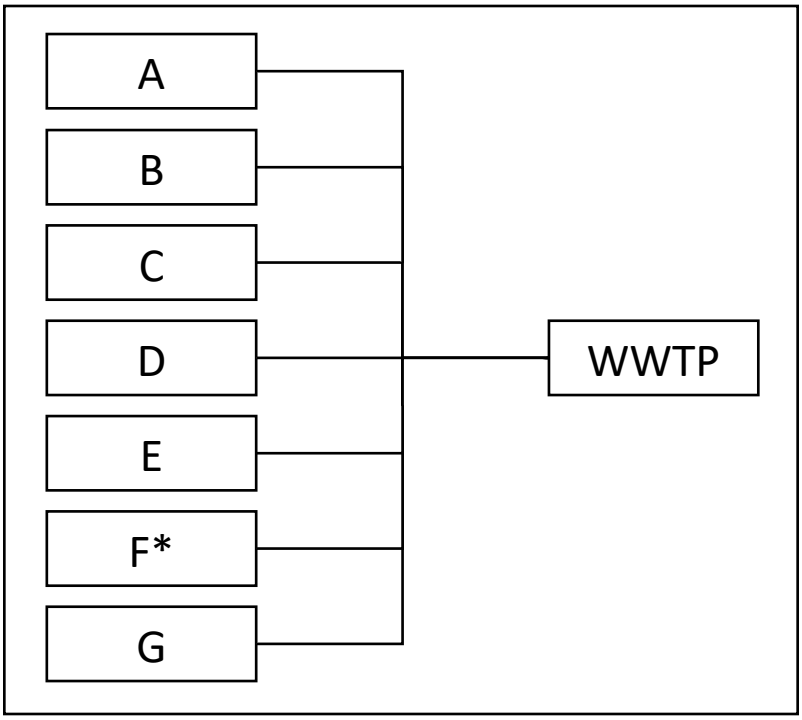

Figure S1. Schematic of connections within sewer system. Trunkline sites A, B, C, D, E, G, plus combined influent and treated effluent of the WWTP were sampled monthly. *F for one less month than other sites and is thus omitted from this analysis.

Liquid Chromatography and Mass Spectrometry

Data was acquired using an Agilent 1260 Infinity HPLC coupled to an Agilent 6530 QTOF-MS. Chromatographic separation was achieved with a Zorbax Eclipse Plus C18 column (100 mm, 2.5 mm, 1.8 µm, Agilent Technologies, Inc.) Ultra-pure water plus 0.1% (v/v) formic acid (A), and acetonitrile plus 0.1% (v/v) formic acid (B) were used as mobile phases for positive electrospray ionization. The initial gradient was held at 2% B for 1.5 minutes, followed by a linear increase to 100% B at 16.5 min and held for 4 min. A postrun column equilibrium time of 3 minutes was used resulting in a total run time of 23.5 minutes. Instrumental parameters are included in Table S1. Data acquisition was done using *All-Ions* fragmentation method (data independent acquisition).

Gas Chromatography Mass Spectrometry

Extracts prepared for analysis on the GC-QTOF-MS (Agilent 7890B GC coupled to an Agilent QTOF/MS 7200B with a HP-5MS 30 m × 0.25 mm, 0.25 µm column, Agilent Technologies, Inc.) were run once in negative chemical ionization (NCI) mode using methane as collision gas and a second time in electron ionization (EI) mode.

Method Evaluation, Quality Assurance, and Quality Control Measures

Prior to applying this analytical method to the wastewater samples tested here, the performance of the method was evaluated for 22 pesticides in wastewater influent samples analyzed in positive electrospray ionization mode. Recoveries of spiked compounds ranged from 57-120% with an average of 89% recovery, and method detection limits were between 4 and 34 ng/L. Quality assurance and quality control measures applied during the study included preparation and analysis of one method blank sample and one matrix spike duplicate in each monthly batch of 8 samples. Performance of the overall method for nontarget feature extraction was evaluated by assessing the ability to align, recover spectra, and identify compounds in the matrix spike samples. When spiked at 100 ppb into wastewater influent, the alignment and deconvolution algorithms correctly isolated and identified 100% of the spiked compounds in for 19/23 compounds, with high detection frequencies for most of the remaining compounds and was over 50% effective for 18/23 compounds spiked at 20 ppb (Table S6).

