Supporting Information

For *Environmental Science & Technology*

Assessing Emissions from Pharmaceutical Manufacturing based on Temporal High-Resolution Mass Spectrometry Data

Sabine Anliker[a,b], Martin Loos[c], Rahel Comte[a,b,§], Matthias Ruff[a,#], Kathrin Fenner[a,b,d], Heinz Singer*[,a]

[a] Eawag, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf 8600, Switzerland
[b] Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, Zürich 8092, Switzerland
[c] enviBee, Dübendorf 8600, Switzerland
[d] Department of Chemistry, University of Zürich, Zürich 8057, Switzerland

[§] Current address: Bachema AG, Analytische Laboratorien, Schlieren 8952, Switzerland

[#] Current address: Gewässer- und Bodenschutzlabor des Kantons Bern, Bern 3014, Switzerland

*E-mail: heinz.singer@eawag.ch. Phone: +41 58 765 55 77.

Number of pages: 35
Number of figures: 12
Number of tables: 7

Contents

Material and methods

SI 1: Chemicals

All organic solvents were of HPLC grade purity (≥ 98%) and were supplied by Fisher Scientific (Wohlen, Switzerland), Sigma Aldrich (Buchs, Switzerland) and Merck (Darmstadt, Germany). Formic acid (≥ 98%) was purchased from Merck (Darmstadt, Germany). Ultrapure water was generated by a laboratory water purification system (Barnstead Nanopure, Thermo Fisher Scientific, San Jose, U.S.). Isotope-labeled internal standards (ISTDs) (purity ≥97%) were obtained from the following suppliers: Sigma Aldrich (Buchs, Switzerland), TRC Canada (Toronto, Canada), Dr. Ehrenstorfer (Augsburg, Germany), TCI Europe (Antwerpen, Belgium), Cambridge Isotope Laboratories (Andover, USA), ReseaLIFEchem GmbH (Burgdorf, Switzerland), CDN Isotopes Inc. (Augsburg, Germany), and Lipomed AG (Arlesheim, Switzerland). Reference material of the target analytes and the three confirmed nontarget substances was purchased as pure substances or concentrated solutions from Sigma Aldrich (Buchs, Switzerland), Dr. Ehrenstorfer (Augsburg, Germany), Toronto Research Chemicals (North York, Canada), ReseaChem (Burgdorf, Switzerland) or Acros Organics (Geel, Belgium) at analytical grade (> 94% purity).

Individual stock solutions of analytes and ISTDs were prepared at a concentration of 1 g/L in ethanol, methanol, acetonitrile, ethanol/water mix, methanol/water mix, dimethyl sulfoxide, ethyl acetate, toluene, acetone, water, ethanol + 0.1 M HCl, or methanol + 0.1 M HCl depending on their solubility and stability. Working standard solutions were produced by mixing the individual stock solutions grouped in substance classes and then diluted with ethanol, at concentrations of 10, 1, 0.1 and 0.01 mg/L. Similarly, mixtures of the ISTDs were prepared in ethanol at a concentration of 0.5 mg/L for each compound. All solutions were stored at -20°C. For quantification the analyte working standard solutions were diluted with ultrapure water to create a calibration curve at 0.1, 0.5, 1, 5 and 10 µg/L.

A large number of 535 target analytes was spiked, particularly pharmaceuticals, pesticides and their metabolites. They were selected based on their frequent detection in WWTP samples, surface and groundwater and alignment with Swiss consumption and sales data. The list of the target compounds including their use classification is provided in SI 2. The list of the 135 ISTDs is in SI 3, and includes pharmaceuticals (81), pesticides (30) and artificial sweeteners (6).

SI 2: List of target analytes

See separate file: es9b07085_si_002.xlsx

SI 3: List of isotope-labeled internal standards

See separate file: es9b07085_si_003.txt

SI 4: Additional information on WWTPs

Table S3: Additional information on WWTPs. Information was provided by the respective cantonal authorities.

WWTP characterization	**WWTP_ind**[a]	**WWTP_dom**[a]
Connected population	33'000	146'000
Design capacity [PE]	200'000	245'000
Hospital beds in the catchment	~110	~150
Wastewater from chemical/ pharmaceutical industry [%]	~60 TOC[b], ~3 vol	-
Sludge retention time [days]	24	10-12
Hydraulic retention time [h]	24 dry weather; 8 rainy weather	20; 6
Wastewater volume [m3/a]	6'902'000	20'294'000
TOC/DOC elimination [%]	95	96
BOD elimination [%]	95	99
Water quality parameters of effluent[c]		
TSS [mg/L]	2-11	1.5-2
TOC [mg/L]	n.a.	6-9.2
DOC [mg/L]	3-15.6	4.6-6
COD [mg/L]	15-42	16-22
P-tot [mg/L]	0.09-0.46	0.12-0.3
BOD5 [mg/L]	2-34	n.a.
BOD7 [mg/L]	n.a.	3-5
NH_4-N [mg/L]	0-5.37	0.01-1.5
NO_3-N [mg/L]	0.1-48.7	8-13.6
NO_2-N [mg/L]	<LOQ-0.47	0.007-0.25
pH	n.a.	6.6-7.2
Temperature [°C]	7-15.2	9.9-17.3

[a] Anonymity was granted to the WWTPs and the connected pharmaceutical company, therefore the exact locations of the sampling sites are not indicated.

[b] The industrial wastewater was collected in storage tanks and directed to WWTP_ind by controlled dosing to prevent exceeding DOC limits in the effluent.

[c] The water quality parameters of the effluent correspond to minimum and maximum values of the measurements performed by the WWTPs during the sampling period, the number of available measurements differed between WWTP and parameter, it ranged from 3 (monthly measurements) to 87 (daily measurements).

BOD5 Biochemical oxygen demand (5 days)

BOD7 Biochemical oxygen demand (7 days)

COD Chemical oxygen demand

DOC	Dissolved organic carbon
LOQ	Limit of quantification
n.a.	Not available
PE	Population equivalents
P-tot	Total phosphorus
TSS	Total suspended solids

SI 5: Analytical instrumentation and method

Samples were measured in three sequences, each containing the samples of one month. To prevent carryover of highly concentrated compounds, expected to be present in the industrial wastewater, analysis was carried out in chronological order for each WWTP separately.

All measurements were performed with the following instrumentation: a PAL HTS-Xt autosampler (CTC Analytics), an Ultimate 3000 RS LC-pump (Thermo Scientific), an Atlantis T3 LC-column (3 µm particle size, 3.0 x 150 mm inner diameter, Waters) guarded with a precolumn of the same material and a precolumn filter, a column oven (Portmann Instruments) and a Q Exactive Plus mass spectrometer (Thermo Scienfific) with and a heated electrospray ion source (HESI II).

100 µL of sample were injected onto the LC column. The LC mobile phase A consisted of ultrapure water and phase B of methanol, both containing 0.1% formic acid. The flow rate was 300 µL/min and the column temperature was set to 30°C. The 29.5 min chromatographic run started with 5% B, which was maintained for 1.5 min, increased linearly to 95% B in 16 min, then stayed at this condition for 7.5 min and finally decreased to 5% B in 0.5min for 4 min re-equilibration.

MS data in positive and negative electrospray ionization mode was acquired in separate runs, i.e. each sample was injected twice. The MS experiment consisted of a full scan followed by five data dependent (dd) MS2 scans. MS2 scans were triggered for the most intense masses detected in a pre-scan. An exclusion list containing background ions and the spiked isotope labeled standard compounds was used, to prevent these masses form triggering MS2 data acquisition. Details on the MS parameter settings are given in **Table S4.**

Table S4: Parameters settings for Q Exactive Plus MS measurement

	Positive mode	Negative mode
Ion source		
Spray voltage [kV]	4	-3
Capillary temperature [°C]	320	
Sheat gas flow(nitrogen) [arb]	40	
Auxiliary gas flow (nitrogen) [arb]	10	
Sweep gas flow [arb]	0	
S-Lense RF level [arb]	50	
MS		
Full MS resolution at m/z 200	280’000	
Full MS scan range [*m/z*]	100-1000	
Full MS AGC target [Arb]	1E6	
Full MS max injection time [ms]	100	
dd-MS2 resolution at *m/z* 200	17’500	
dd-MS2 scan range [*m/z*]	200-2000	
dd-MS2 AGC target [arb]	5E5	
dd-MS2 max injection time [ms]	100	
dd-MS2 isolation window [*m/z*]	1	
dd-MS2 stepped NCE [arb]	20, 50, 80	
dd-MS2 dynamic exclusion [s]	6	

arb: arbitrary units

SI 6: Workflow steps included in enviMass data processing

Data processing using enviMass (version 4.0)[1] included the following 13 steps: (1) centroidization and conversion of the raw data files into mzXML file format via the msconvert tool from ProteoWizard[2] (version 3.0.11417); (2) peak picking; (3) mass recalibration based on ISTD compounds; (4) retention time alignment; (5) annotation of peaks that are also present in blank samples; (6) profile extraction; (7) limit of detection (LOD) interpolation; (8) screening for compounds on the ISTD and the target compound list; (9) intensity normalization based on ISTD compounds; (10) extracted ion chromatogram (EIC) correlation; (11) file-wise componentization including isotopologue and adduct grouping; (12) profile componentization; and (13) calculation of sample to blank peak intensity ratios across each profile.

SI 7: Parameter settings for enviMass v.4.0 workflow

Table S5: Parameter settings for enviMass v.4.0 workflow

Instrument/Resolution	
	Q-Exactive, QExactivePlus_280K@200
Peak Picking	
Data filtering	
Filter range?	No
Lower RT bound [minutes]	0
Upper RT bound [minutes]	25
Filter mass range?	No
Lower m/z bound	0
Upper m/z bound	2000
Parameter estimation	
Include estimation?	no
Extraction of ion chromatogram	
Max. retention time gap in EIC [s]	300
Max. m/z deviation of a centroid data point from its mean [ppm]	10
Peak picking	
Min. number of centroid data points per peak	4
…within a given RT window [s]	10
Max. RT gap length to be interpolated [s]	10
Max. RT width of a single peak [s]	120
Min. log10 intensity threshold	5
Minimum signal/noise	5
Minimum signal to base	2
Maximum possible number of peaks within a single EIC	4

Peak intensity: use peak area or peak intensoid?	Intensoid (max. int)
Peak mass definition	Weighted mean
Advanced settings	
Upper log10 intensity safety threshold	12
How often can peak detection fail to end the recursion?	1
Weight for assigning centroid data points to a peak	1
Percentage of low-intensity data point do discard	0
Mass recalibration	
Include mass recalibration for positive/negative ion mode files?	yes
Reference compounds	Internal standards
± m/z tolerance	1
Maximum allowable m/z correction	1
… given in	Absolute [mmu]
RT tolerance [s]	30
Alignment	
Include retention time alignment for positive/negative ion mode files?	Yes
+/- m/z tolerance for peak matches	2
… given in:	ppm
Advanced settings	
Reference peaks/masses	All peaks (recommended)
Maximum number of most intense reference peaks to include	2000
Maximum number of iteration for match window adaption	5
Only include replicable peaks	Yes
Blind	
+/- m/z tolerance	2
… given in:	ppm
RT tolerance [s]	30
Select blind/blank files for subtraction	Subtract with the next blank/blind file preceding a sample by its date & time
Screening	
IS	
RT tolerance of peaks relative to their expected RT [s]	30
RT tolerance of peaks within an isotope pattern [s]	4
± m/z tolerance	2
…given in:	ppm

Intensity tolerance %	30
Lower intensity threshold (if LOD interpolation disabled)	50000
Restrict screening to latest files (covered during profiling) Include?	FALSE
Cutoff score [0,1]	0.8
Exclude matches below cutoff score	TRUE
Screen only most intense isotopologue peak?	FALSE
Targets & Suspects	
RT tolerance of peaks relative to their expected RT [s]	30
RT tolerance of peaks within an isotope pattern [s]	4
± m/z tolerance…	2
…given in:	ppm
Intensity tolerance %	30
Lower intensity threshold (if LOD interpolation disabled)	50000
Restrict screening to latest files (covered during profiling) Include?	FALSE
Cutoff score [0,1]	0.8
Exclude matches below cutoff score	TRUE
Screen only most intense isotopologue peak?	FALSE
Adducts	
Positive ions:	M+H, M+NH4, M+Na, M+K, M+, 2M+H, 2M+NH4, 2M+Na, 2M+K
Negative ions:	M-H, M+FA-H, M+Hac-H, M-, 2M-H, 2M+FA-H, 2M+Hac-H
Normalization	
Include normalization for positive ion mode files?	Yes
Minimum of screened files covered by each IS profile [%]	90
Screening threshold	0.8
Minimum number of IS profile peaks	15
Show median deviation of blank/blind profiles	Yes
Use subsampling	Yes
Number of blank/blind profiles in subsample	100
Show median deviation of sample (i.e. non-blank) profiles?	Yes
Use subsampling	Yes
Number of profiles in subsample	100
File-wise componentization	
Isotopologue grouping	

+/- m/z tolerance…	2
… given in:	ppm
RT tolerance of peaks within an isotopologue pattern [s]	4
Intensity tolerance %	30
Run atom bound estimation?	FALSE
Adduct grouping	
+/- m/z tolerance…	2
… given in:	ppm
RT tolerance of peaks within an adduct pattern [s]	4
Positive mode:	M+H, M+NH4, M+Na, M+K, M+, 2M+H, 2M+NH4, 2M+Na, 2M+K
Negative mode	M-H, M+FA-H, M+Hac-H, M-, 2M-H, 2M+FA-H, 2M+Hac-H
Peak shape correlation	
RT tolerance window for candidate peak pairs [s]	4
Min. number of MS1 scans over which peak pairs co-elute for their peak shape correlation:	15
Min. Pearson correlation [0,1] coefficient	0.95
Profile componentization	
Minimum number of files over which peaks of different profiles have to co-occur to check their intensity correlation:	5
Minimum Pearson profile intensity correlation:	0.8
RT tolerance window for co-occurring peaks of different profiles [s]:	4
Restrict profile componentization to isotopologue and selected adduct relations only?	False
Restrict profile componentization to a set of latest files only?	False
Filter positive/negative mode components?	True
Set standard profiles by:	All profiles

SI 8: Results of intensity normalization

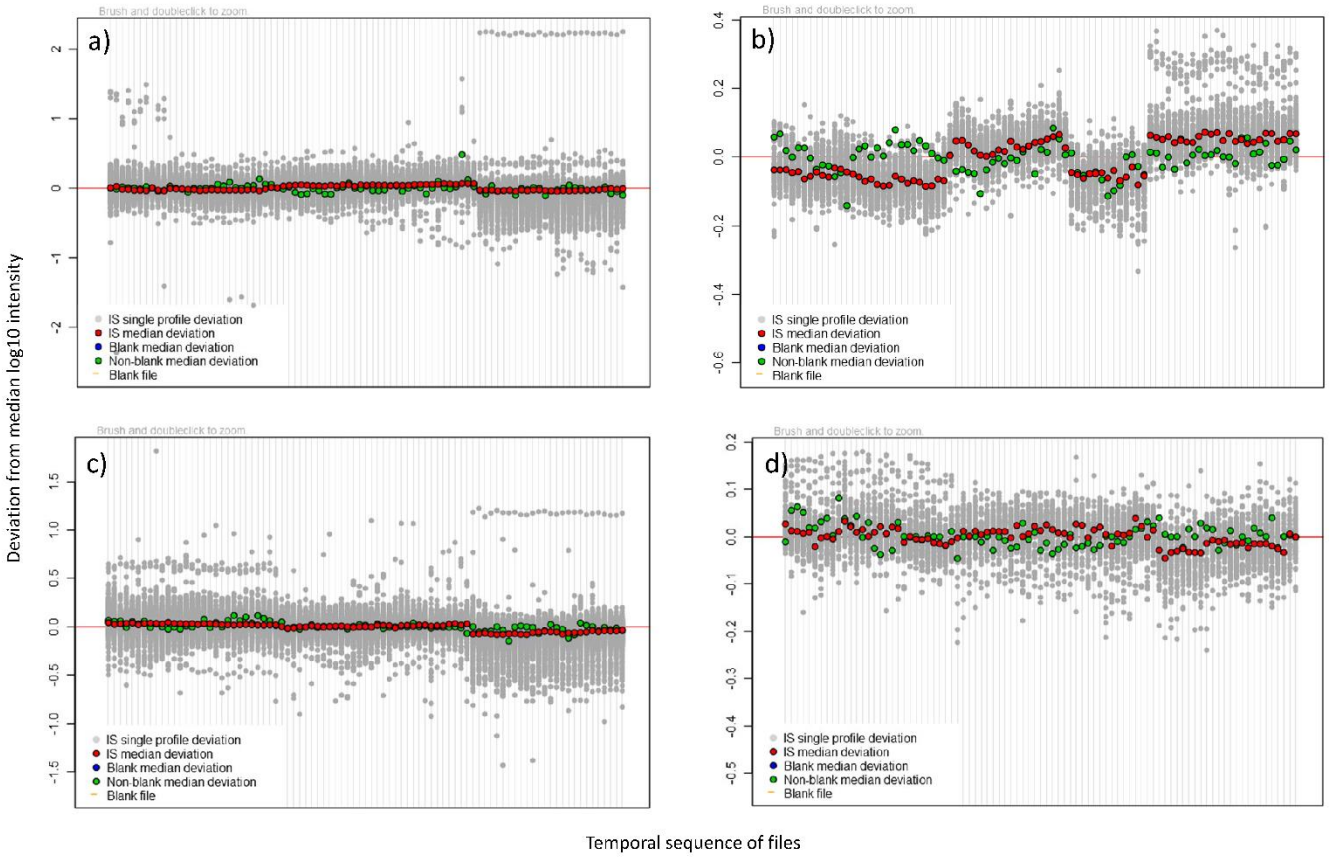


Figure S1: enviMass visualization of the outcome of the intensity normalization, in a) and b) WWP_ind and in c) and d) WWTP_dom. Figures a) and c) display the positive and figures b) and d) the negative ion mode data. The plots show the deviation of a peak intensity from the median intensity of its time profile (gray dots), for each ISTD and over all the samples. The deviation is expressed as intensity ratio between peak and median intensity; positive deviations indicate ISTD peak intensities above their profile median. The red dots indicate the median deviation over all ISTDs for each file; this value was used for the intensity normalization of all peaks derived from a file. The green dots show the median intensity deviations from the individual profile medians for randomly sampled non-ISTD time profiles, which were not found in blank samples. Files are ordered by their date. It should be noted that the y-axis is given on a log scale and differs for each of the figures a) - d).

SI 9: Rationale for missing data points, problems with time profile building and failed profile componentization

Problems with profile building became evident in the form of incomplete time series. Missing data points in a time series can either be caused by no signal, a signal below the limit of detection (LOD) or an error in measurement (i.e. missing at random). However, they may also originate from inconsistent profile building by the automated workflow. There are two main reasons for this. First, the retention time and/or the mass tolerance specified in the workflow settings were too restrictive for a certain compound. Second, chromatographic peaks were associated to the wrong time profile, e.g., in case of closely eluting isobaric compounds, peak tailing, double peaks and unclean extracted ion chromatograms of signals close to the LOD. The latter problems, regarding chromatographic quality, were partially attributable to matrix interferences, which could not be corrected by intensity normalization and retention time alignment.

Apart from ionization in both positive and negative mode, these inconsistencies in time profile building resulted in duplicated entries in the time profile inventory for one single compound. Moreover, the setting of the "minimum Pearson profile intensity correlation" of the enviMass workflow is critical for time profile componentization. If the observed correlation coefficient of time series of different adducts or isotopologues of a single compound fall below the specified threshold value, they will not be aggregated and hence different profiles for the respective compound will appear in the final profile list. This may especially be important for different adducts of one single compound, which in some cases do not display nicely correlating time series due to varying matrix effects in different samples.

SI 10: Data filtering

To enhance the data quality, four data reduction filters were applied: (i) Time profiles that originate from the same chemical compound (e.g., related to adducts or isotopes); (ii) the results matrix was filtered for time profiles of an average sample to blind ratio > 10; (iii) only time profiles of features that eluted after the dead volume of the analytical system (RT ≥ 3 min for the WWTP samples and ≥ 1 min for the river samples) were considered; (iv) results were restricted to features that were detected on at least three consecutive days. The reason for restriction (iv) was twofold. First, even for short-term emissions signals were expected to occur over several days in the WWTP effluent due to hydraulic retention in the WWTP and to controlled dosing of industrial wastewater from stacking containers during several days. Second, this filtering step removed false positive time series consisting of only sporadically detected features. In this way, samples collected on consecutive days acted as an alternative to replicate measurements. **Table S4** summarizes the effects of the different data reduction steps.

Table S6: Effects of data filtering steps on the number of time profiles per inventory

Filter criteria	WWTP_ind	WWTP_dom
Initial number of profiles	173’036	115’551
RF by sample/blank > 10	1.10	1.15
RF by componentization	1.08	1.19
RF by retention time threshold	1.78	2.61
RF by min 3 consecutive detects	3.38	5.16
Final number of profiles	24’245	7’200

RF: reduction factor of the respective filtering step in relation to the number of profiles of the previous reduction step

SI 11: Selection of retention time (RT) and *m/z* window for correlation analysis

For correlation analysis of time series of the same compounds between the different sampling sites appropriate RT and *m/z* windows had to be determined. For this purpose we assessed the deviation of the measured RTs and *m/z* values between the different sampling sites based on the ISTDs (cf. **Figures S2** and **S3**). Mean values of the respective time profile pairs were considered. Finally, the mass window was set to ± 2 ppm and the RT tolerance to 0 to -30 sec for correlations between time profiles in WWTP_ind and WWTP_dom, whereas the mass window was set to ± 5 ppm and the RT tolerance to +5 to +10 min for correlations between WWTP and river time profiles measured at the international Rhine monitoring station (RUES). Since the data of the WWTPs and the RUES was acquired with different analytical methods, much larger RT and *m/z* deviations were observed for corresponding ISTDs between these datasets compared to the deviations between the two WWTPs.

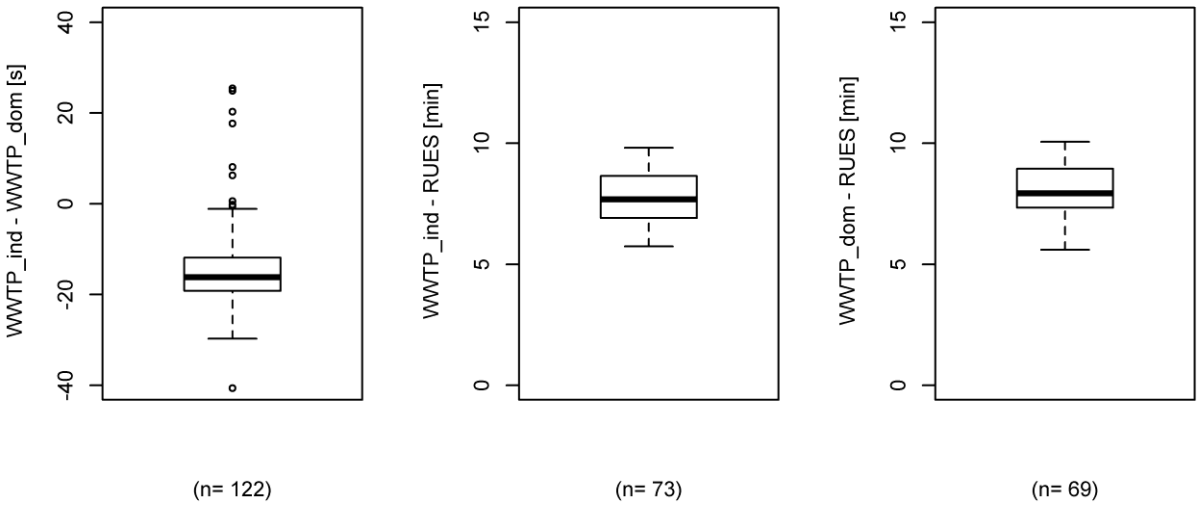


Figure S2: Retention time difference for ISTDs between sampling sites. In brackets below the plot the number of ISTD pairs is indicated.