Table S1: LC-QTOF-MS parameters for All Ions acquisition and targeted MS/MS (tMS/MS) acquisition

Agilent 6530 QTOF	
Injection volume (µL)	20
LC settings	
Mobile phase	
A (pos)	milliQ water + 0.1% formic acid
B (pos)	Acetonitrile + 0.1% formic acid
Solvent flow (µL/min)	350
Gradient	
	2% B for 1.5 min
	2-100% B for 15 min
	100% B for 5 min
	Equilibration to initial conditions for 3 min
Column	Zorbax Eclipse Plus (2.5 mm ID, 1.8 µm particle size)
Guard-column	Zorbax Eclipse Plus (2.5 mm ID, 1.8 µm particle size)
Column temperature (°C)	30
Source parameters	
Gas temperature (°C)	300
Gas flow rate (L/min)	12
Nebulizer (psi)	25
Sheath gas temperature (°C)	350
Sheath gas flow rate (L/min)	11
Capillary (V)	3000
Nozzle voltage (V)	1500
Fragmentor (V)	110
Skimmer (V)	65
Cell RF (eV)	0,10, 20, 40
Octopole RF (V)	750
MS Settings	
Gas temperature (°C)	300
Drying gas flow rate (L/min)	12
Nebulizer (psig)	25
Sheath gas temperature (°C)	350
Sheath gas flow rate (L/min)	11
Vcap	3500
Fragmentor (V)	110

Reference mass correction	121.0509, 922.0098
All Ions Data independent acquisition (DIA)	
Scan range	50-1050 m/z
Scan speed	4 spectra/s
Collision energies (eV)	0, 10, 20, 40
Targeted MS/MS Data dependent acquisition (DDA)	
MS1	
Scan range	30-1050 m/z
Scan speed	4 spectra/s
Collision energies (eV)	0
Maximum time between MS (s)	3
MS2	
Scan range MS2	30-1050 M/Z
Scan speed	6 spectra/s
Collision energies (eV)	0, 10, 20, 40
Retention time window (min)	0.8
Isotopic width	Narrow (1.3 m/z)
Z	1

Table S2: General MS-DIAL alignment parameters for tMS/MS and All Ions data

	tMS/MS	**All Ions**
Project		
MS1 Data type	Profile	Profile
MS2 Data type	Centroid	Profile
Ion mode	Positive	Positive
Target	Metabolomics	Metabolomics
Mode	ddMSMS	diMSMS
Data collection parameters		
Retention time begin	4	0
Retention time end	100	100
Mass range begin	0	0
Mass range end	2000	5000
MS2 mass range begin	0	0
MS2 mass range end	2000	0
Centroid parameters		
MS1 tolerance	0.01	0.01
MS2 tolerance	0.025	0.025
Isotope recognition		
Maximum charged number	2	2
Data processing		
Number of threads	5	5
Peak detection parameters		
Smoothing method	LinearWeightedMovingAverage	LinearWeightedMovingAverage

Smoothing level	3	3
Minimum peak width	5	5
Minimum peak height	3000	3000
Peak spotting parameters		
Mass slice width	0.1	0.1
Exclusion mass list (mass & tolerance)		
Deconvolution parameters		
Sigma window value	0.5	0.5
MS2Dec amplitude cut off	0	0
Exclude after precursor	TRUE	TRUE
Keep isotope until	0.5	0.5
Keep original precursor isotopes	FALSE	FALSE
MSP file and MS/MS identification setting		
MSP file	MergedPCDL_AllSpectra_Positive.msp	MergedPCDL_AllSpectra_Positive.msp
Retention time tolerance	100	100
Accurate mass tolerance (MS1)	0.01	0.01
Accurate mass tolerance (MS2)	0.05	0.05
Identification score cut off	80	80
Using retention time for scoring	FALSE	TRUE
Using retention time for filtering	FALSE	FALSE
Text file and post identification (retention time and accurate mass based) setting		
Text file		
Retention time tolerance	0.1	0.1
Accurate mass tolerance	0.01	0.01
Identification score cut off	85	85
Advanced setting for identification		
Relative abundance cut off	0	0
Top candidate report	FALSE	TRUE
Adduct ion setting		
[M+H]+		