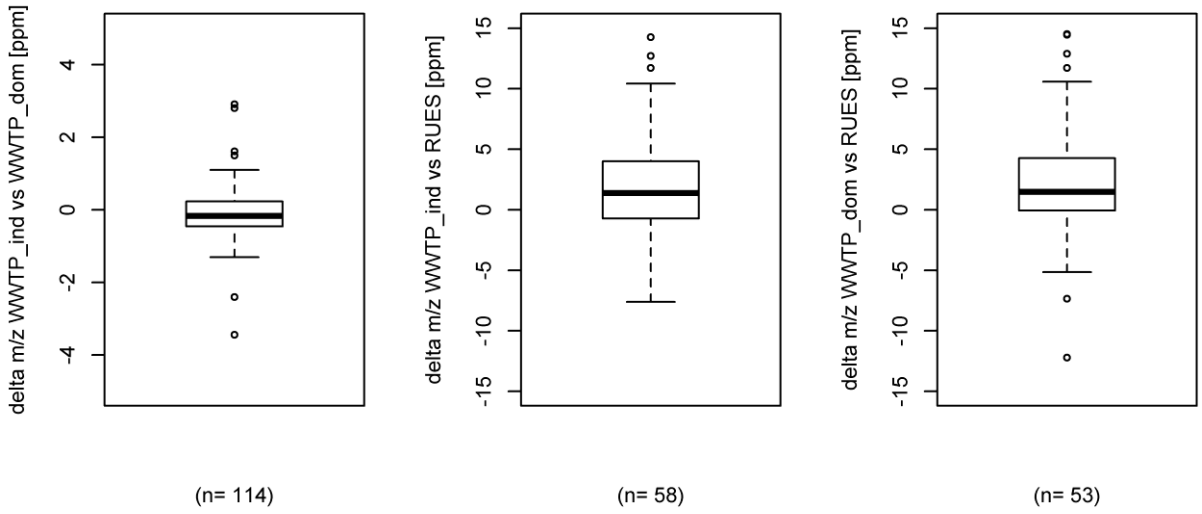
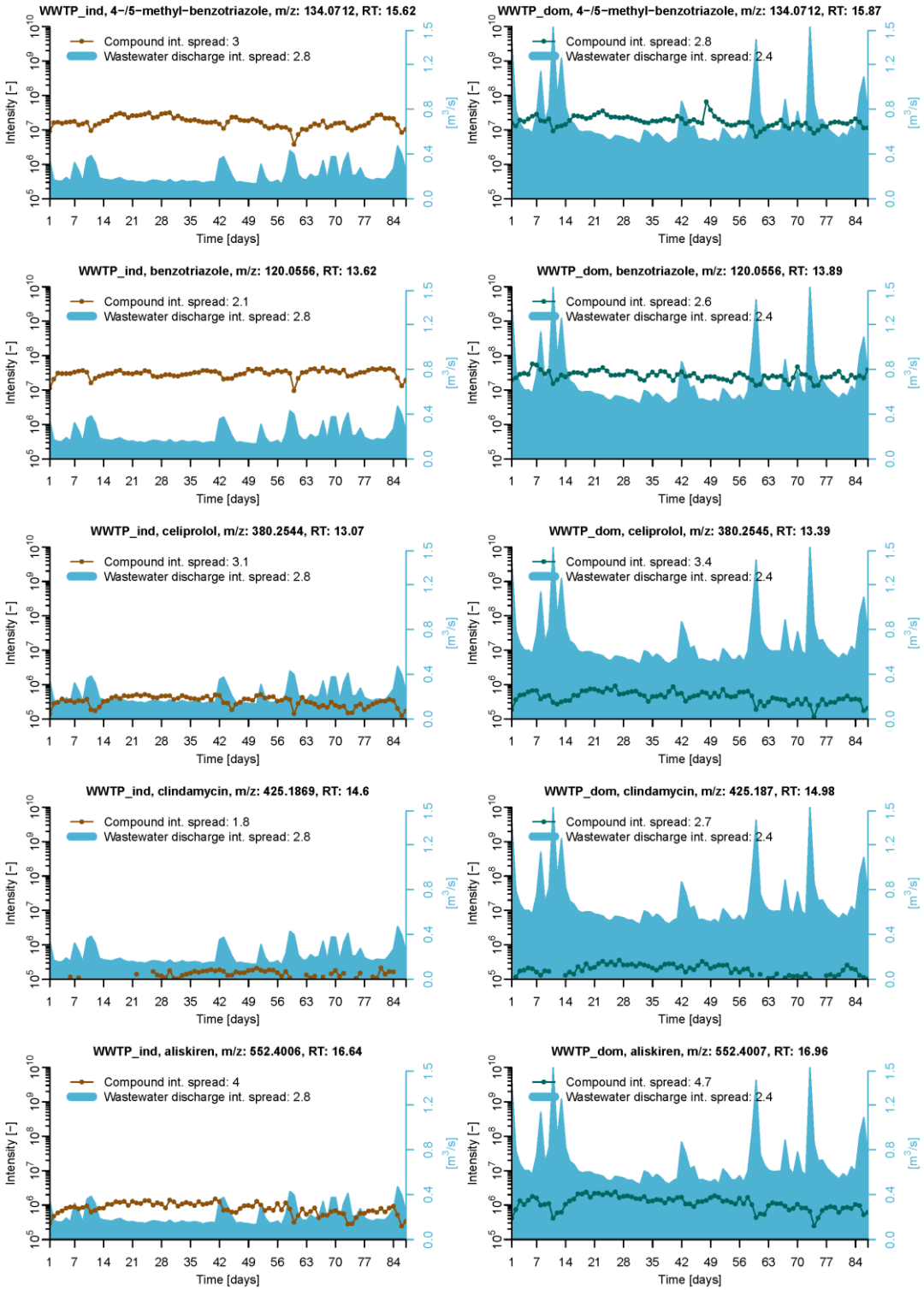
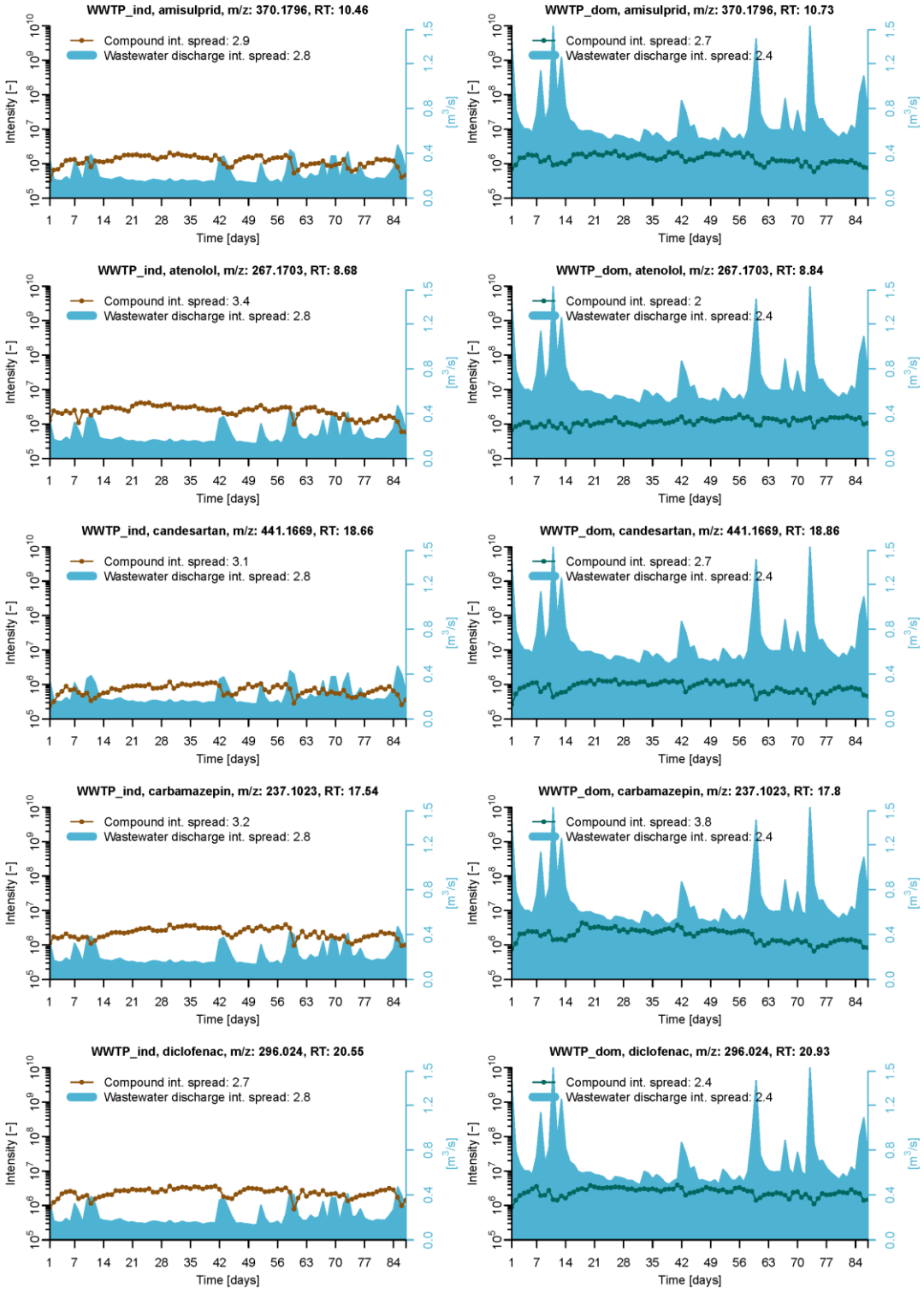


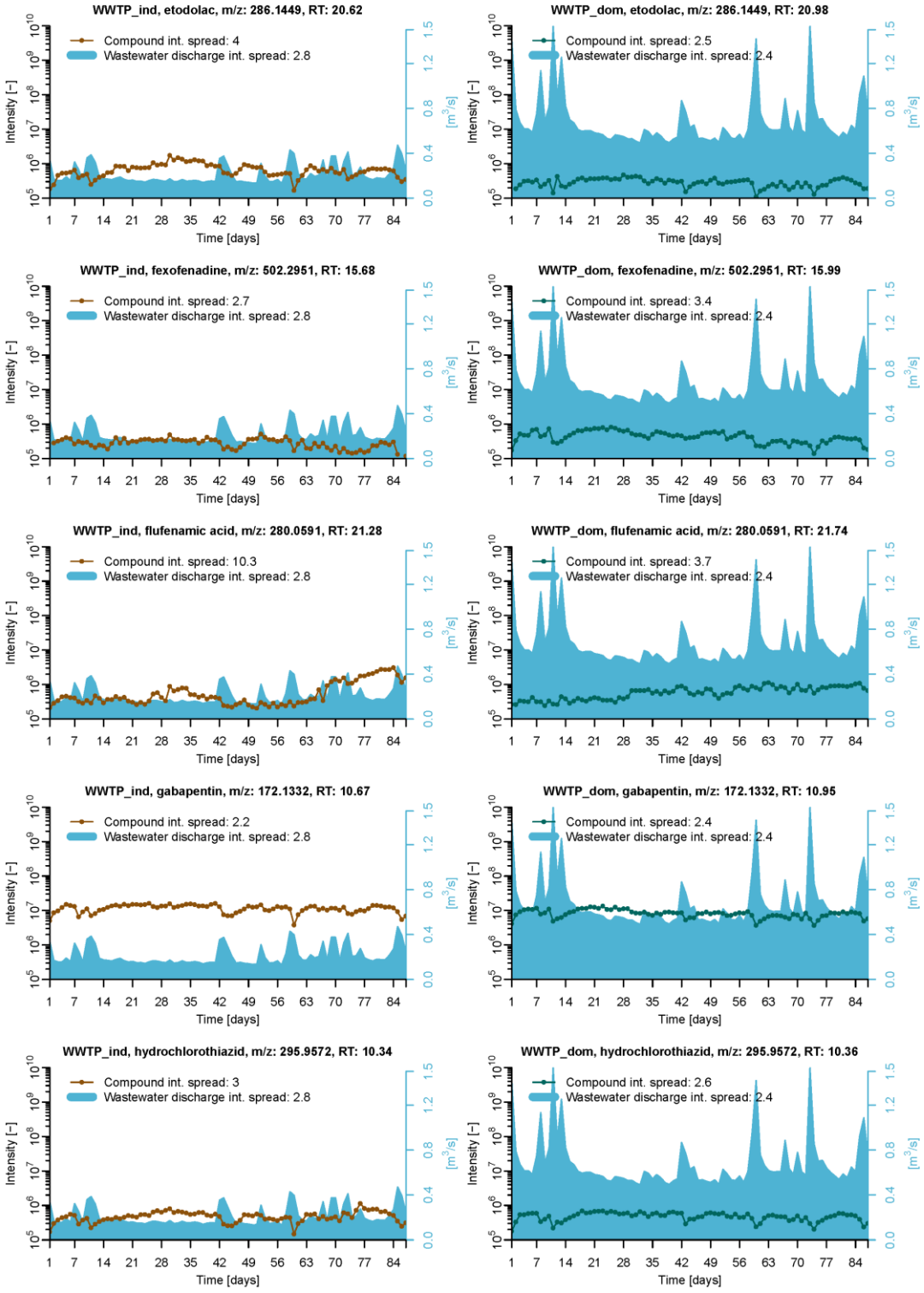
Figure S3: Mass difference for ISTDs between sampling sites. In brackets below the plot the number of considered ISTD pairs is indicated.