[M+NH4]+		
[M+Na]+		
Alignment parameters setting		
Reference file	(varies for alignment)	(varies for alignment)
Retention time tolerance	0.05	0.05
MS1 tolerance	0.015	0.015
Retention time factor	0.5	0.5
MS1 factor	0.5	0.5
Peak count filter	0	0
N% detected in at least one group	0	0
Remove feature based on peak height fold-change	FALSE	FALSE
Sample max / blank average	5	5
Sample average / blank average	5	5
Keep identified and annotated metabolites	TRUE	TRUE
Keep removable features and assign the tag for checking	TRUE	TRUE
Gap filling by compulsion	FALSE	TRUE
Tracking of isotope labels		
Tracking of isotopic labels	FALSE	FALSE
Ion mobility		
Ion mobility data	FALSE	FALSE

Table S3: Labelled internal standards used for retention time alignment in MS-DIAL

Compound	Rt (min)	Rt tol. (min)	m/z	m/z tol. (Da)	Min. Height	Use
Methomyl-D3	6.65	0.2	166.0721	0.025	5000	T
Simazine-D5	9.5	0.2	207.1163	0.025	5000	T
Dimethoate-D6	8.16	0.2	236.0443	0.025	5000	T
Diuron-D6	10.89	0.2	239.0618	0.025	5000	T
Imidacloprid-D4	8.01	0.2	260.0858	0.025	5000	T
Pendimeth-D5	15.9	0.2	287.1775	0.025	5000	T
Boscalid-D4	12.69	0.2	347.0651	0.025	5000	T

Table S4: MSFINDER settings

Formula finder parameters	
LEWIS and SENIOR CHECK	Yes
Ms1 Tolerance	10
Isotopic Abundance Tolerance	20
Mass tolerance type	ppm
Element Ratio Check	Common Range
Extended Range	FALSE
Extreme Range	FALSE
Element Probability Check	Yes
Element selection	O, N, P, S, F, Cl, Br, I
Structure finder parameters	
TreeDepth	2
MS2 tolerance	20
Relative Abundance Cut Off	5%
Data source	
MINEs (Metabolic In Silico Network Expansions) setting	Never use it
PubChem Online setting	Only use when there is no query in local databases
Local Databases	
HMDB (Human)	
YMDB (Yeast)	
PubChem	
SMPDB (Human)	
UNPD (Natural Product)	
ChEBI (Biomolecules)	
PlantCyc (Plant)	
KNApSAcK (Natural Product)	
BMDB (Bovine)	
FooDB (Food)	
ECMDB (E.coli)	
DrugBank (Drug)	
T3DB (Toxin)	
STOFF (Environment)	
NANPDB (Natural Product)	
LipidMAPS (Lipids)	
Urine (Human)	
Saliva (Human)	
Feces (Human)	
Serum (Human)	
CSF (Human)	
User Defined DB: PubChemLite	

Table S5: GC-QTOF-MS instrumental parameters

GC-NCI-MS Method	
Injection Volume	2.5 µL
Injection Mode	splitless
Purge Flow to Split Vent	33 mL/min at 0.75 min
Inlet Temperature	280 °C
GC Settings	
Column	HP-5MS (30m x 0.25mm, 025 µm)
Initial Oven Temperature	100 °C, hold 1 min
Ramp 1	15°C/min to 200 °C
Ramp 2	3.8 °C/min to 290 °C
Ramp 3	10 °C/min to 300 °C, hold 4 min
He Flow	1.35 mL/min, constant flow
Transfer Line Temperature	300 °C
MS Settings	
N2 Collision Gas	1.5 ml/min
Reactant Gas (Methane)	40%
Source Temperature	200 °C
Emmission Current Filament	90 µA
Electron Energy	70 eV
Scan Range	35-1000 m/z
Scan Speed	3 spectra/sec
Reference Mass Correction	internal mass correction after every second sample
GC-EI-MS Method	
Injection Volume	2.5 µL
Injection Mode	splitless
Purge Flow to Split Vent	33 mL/min at 0.75 min
Inlet Temperature	280 °C
GC Settings	
Column	HP-5MS (30m x 0.25mm, 025 µm)
Initial Oven Temperature	60 °C, hold 1 min
Ramp 1	40 °C/min to 120 °C
Ramp 2	5 °C/min to 310 °C
Optimized He Flow for RT locking	0.776 mL/min, constant flow
Transfer Line Temperature	280 °C
MS Settings	