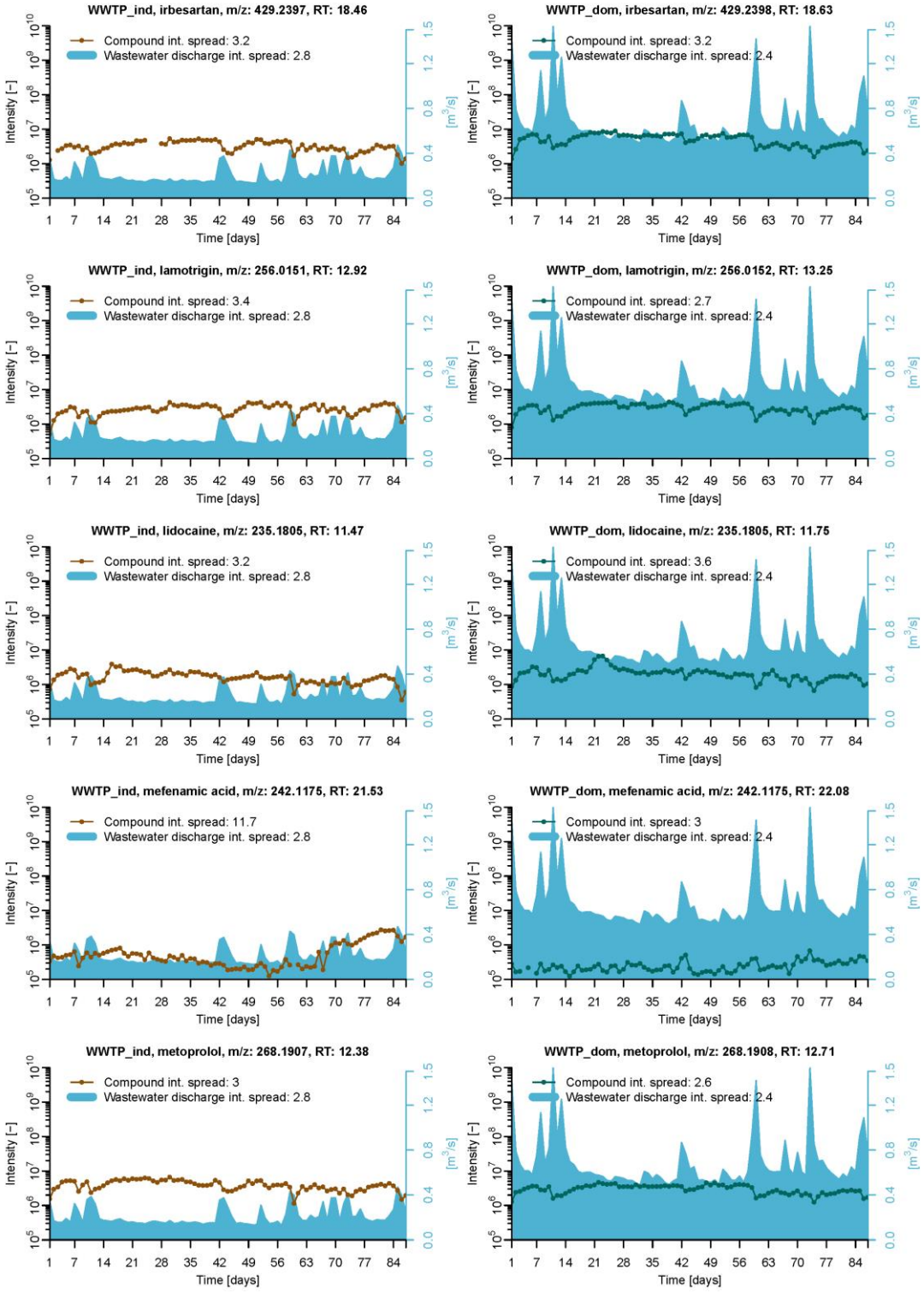
Results and discussion

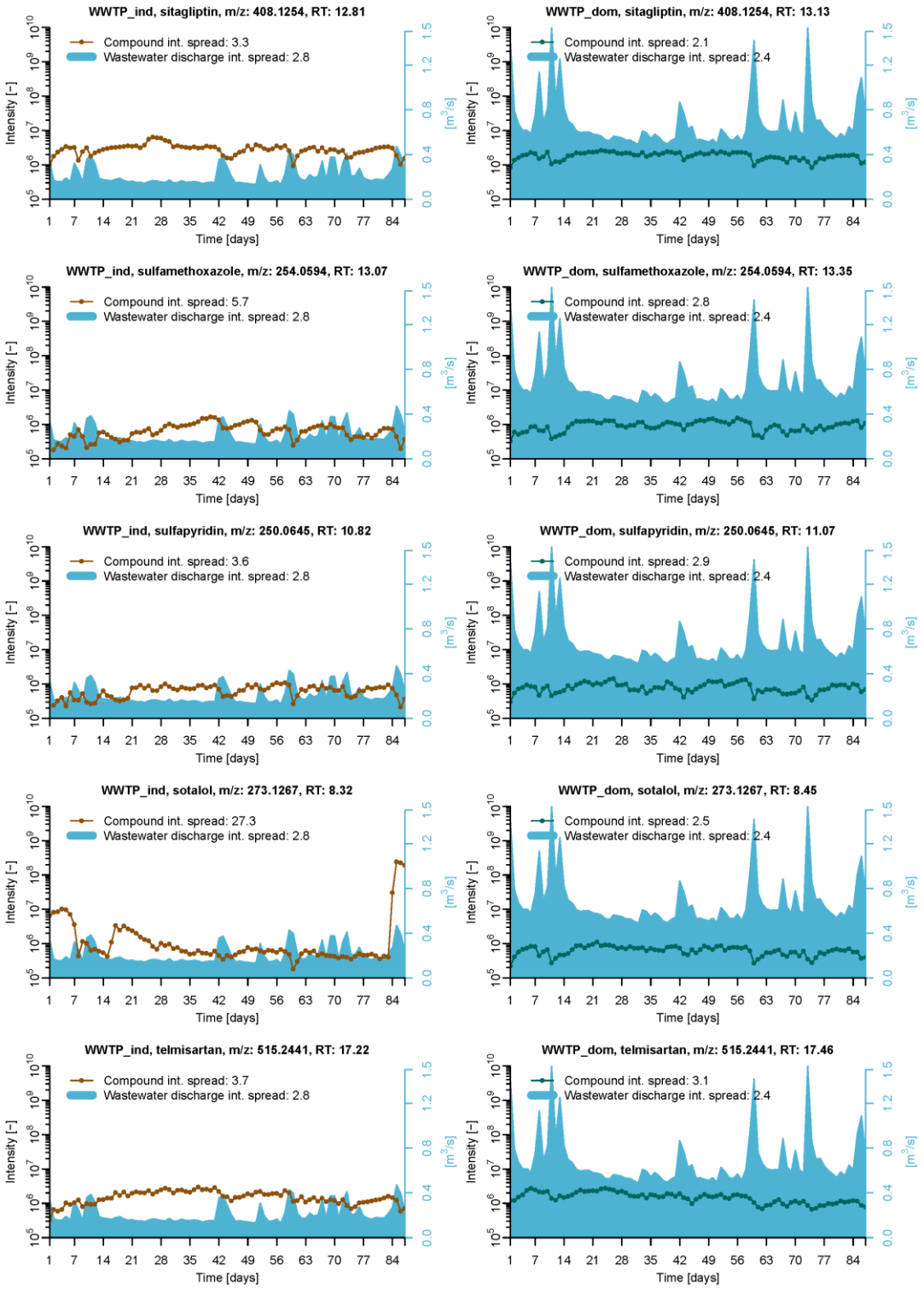
SI 12: Time profiles of tracer compounds for domestic wastewater




WWTP_ind, amisulprid, m/z: 370.1796, RT: 10.46
Compound int. spread: 2.9
Wastewater discharge int. spread: 2.8
WWTP_dom, amisulprid, m/z: 370.1796, RT: 10.73
Compound int. spread: 2.7
Wastewater discharge int. spread: 2.4
WWTP_ind, atenolol, m/z: 267.1703, RT: 8.68
Compound int. spread: 3.4
Wastewater discharge int. spread: 2.8
WWTP_dom, atenolol, m/z: 267.1703, RT: 8.84
Compound int. spread: 2
Wastewater discharge int. spread: 2.4
WWTP_ind, candesartan, m/z: 441.1669, RT: 18.66
Compound int. spread: 3.1
Wastewater discharge int. spread: 2.8
WWTP_dom, candesartan, m/z: 441.1669, RT: 18.86
Compound int. spread: 2.7
Wastewater discharge int. spread: 2.4
WWTP_ind, carbamazepin, m/z: 237.1023, RT: 17.54
Compound int. spread: 3.2
Wastewater discharge int. spread: 2.8
WWTP_dom, carbamazepin, m/z: 237.1023, RT: 17.8
Compound int. spread: 3.8
Wastewater discharge int. spread: 2.4
WWTP_ind, diclofenac, m/z: 296.024, RT: 20.55
Compound int. spread: 2.7
Wastewater discharge int. spread: 2.8
WWTP_dom, diclofenac, m/z: 296.024, RT: 20.93
Compound int. spread: 2.4
Wastewater discharge int. spread: 2.4
Intensity [-]
[m3/s]
Time [days]


WWTP_ind, etodolac, m/z: 286.1449, RT: 20.62
Compound int. spread: 4
Wastewater discharge int. spread: 2.8
WWTP_dom, etodolac, m/z: 286.1449, RT: 20.98
Compound int. spread: 2.5
Wastewater discharge int. spread: 2.4
WWTP_ind, fexofenadine, m/z: 502.2951, RT: 15.68
Compound int. spread: 2.7
Wastewater discharge int. spread: 2.8
WWTP_dom, fexofenadine, m/z: 502.2951, RT: 15.99
Compound int. spread: 3.4
Wastewater discharge int. spread: 2.4
WWTP_ind, flufenamic acid, m/z: 280.0591, RT: 21.28
Compound int. spread: 10.3
Wastewater discharge int. spread: 2.8
WWTP_dom, flufenamic acid, m/z: 280.0591, RT: 21.74
Compound int. spread: 3.7
Wastewater discharge int. spread: 2.4
WWTP_ind, gabapentin, m/z: 172.1332, RT: 10.67
Compound int. spread: 2.2
Wastewater discharge int. spread: 2.8
WWTP_dom, gabapentin, m/z: 172.1332, RT: 10.95
Compound int. spread: 2.4
Wastewater discharge int. spread: 2.4
WWTP_ind, hydrochlorothiazid, m/z: 295.9572, RT: 10.34
Compound int. spread: 3
Wastewater discharge int. spread: 2.8
WWTP_dom, hydrochlorothiazid, m/z: 295.9572, RT: 10.36
Compound int. spread: 2.6
Wastewater discharge int. spread: 2.4
Intensity [-]
[m3/s]
Time [days]


WWTP_ind, irbesartan, m/z: 429.2397, RT: 18.46
Compound int. spread: 3.2
Wastewater discharge int. spread: 2.8
WWTP_dom, irbesartan, m/z: 429.2398, RT: 18.63
Compound int. spread: 3.2
Wastewater discharge int. spread: 2.4
WWTP_ind, lamotrigin, m/z: 256.0151, RT: 12.92
Compound int. spread: 3.4
Wastewater discharge int. spread: 2.8
WWTP_dom, lamotrigin, m/z: 256.0152, RT: 13.25
Compound int. spread: 2.7
Wastewater discharge int. spread: 2.4
WWTP_ind, lidocaine, m/z: 235.1805, RT: 11.47
Compound int. spread: 3.2
Wastewater discharge int. spread: 2.8
WWTP_dom, lidocaine, m/z: 235.1805, RT: 11.75
Compound int. spread: 3.6
Wastewater discharge int. spread: 2.4
WWTP_ind, mefenamic acid, m/z: 242.1175, RT: 21.53
Compound int. spread: 11.7
Wastewater discharge int. spread: 2.8
WWTP_dom, mefenamic acid, m/z: 242.1175, RT: 22.08
Compound int. spread: 3
Wastewater discharge int. spread: 2.4
WWTP_ind, metoprolol, m/z: 268.1907, RT: 12.38
Compound int. spread: 3
Wastewater discharge int. spread: 2.8
WWTP_dom, metoprolol, m/z: 268.1908, RT: 12.71
Compound int. spread: 2.6
Wastewater discharge int. spread: 2.4
Intensity [-]
[m³/s]
Time [days]


WWTP_ind, sitagliptin, m/z: 408.1254, RT: 12.81
Compound int. spread: 3.3
Wastewater discharge int. spread: 2.8
WWTP_dom, sitagliptin, m/z: 408.1254, RT: 13.13
Compound int. spread: 2.1
Wastewater discharge int. spread: 2.4
WWTP_ind, sulfamethoxazole, m/z: 254.0594, RT: 13.07
Compound int. spread: 5.7
Wastewater discharge int. spread: 2.8
WWTP_dom, sulfamethoxazole, m/z: 254.0594, RT: 13.35
Compound int. spread: 2.8
Wastewater discharge int. spread: 2.4
WWTP_ind, sulfapyridin, m/z: 250.0645, RT: 10.82
Compound int. spread: 3.6
Wastewater discharge int. spread: 2.8
WWTP_dom, sulfapyridin, m/z: 250.0645, RT: 11.07
Compound int. spread: 2.9
Wastewater discharge int. spread: 2.4
WWTP_ind, sotalol, m/z: 273.1267, RT: 8.32
Compound int. spread: 27.3
Wastewater discharge int. spread: 2.8
WWTP_dom, sotalol, m/z: 273.1267, RT: 8.45
Compound int. spread: 2.5
Wastewater discharge int. spread: 2.4
WWTP_ind, telmisartan, m/z: 515.2441, RT: 17.22
Compound int. spread: 3.7
Wastewater discharge int. spread: 2.8
WWTP_dom, telmisartan, m/z: 515.2441, RT: 17.46
Compound int. spread: 3.1
Wastewater discharge int. spread: 2.4
Intensity [-]
[m3/s]
Time [days]

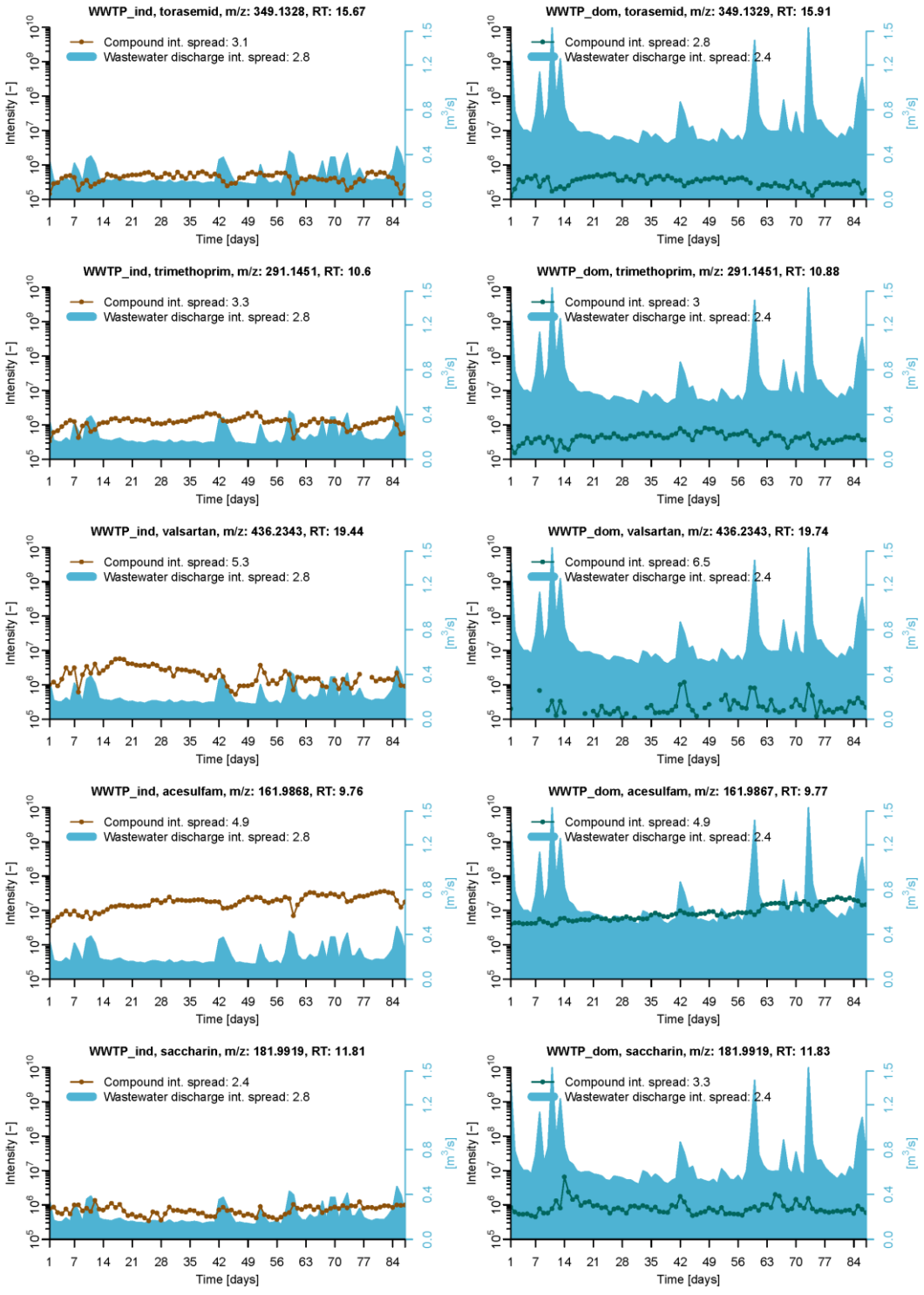
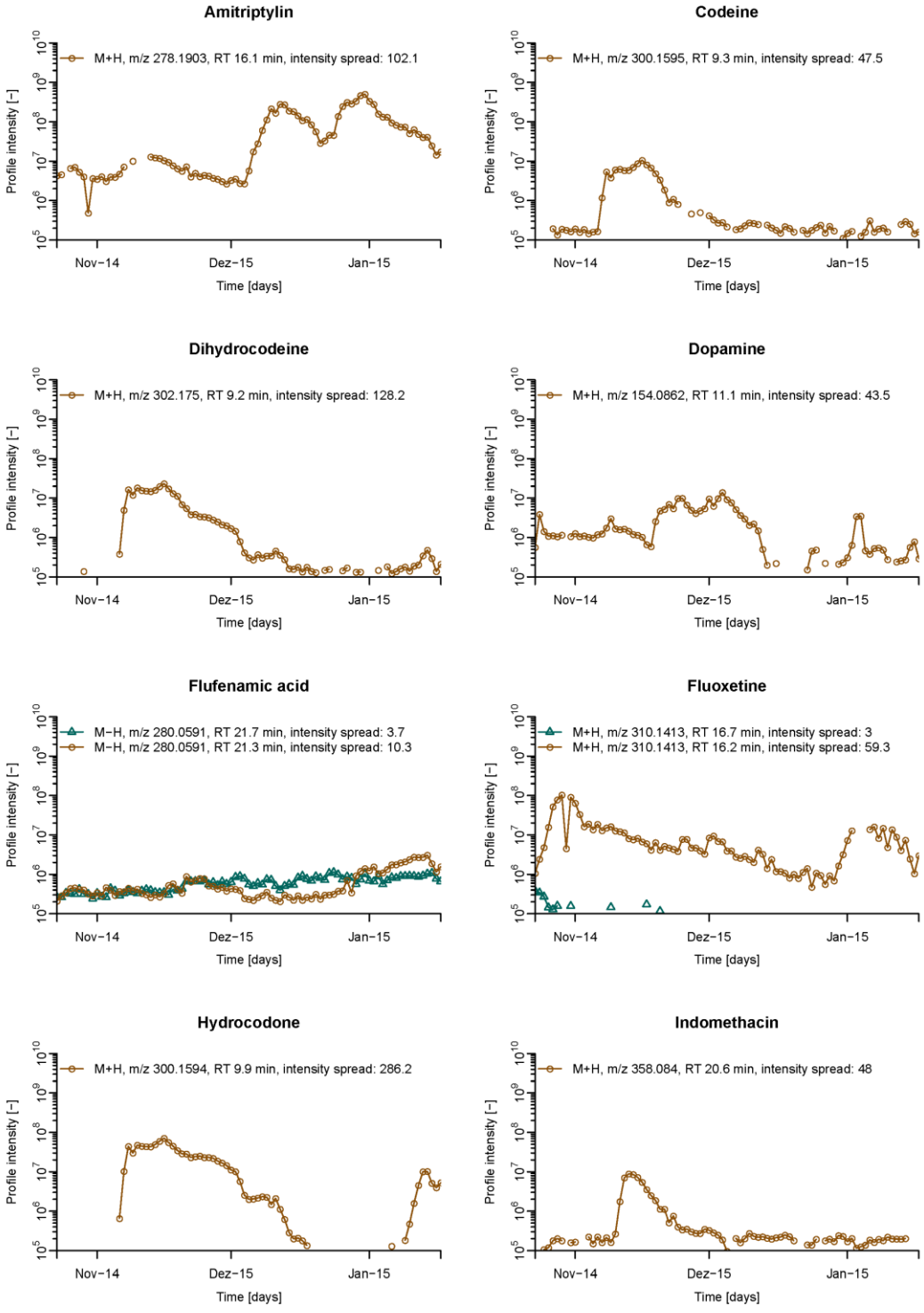
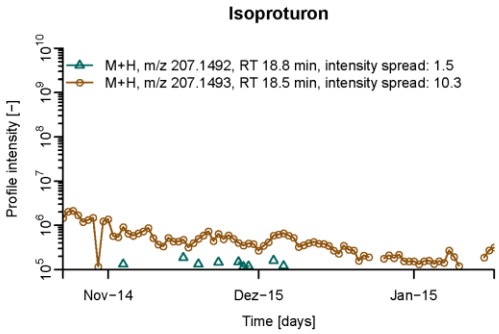
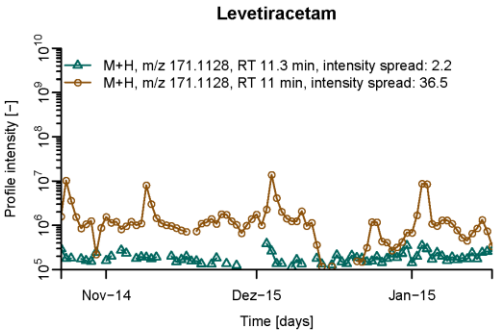


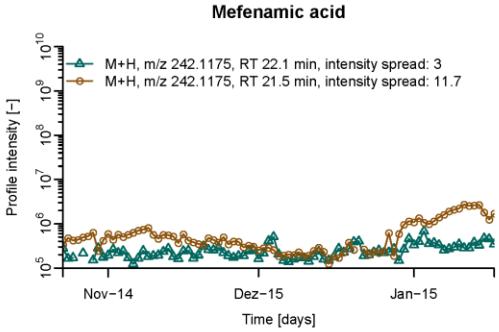
Figure S4: Intensity time profiles of 30 domestic wastewater tracer compounds. The time pattern of the WWTP discharge is plotted as blue area in the background. For each compound, the time profile detected at WWTP_ind is shown on the right and the one detected at WWTP_dom on the left. The intensity spread of the time profile intensity and the WWTP discharge was calculated as the 95% quantile/ 5% quantile ratio of the intensity values. Please note the logarithmic scale of the y-axis.

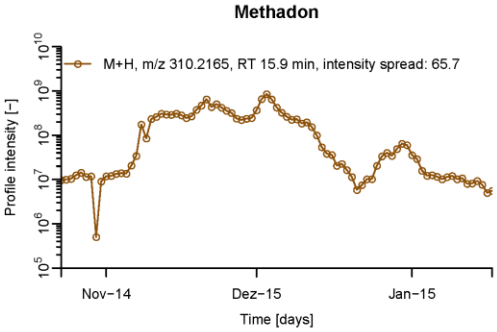
SI 13: Time profiles of target compounds classified as potential industrial emissions

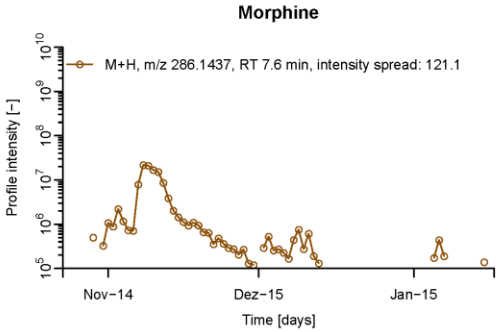


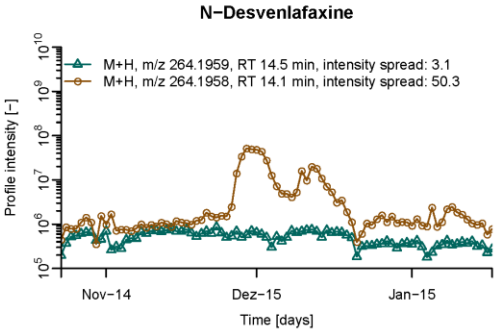

Isoproturon
M+H, m/z 207.1492, RT 18.8 min, intensity spread: 1.5
M+H, m/z 207.1493, RT 18.5 min, intensity spread: 10.3
Profile intensity [-]
Nov-14
Dez-15
Jan-15
Time [days]

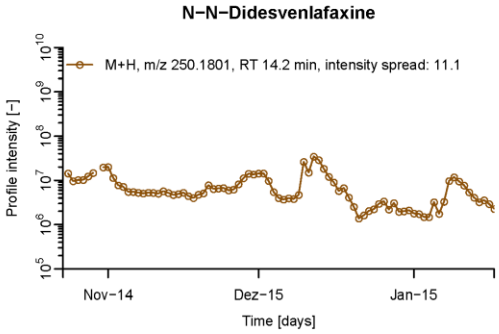

Levetiracetam
M+H, m/z 171.1128, RT 11.3 min, intensity spread: 2.2
M+H, m/z 171.1128, RT 11 min, intensity spread: 36.5
Profile intensity [-]
Nov-14
Dez-15
Jan-15
Time [days]

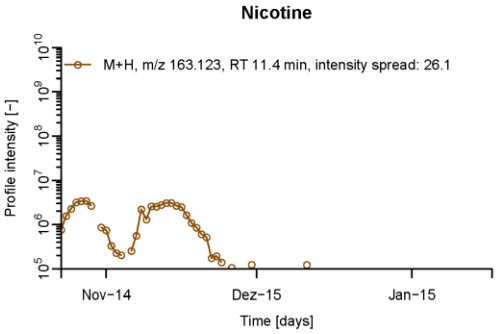

Mefenamic acid
M+H, m/z 242.1175, RT 22.1 min, intensity spread: 3
M+H, m/z 242.1175, RT 21.5 min, intensity spread: 11.7
Profile intensity [-]
Nov-14
Dez-15
Jan-15
Time [days]


Methadon
M+H, m/z 310.2165, RT 15.9 min, intensity spread: 65.7
Profile intensity [-]
Nov-14
Dez-15
Jan-15
Time [days]


Morphine
M+H, m/z 286.1437, RT 7.6 min, intensity spread: 121.1
Profile intensity [-]
Nov-14
Dez-15
Jan-15
Time [days]


N-Desvenlafaxine
M+H, m/z 264.1959, RT 14.5 min, intensity spread: 3.1
M+H, m/z 264.1958, RT 14.1 min, intensity spread: 50.3
Profile intensity [-]
Nov-14
Dez-15
Jan-15
Time [days]


N-N-Didesvenlafaxine
M+H, m/z 250.1801, RT 14.2 min, intensity spread: 11.1
Profile intensity [-]
Nov-14
Dez-15
Jan-15
Time [days]


Nicotine
M+H, m/z 163.123, RT 11.4 min, intensity spread: 26.1
Profile intensity [-]
Nov-14
Dez-15
Jan-15
Time [days]