N2 Collision Gas	1.5 ml/min
Source Temperature	300 °C
Emmission Current Filament	35 µA
Electron Energy	70 eV
Scan Range	35-1000 m/z
Scan Speed	4 spectra/sec
Reference Mass Correction	internal mass correction after every second sample

Supplemental Results

Table S6: Target compound detection in standards and spiked wastewater after MS-DIAL alignment

Compound	Detection Frequency in 100 ppb Standards[1] (%) (n = 4)	Detection Frequency in 20 ppb Spikes[2] (%) (n = 10)	Detection Code[3]
Azoxystrobin	100	90	C
Boscalid	100	90	U
Chlorantraniliprole	100	80	C
Clomazone	100	90	C
Cyprodinil	100[4]	100[4]	U
DEET	100	90	C
Difenoconazole	100	90	C
Dimethoate	100	90	U
Diuron	100	90	U
Hexazinone	100	90	C
Imidacloprid	100	90	U
Methomyl	100	60	U
Methoxyfenozide	75	60	U
Metolachlor	100	90	C
Pendimethalin	100	40	U
Propanil	100	90	C
Propoxur	100	80	C
Pyriproxyfen	100	80	C
Simazine	100	90	C
Thiacloprid	100	90	U
Thiamethoxam	100	80	U
Thiobencarb	100	70	I
Triclocarban	100	40	U

[1] Triclosan concentration: 10 ppb.
[2] Fipronil concentration: 4 ppb. Pyriproxyfen concentration: 24 ppb. Triclosan concentration: 2 ppb.
[3] C: Compounds Identified correctly; I: Compounds identified as isomers; U: Compounds identified as Unknown but the correct compounds are in the top five hits under Compound Search.

Table S7: GC target compounds detection in standards and spikes

Compounds	Ion Mode	Detection Frequency in 250 ppb Standards (%)	Detection Frequency in 100 ppb Spikes[1] (%)	Detection Code[2]
Non-pyrethroid pesticides				
Chlorothalonil	GC-EI	67	0	C
Chlorpyrifos	GC-EI	100	30	C
Pyriproxyfen	GC-EI	100	90[3]	C
Pyrethroid Insecticides				
Bifenthrin	GC-EI	0	0	N
Bioallethrin	GC-EI	0	0	N
Cyhalothrin	GC-EI	100	20	C
Cypermethrin	GC-EI	67	0	C
Deltamethrin	GC-EI	0	0	N
Esfenvalerate	GC-EI	100	0	C
Etofenprox	GC-EI	0	0	N
Imiprothrin	GC-EI	0	0	N
Permethrin	GC-EI	100	70	C
Phenothrin	GC-EI	100	50	C
Prallethrin	GC-EI	0	0	N
Resmethrin	GC-EI	100	50	C
Tetremethrin	GC-EI	0	0	N

[1]Pyriproxyfen concentration: 600 ppb.
[2]C: Compounds identified correctly. N: Compounds that are not identified.
[3]More than one entry. Summed the numbers detected in all entries. (Total: 3 standards, 10 spikes)

Table S8: Suspect-annotated LC compounds with agreement between MS-DIAL and *Qualitative Analysis* and level of identification confidence, if achieved

Compound	**Use Category**	**ID Level of Confidence**	**Detection frequency[1] (n = 56)**
DEET / Diethyltoluamide	Pesticide	1	89%
Valsartan	Pharmaceutical- anti-hypertensive	1	73%
Caffeine	Food	1	68%
Oleamide	Multi- food packaging; lubricants	1	61%
Acetaminophen	Pharmaceutical- NSAID	2a	57%
Mycophenolic acid	Pharmaceutical- immunosuppressant	2a	54%
Hydrocortisone (Cortisol)	Pharmaceutical		43%
BTA / Benzotriazole[2]	Cleaning product		41%
Benzoylecgonine	Metabolite- cocaine		36%

1-(3-Trifluoromethylphenyl)piperazine	Drug		32%
DEP / Diethyl phthalate	Plasticizer	1	32%
Gabapentin	Pharmaceutical		29%
Fexofenadine	Pharmaceutical- antihistamine	1	25%
Iohexol	Iodinated Xray Contrast	1	25%
Piperine	Natural product	2a	25%
2,6-Xylidine	Metabolite		20%
TBEP / Tris(2-butoxyethyl) phosphate	Flame retardant	1	16%
Octyl methoxycinnamate	Fragrance		14%
Bis(2-ethylhexyl) phthalate (DEHP)	Plasticizer	1	13%
Sulfamethoxazole	Pharmaceutical- antibiotic	1	13%
Metoprolol	Pharmaceutical- beta blocker	1	11%
Trimethoprim	Pharmaceutical- antibiotic	1	9%
Ivermectin B1a	Vet		7%
Diethofencarb[2]	Fungicide		5%
Carbamazepine	Pharmaceutical- anti-seizure	1	4%
O-DT / O-Desmethyltramadol	Metabolite	1	2%
TEP / Triethyl phosphate	Flame retardant		2%

[1] In trunkline, WWTP influent and effluent

[2] Compared against analytical standards but not confirmed.

Table S9: GC suspect-identified compounds and detection frequencies

Compound	**Use Category**	**Mode**	**Detection frequency[1] (n = 56)**	**Groomer (n = 4)**	**Laundry (n = 4)**	**PCO (n = 4)**
Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	Consumer product antioxidant	EI	39.3%	25%	0%	25%
Indole, 3-methyl-	Endogenous	EI	91.1%	25%	25%	0%
p-Cresol	Endogenous	EI	60.7%	25%	0%	0%
Hippuric acid	Endogenous	EI	44.6%	0%	25%	0%
Allopregnane-3.alpha.,20.alpha.-diol	Endogenous	EI	35.7%	0%	25%	75%
Cholestan-3-ol, (3.beta.,5.beta.)-	Endogenous	EI	30.4%	100%	75%	75%
TCPP	Flame retardant	EI	75.0%	50%	75%	0%
Oxybenzone	Flavoring; Fragrance; Personal care	EI	96.4%	25%	0%	0%
Benzoic acid	Flavoring; Fragrance; Personal care	EI	76.8%	25%	0%	0%

Heptasiloxane, hexadecamethyl-	Flavoring; Fragrance; Personal care	EI	75.0%	50%	100%	50%
Triclosan	Flavoring; Fragrance; Personal care	EI	64.3%	0%	25%	0%
o-Cymene	Flavoring; Fragrance; Personal care	EI	60.7%	0%	0%	0%
Methylparaben	Flavoring; Fragrance; Personal care	EI	50.0%	0%	25%	0%
Dimethyl phthalate	Flavoring; Fragrance; Personal care	EI	44.6%	25%	25%	50%
Methyl tetradecanoate	Flavoring; Fragrance; Personal care	EI	44.6%	100%	100%	50%
Benzene, 1-(1,1-dimethylethyl)-2-methoxy-4-methyl-	Flavoring; Fragrance; Personal care	EI	37.5%	0%	25%	0%
Benzoic acid, 2-hydroxy-, pentyl ester	Flavoring; Fragrance; Personal care	EI	37.5%	25%	0%	0%
Tetrasiloxane, decamethyl-	Flavoring; Fragrance; Personal care	EI	33.9%	25%	0%	25%
Isobutyl paraben	Flavoring; Fragrance; Personal care	EI	32.1%	0%	0%	0%
Ethanol, 2-(4-chlorophenoxy)-	Flavoring; Fragrance; Personal care	EI	30.4%	25%	75%	50%
1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-	Flavoring; Fragrance; Personal care	EI	25.0%	50%	50%	25%
2(3H)-Furanone, 5-heptyldihydro-	Flavoring; Fragrance; Personal care	EI	21.4%	0%	25%	25%
D-Limonene	Food	EI	94.6%	25%	50%	0%
aR-Turmerone	Food	EI	35.7%	100%	75%	50%
Stigmasta-5,24(28)-dien-3-ol, (3.beta.,24Z)-	Food	EI	25.0%	25%	0%	0%
Phenol, 3-methyl-	Human xenobiotic metabolite	EI	96.4%	75%	100%	75%
2-Naphthalenol	Human xenobiotic metabolite	EI	30.4%	0%	50%	0%