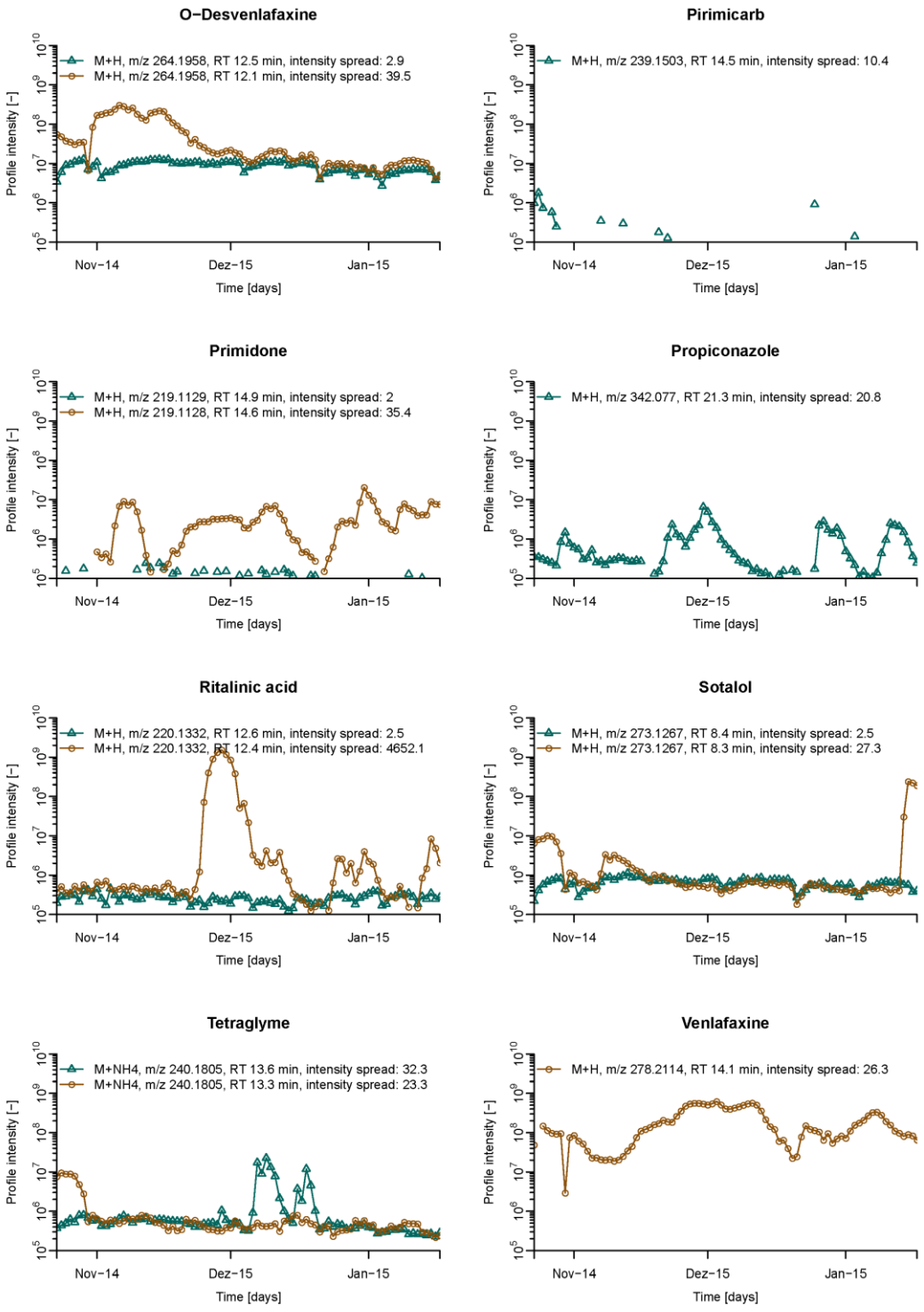


Figure S5: Intensity time profiles of the 24 target compounds that were detected among the potential industrial emissions (intensity spread > 10) either at WWTP_ind (brown profiles) or at WWTP_dom (blue-green profile). The intensity spread of the time profile was calculated as the 95% quantile/ 5% quantile ratio of the intensity values. Please note the logarithmic scale of the y-axis.

SI 14: Quantification results

Table S7: Quantification results

		WWTP_ind			WWTP_dom		
Substance[a]	**LOQ[b] [µg/L]**	**min. - max.[c] [µg/L]**	**Average [µg/L]**	**n[d]**	**min. - max.[c] [µg/L]**	**Average [µg/L]**	**n[d]**
Amitriptyline & Maprotiline*	2	< LOQ – 44.47	13.9	59	< LOQ	NA	0
Codeine	0.1	< LOQ – 0.11	0.11	3	< LOQ	NA	0
Dihydrocodeine	0.1	< LOQ - 4.56	1.84	14	< LOQ	NA	0
Dopamine	1	< LOQ - 41.5	13.7	27	< LOQ	NA	0
Fufenamic acid	0.1	< LOQ - 0.28	0.18	18	< LOQ - 0.13	0.11	19
Fluoxetine	1	< LOQ - 18.7	3.5	67	< LOQ	NA	0
Hydrocodone	0.1	< LOQ - 24.44	3.72	64	< LOQ – 0.13	0.10	7
Indomethacine	0.1	< LOQ - 6.21	0.57	81	< LOQ - 0.11	0.10	15
Isoproturon	0.1	< LOQ - 0.22	0.15	11	< LOQ	NA	0
Levetiracetam	0.5	< LOQ - 14.25	1.52	50	< LOQ	NA	0
Mefenamic acid	0.1	< LOQ - 0.47	0.19	50	< LOQ – 0.15	0.12	6
Methadone	1	< LOQ - 31.9	9.0	83	< LOQ -1.5	NA	1
Morphine	0.1	< LOQ - 6.58	0.87	42	< LOQ	NA	0
N-Desvenlafaxine	0.1	< LOQ - 5.31	0.94	75	< LOQ	NA	0
Nicotine	0.5	< LOQ - 0.82	0.68	16	< LOQ	NA	0
N-N-Didesvenlafaxine	0.1	0.2 – 5.24	1.39	87	< LOQ	NA	0
O-Desvenlafaxine & Tramadol*	0.2	< LOQ - 25.07	3.2	86	0.33 – 1.21	0.88	87
Pirimicarb	0.1	< LOQ	NA	0	< LOQ - 0.10	NA	1
Primidone	0.5	< LOQ - 11.60	3.49	57	< LOQ	NA	0
Propiconazole	0.1	< LOQ	NA	0	< LOQ - 1.34	0.31	55
Ritalinic acid	0.1	< LOQ - 214.89	36.2	25	< LOQ	NA	0
Sotalol	0.1	< LOQ - 45.31	1.98	76	< LOQ - 0.2	0.15	82
Tetraglyme	0.1	< LOQ - 0.75	0.57	7	< LOQ - 1.82	0.69	10
Venlafaxine	0.1	1.25 – 65.38	18.55	87	0.12 – 0.49	0.25	87

[a] For compounds with bolded names an isotope-labeled analogue was available as internal standard for quantification. Analytes without isotope labeled analogue were quantified with the isotope-labeled standard with the closest retention time.

[b] Limit of quantification

[c] Maximum and average concentrations above 10 µg/L are outside of the calibration range and thus estimates.

[d] Number of samples in which the respective compound was detected > LOQ. The total number of samples was 87.

* Isobaric co-eluting substance pairs that were quantified as the sum of two respective compounds.

NA Not assignable

LOQ Limit of quantification

SI 15: Time profiles of suspect compounds classified as industrial emissions

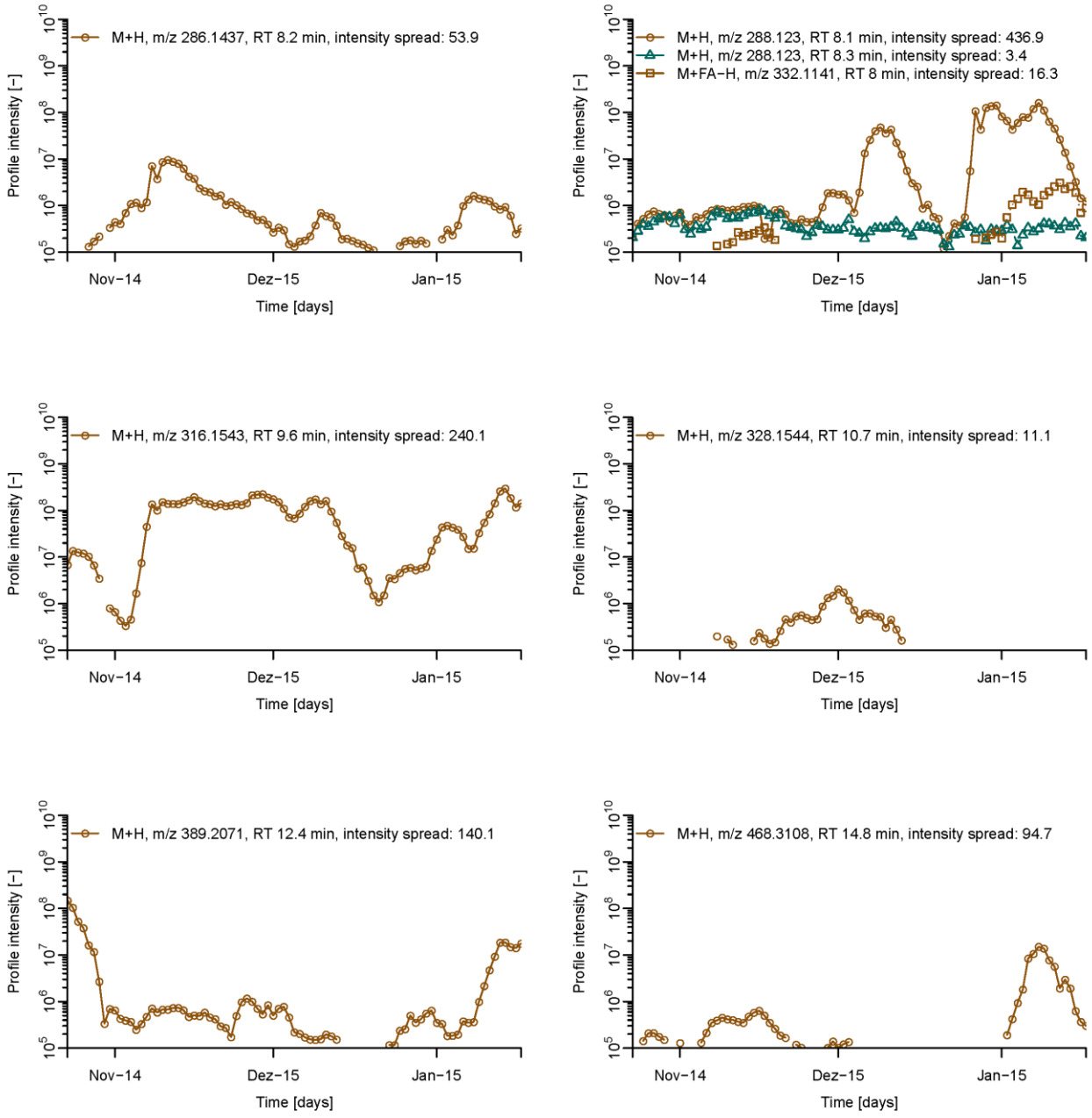


Figure S6: Intensity time profiles of the six suspect compounds classified as industrial emissions at WWTP_ind (brown profiles). Only one of the compounds was also detected at WWTP_dom (blue-green profile), the respective compound was detected in positive (M+H) and negative ion (M+FA-H) mode at WWTP_ind. The intensity spread of the time profile was calculated as the 95% quantile/ 5% quantile ratio of the intensity values. Please note the logarithmic scale of the y-axis.

SI 16: Identified nontarget compounds

SI 16.1: Time profiles

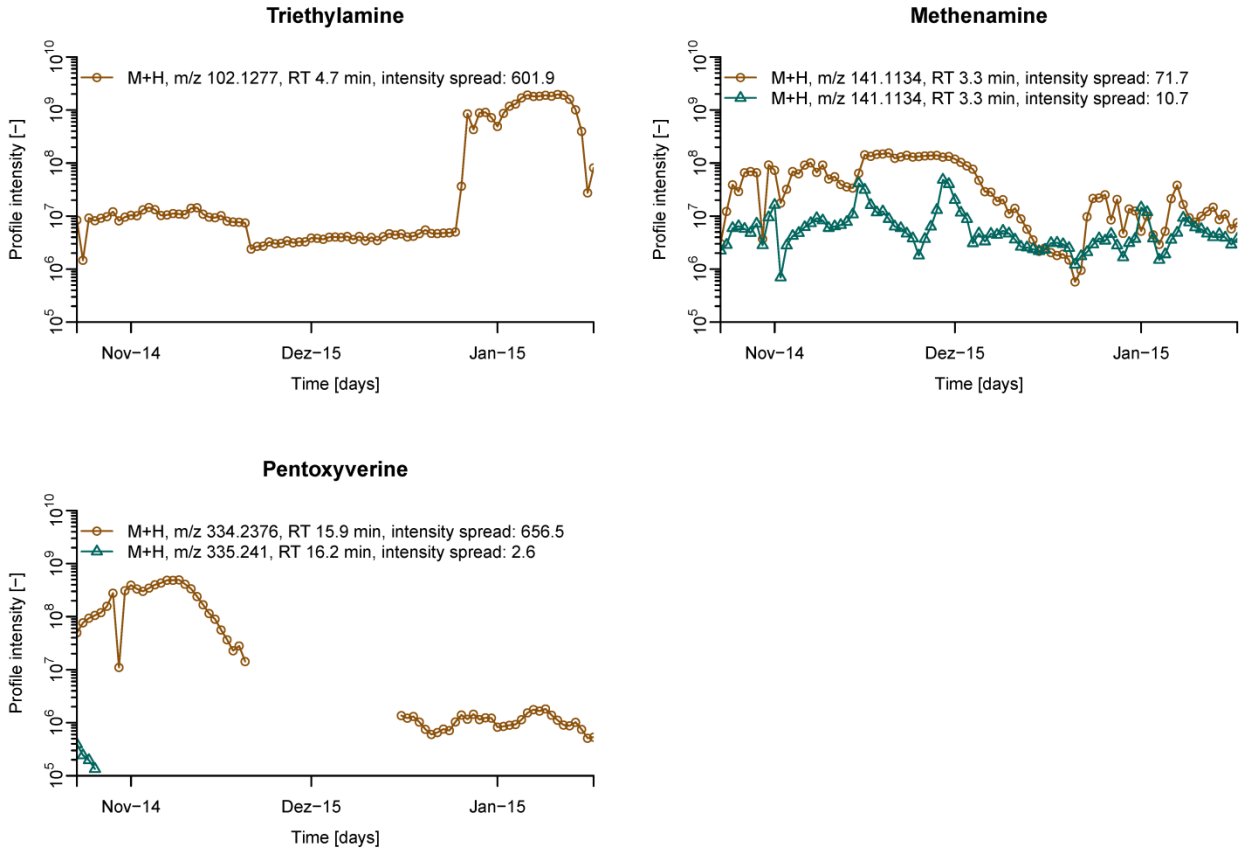


Figure S7: Time profiles of the three identified nontarget compounds (level 1 structure confirmation). Brown time profiles were detected at WWTP_ind, blue-green profiles were detected at WWTP_dom. The intensity spread of the time profile was calculated as the 95% quantile/ 5% quantile ratio of the intensity values. Please note the logarithmic scale of the y-axis.

SI 16.2: Confirmation

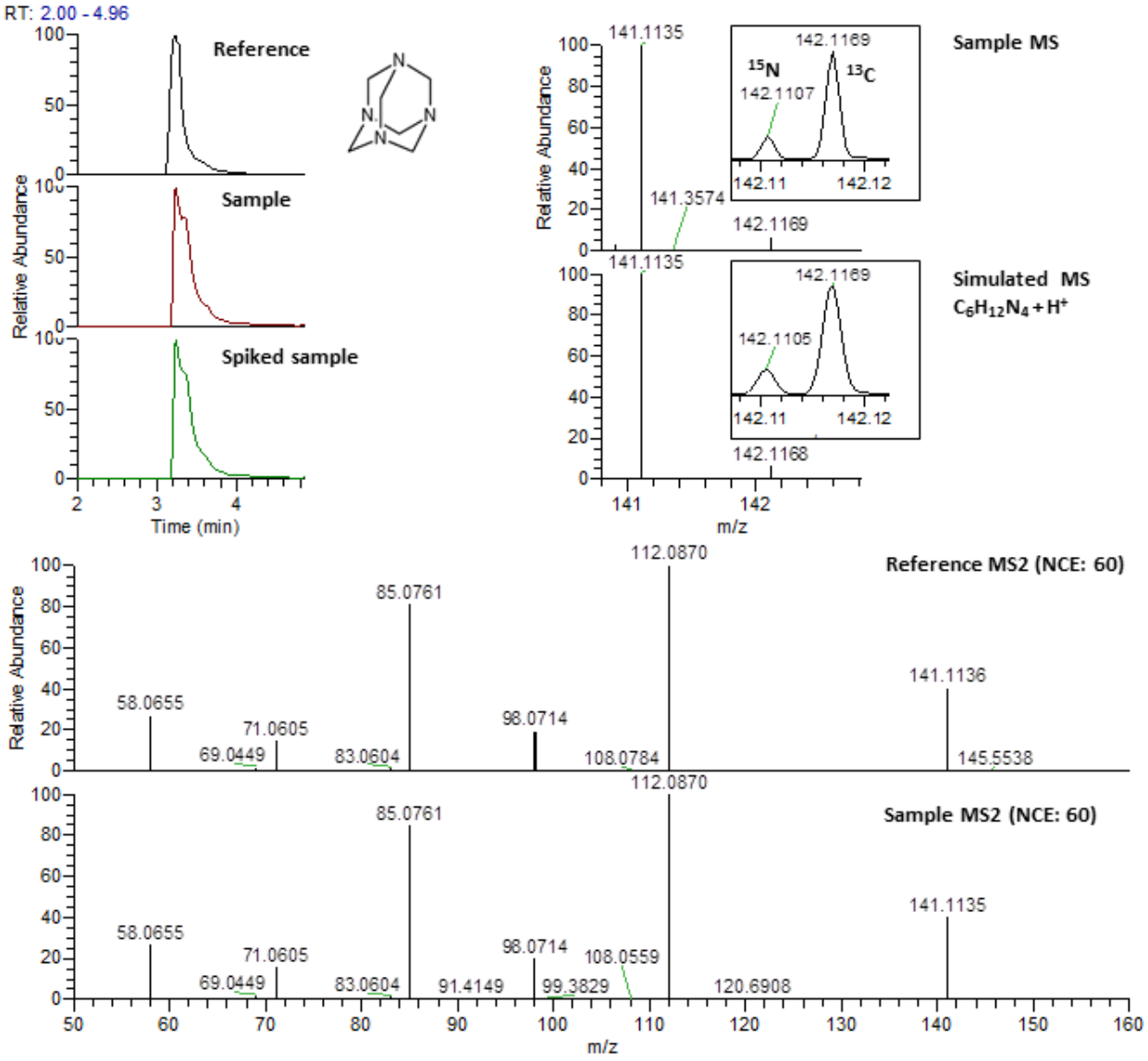


Figure S8: Methenamine (International Chemical Identifier (InChI): 1S/C6H12N4/c1-7-2-9-4-8(1)5-10(3-7)6-9/h1-6H2), $C_6H_{12}N_4$, *m/z* 141.1134, RT 3.3 min, positive ion mode; extracted ion chromatogram with 5 ppm window; NCE: normalized collision energy for MS2 fragmentation in HCD cell.

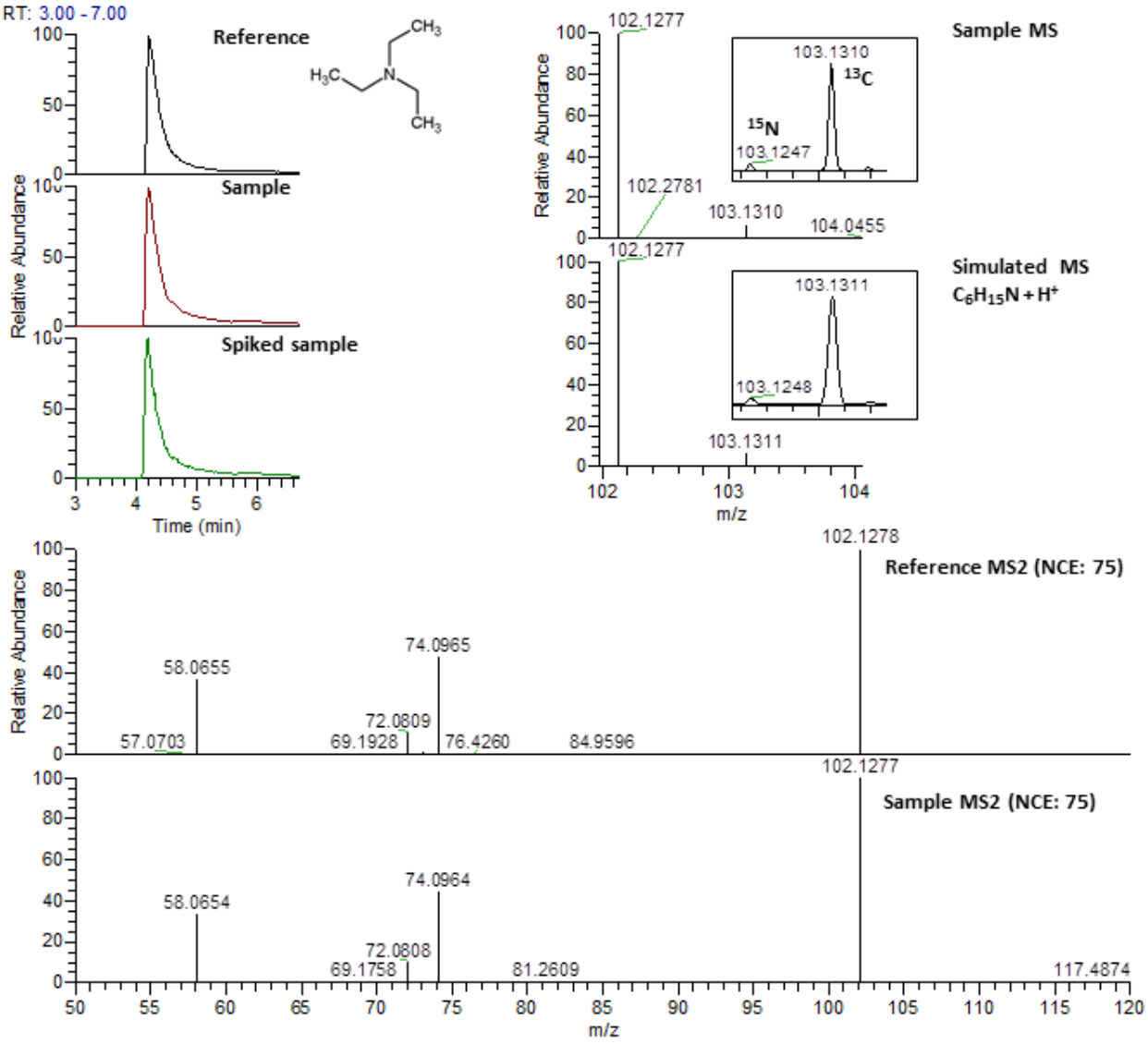


Figure S9: Triethylamine (InCh: 1S/C6H15N/c1-4-7(5-2)6-3/h4-6H2,1-3H3), $C_6H_{15}N$, *m/z* 102.1277, RT 4.7 min, positive ion mode; extracted ion chromatogram with 5 ppm window; NCE: normalized collision energy for MS2 fragmentation in HCD cell.