1,2-Dichlorobenzene	Industrial	NCI	39.3%	50%	50%	50%
Lilial	Pesticide	EI	55.4%	0%	75%	0%
o-Hydroxybiphenyl	Pesticide	EI	50.0%	0%	0%	0%
Dichlorvos	Pesticide	NCI	48.2%	100%	100%	75%
Parathion-methyl	Pesticide	NCI	21.4%	25%	0%	25%
Etiracetam	Pharmaceutical	EI	82.1%	75%	100%	25%
Iminostilbene	Pharmaceutical	EI	76.8%	25%	25%	0%
Ibuprofen	Pharmaceutical	EI	51.8%	100%	100%	75%
Gabapentin	Pharmaceutical	EI	41.1%	0%	0%	0%
Guaifenesin	Pharmaceutical	EI	28.6%	0%	0%	0%
Stigmastanol	Pharmaceutical	EI	26.8%	0%	0%	0%
9,12,15-Octadecatrienol	Plastics	EI	69.6%	50%	50%	25%

[1] Trunkline, influent, & effluent

Targeted MS/MS structure results

Acetaminophen

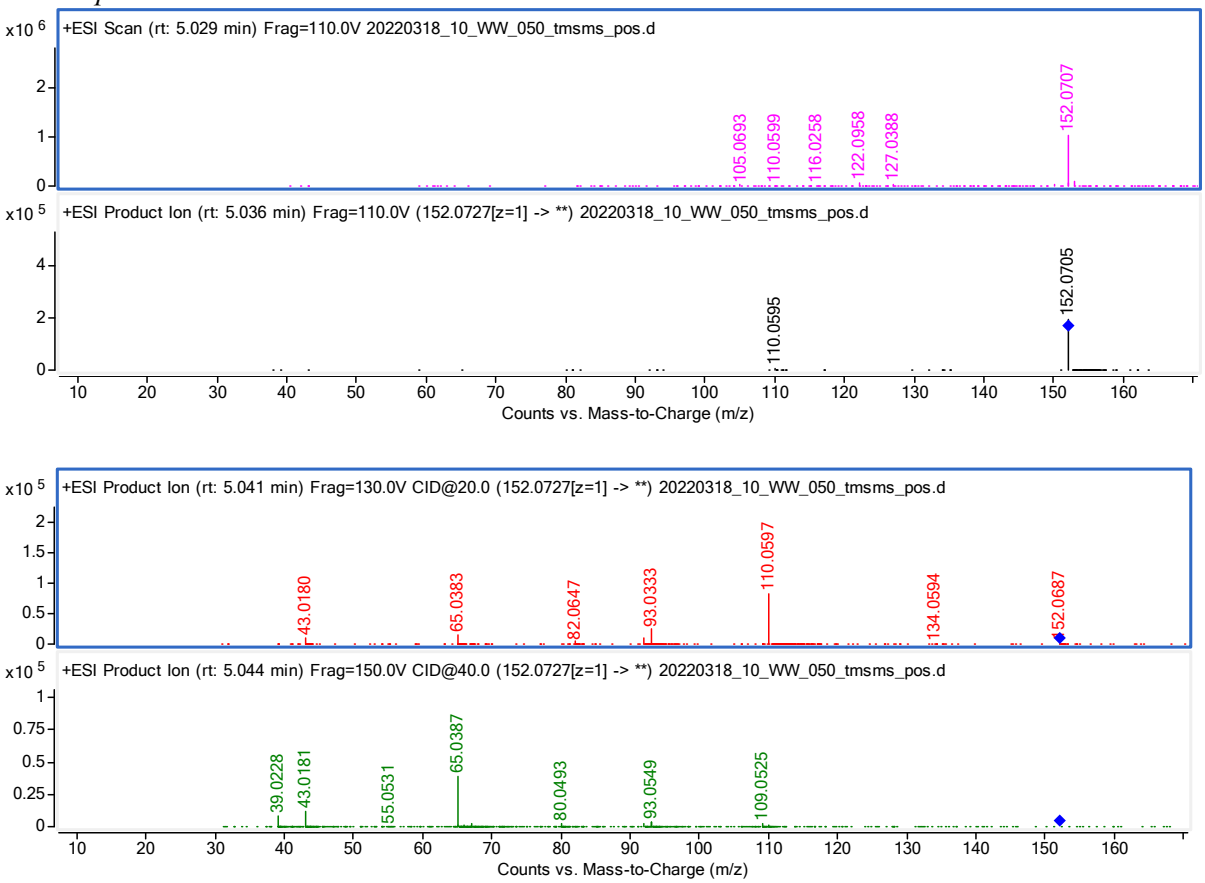

Figure S2. tMS/MS at collision energies of 0, 10, 20, and 40 eV for the feature identified at 2a confidence level as acetaminophen.

Table S10: Acetaminophen: library fragments and average mass error

Library Fragment m/z*	Average mass error (ppm)
152.0716	-6.58
110.0622	-23.62
65.0389	-6.15

*from MassBank Europe: https://massbank.eu/MassBank/Result.jsp?inchikey=RZVAJINKPMORJF-UHFFFAOYSA-N

Mycophenolic acid

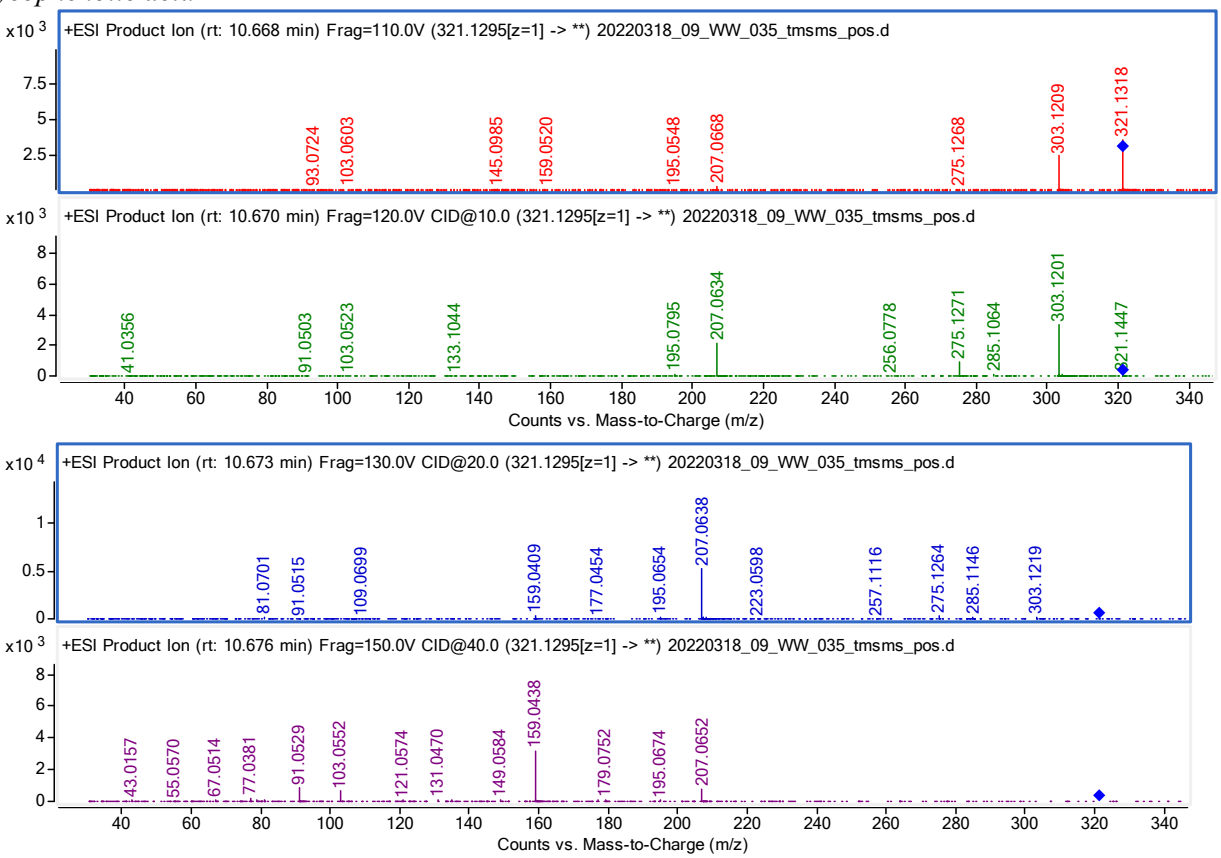

Figure S3. tMS/MS at collision energies of 0, 10, 20, and 40 eV for the feature identified at 2a confidence level as mycophenolic acid.