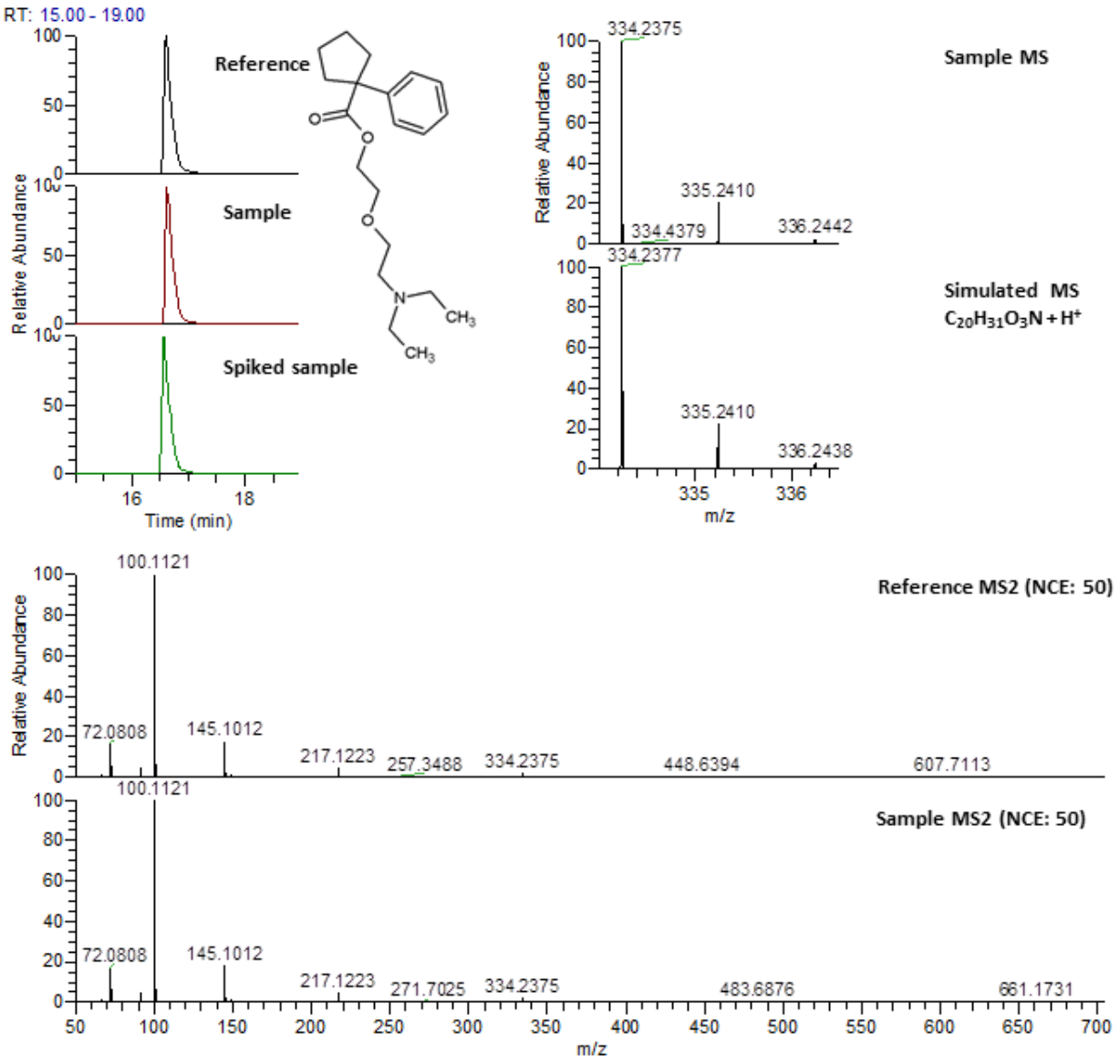
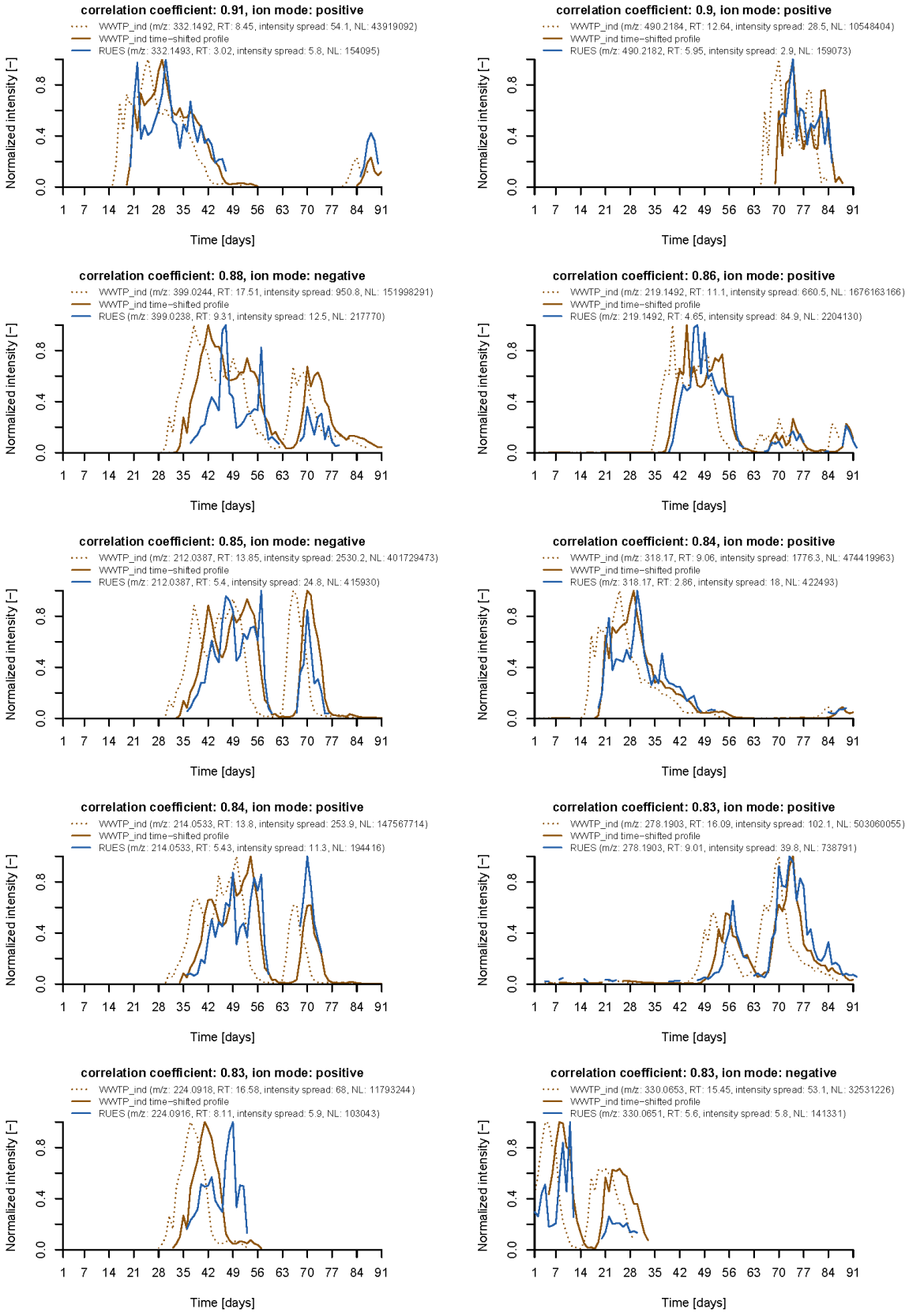


Figure S10: Pentoxyverine (InChI: 1S/C20H31NO3/c1-3-21(4-2)14-15-23-16-17-24-19(22)20(12-8-9-13-20)18-10-6-5-7-11-18/h5-7,10-11H,3-4,8-9,12-17H2,1-2H3), $C_{20}H_{31}O_3N$, *m/z* 334.2376, RT 15.9 min, positive ion mode; extracted ion chromatogram with 5 ppm window; NCE: normalized collision energy for MS2 fragmentation in HCD cell.

SI 17: Correlating time profiles between WWTP_ind and RUES



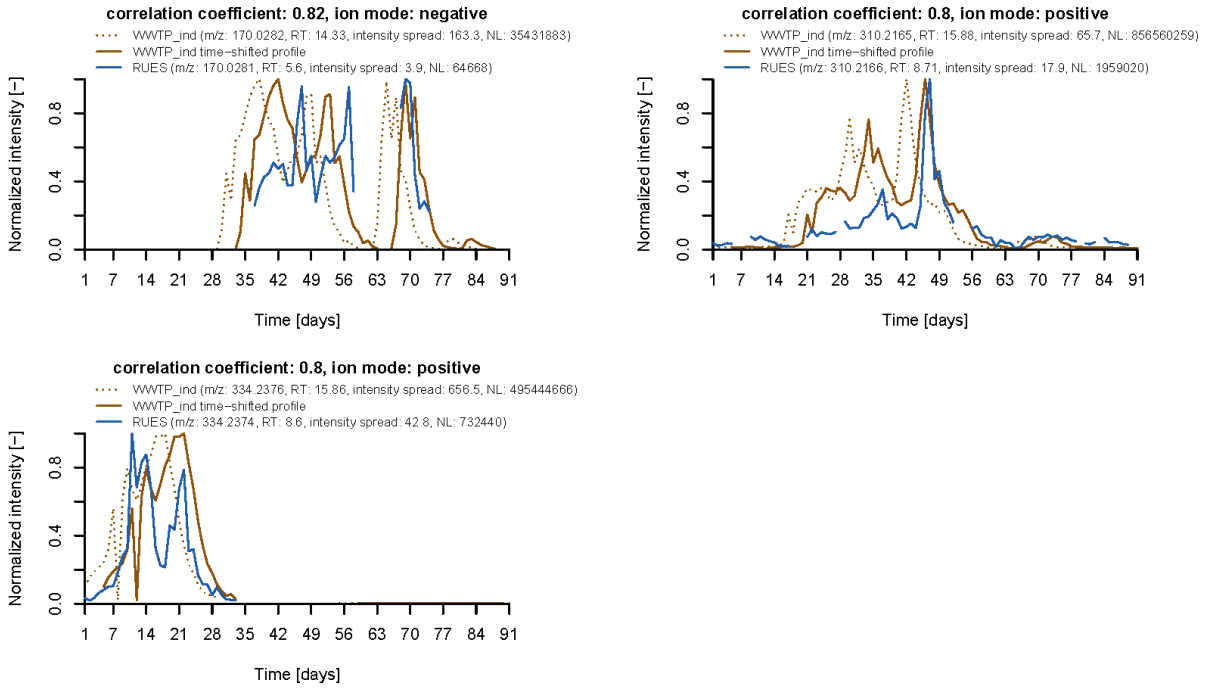


Figure S11: Correlating time profiles between WWTP_ind and the river Rhine at the international Rhine monitoring station (RUES). For WWTP_ind the original (dotted brown line) as well as the 4-day time-shifted profile is shown (solid brown line); the latter overlaps with the time profile measured at the RUES (blue line). Intensity time profiles were normalized by dividing all intensities by the maximum intensity of the respective time profile, the respective normalization level (NL) is indicated in the plots. Two profiles belong to the same compound that ionized in positive ESI (*m/z* 214.0533) and in negative ESI (*m/z* 212.0387) mode.

SI 18: Time profiles displaying recurring phases of high signal intensities at RUES

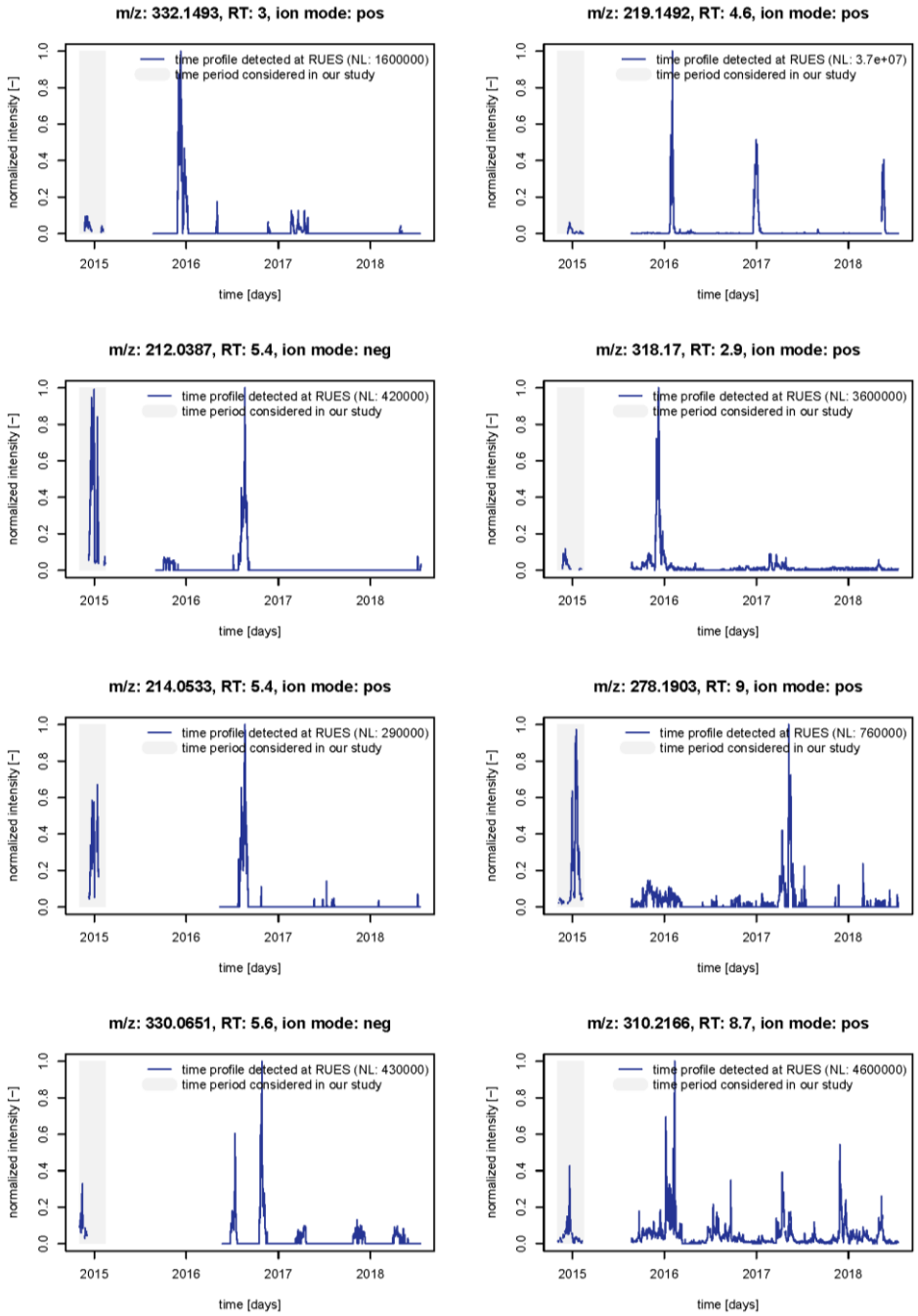


Figure S12: Long–term time profiles extracted from the daily LC-HRMS monitoring data of the RUES. The extracted time profiles correspond to compounds for which a highly correlating time profile between WWTP_ind and the RUES was found (cf. **SI 17**) and for which recurring phases of high signal intensities were observed in the RUES data. The time profiles were normalized by dividing all intensities by the maximum intensity of the respective time profile, the respective normalization level (NL) is indicated in the plots. Two profiles belong to the same compound that ionized in positive ESI (*m/z* 214.0533) as well as in negative ESI (*m/z* 212.0387) mode.

References

(1) Loos, M., enviMass version 4.0. Zenodo, 2018.

(2) Chambers, M. C.; Maclean, B.; Burke, R.; Amodei, D.; Ruderman, D. L.; Neumann, S.; Gatto, L.; Fischer, B.; Pratt, B.; Egertson, J.; Hoff, K.; Kessner, D.; Tasman, N.; Shulman, N.; Frewen, B.; Baker, T. A.; Brusniak, M.-Y.; Paulse, C.; Creasy, D.; Flashner, L.; Kani, K.; Moulding, C.; Seymour, S. L.; Nuwaysir, L. M.; Lefebvre, B.; Kuhlmann, F.; Roark, J.; Rainer, P.; Detlev, S.; Hemenway, T.; Huhmer, A.; Langridge, J.; Connolly, B.; Chadick, T.; Holly, K.; Eckels, J.; Deutsch, E. W.; Moritz, R. L.; Katz, J. E.; Agus, D. B.; MacCoss, M.; Tabb, D. L.; Mallick, P., A cross-platform toolkit for mass spectrometry and proteomics. *Nature Biotechnology* **2012,** *30*, 918.