Table S11: Mycophenolic acid: library fragments and average mass error

Library Fragment m/z*	Average mass error (ppm)
321.1299	6.066082
303.1225	-6.48913
207.0644	-3.97461
159.0397	16.9266

*Library spectrum from Human Metabolome Database: https://hmdb.ca/metabolites/HMDB0015159#spectra

Piperine

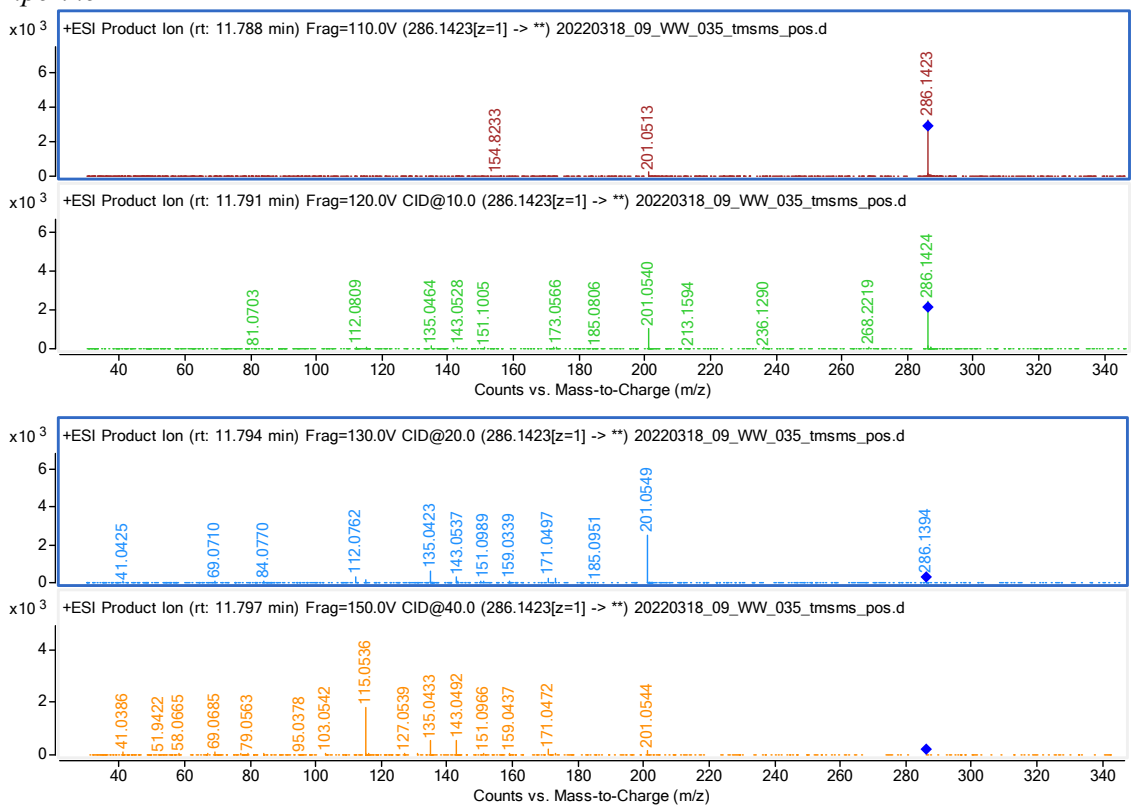

Figure S4. tMS/MS at collision energies of 0, 10, 20, and 40 eV for the feature identified at 2a confidence level as piperine.

Table S12: Piperine: library fragments and average mass error

Library Fragment m/z*	Average mass error (ppm)
286.1437	-4.71791
201.054	2.238205
135.0431	-2.22151

*Library spectrum from MassBank Europe: https://massbank.eu/MassBank/Result.jsp?inchikey=MXXWOMGUGJBKIW-YPCIICBESA-N