Assessment of highly polar chemicals in Dutch and Flemish drinking water and its sources: presence and potential risks

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- **Summary of data processing parameters** and Compound Discoverer 2.1 settings

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Table S1. Method performance characteristics for HILIC-MS screening method for 32 highly polar compounds in drinking and surface water

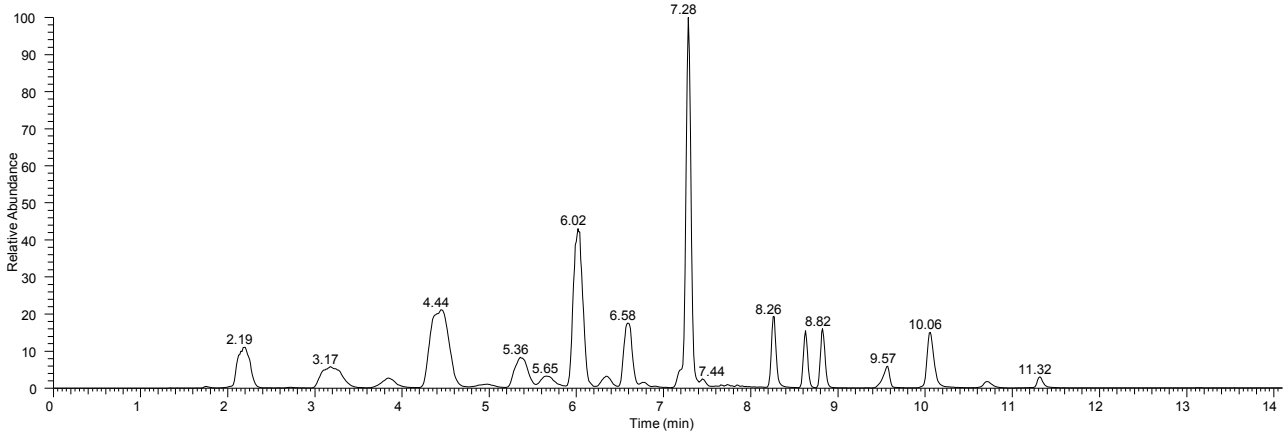
							Drinking water				Surface Water			
Compound	Log D at pH7.4[a]	Ionisation mode	Ion	Accurate Mass (m/z)	MS/MS Fragment Ion (m/z)	t_R (min)	LOD (µg/L)	LOQ (µg/L)	RSD 1 µg/L (%) (n=8)	Recovery 1 µg/L (%) (n=8)	LOD (µg/L)	LOQ (µg/L)	RSD 1 µg/L (%) (n=8)	Recovery 1 µg/L (%) (n=8)
5-Fluorocystosine	-2.16	pos	$[M+H]^+$	130.04112	113.01457	5.13	0.003	0.008	7.2	102.9	0.002	0.006	3.8	84.5
Acephate	-0.53	pos	$[M+H]^+$	184.01918	142.99263	2.10	0.20	0.59	6.8	93.1	0.16	0.47	8.2	62.1
Ammelide	-4.29	pos	$[M+H]^+$	129.04070	87.01890	3.66	0.028	0.085	14.1	58.7	0.009	0.026	11.0	14.5
Ammeline	-2.76	pos	$[M+H]^+$	128.05669	86.03489	7.01	0.012	0.036	7.7	97.1	0.019	0.056	5.3	114.6
Amprolium	-2.86	pos	$[M]^+$	243.16042	150.10257	10.70	0.018	0.053	11.8	85.3	0.084	0.25	3.9	89.0
Chlormequat	-3.04	pos	$[M]^+$	122.07310	58.06513	8.80	0.005	0.016	8.5	102.7	0.005	0.014	9.7	105.1
Chlormequat-d_9 (ISTD)	-	pos	$[M]^+$	131.12959	-	8.80	-	-	-	-	-	-	-	-
Cotinine	0.17	pos	$[M+H]^+$	177.10224	80.04948	4.32	0.003	0.010	7.0	109.4	0.003	0.009	10.9	77.1
Cyromazin	0.23	pos	$[M+H]^+$	167.10397	85.05087	5.71	0.004	0.011	3.2	107.5	0.002	0.005	3.2	107.8
Cytarabine	-2.18	pos	$[M+H]^+$	244.09280	112.05054	6.56	0.024	0.071	9.4	93.0	0.088	0.26	5.3	107.7
Diatrizoic acid	-1.00	pos	$[M+H]^+$	614.77690	233.05568	7.10	0.087	0.26	13.8	114.5	0.093	0.28	8.9	99.7
Gabapentin	-1.40	pos	$[M+H]^+$	172.13321	154.12264	8.81	0.01	0.031	6.0	99.4	0.087	0.26	7.8	97.2
Gemcitabine	-1.36	pos	$[M+H]^+$	264.07904	112.05054	3.08	0.003	0.008	7.1	108.7	0.007	0.021	5.6	22.1
Guanylurea	-1.82	pos	$[M+H]^+$	103.06144	60.05562	7.30	0.022	0.067	6.0	85.4	0.12	0.35	2.9	112.0
Iohexol	-3.17	pos	$[M+H]^+$	821.88761	803.87702	7.10	0.24	0.73	7.7	104.5	0.20	0.61	6.3	101.2
Iopamidol	-2.31	pos	$[M+H]^+$	777.86140	558.88575	4.70	0.082	0.25	7.0	88.5	0.11	0.33	9.4	92.6
Iopromide	-2.12	pos	$[M+H]^+$	791.87705	572.90140	4.75	0.046	0.14	9.4	102.2	0.11	0.33	8.8	88.1
Maleic hydrazide	-3.06	pos	$[M+H]^+$	113.03455	85.03964	2.66	0.13	0.40	27.0	16.2	0.071	0.50	6.0	38.0
Melam	-2.15	pos	0.15$[M+H]^+$	236.11152	152.06792	8.19	0.002	0.006	13.1	101.6	0.003	0.009	8.4	90.6
Melamine	-1.18	pos	$[M+H]^+$	127.07267	85.05087	6.38	0.19	0.57	4.7	118.5	0.19	0.56	3.0	96.4
Melem	-4.18	pos	$[M+H]^+$	219.08497	177.06317	6.16	0.023	0.068	10.2	97.4	0.020	0.061	15.6	97.8
Mepiquat	-2.34	pos	$[M]^+$	114.12773	98.09643	10.05	0.006	0.019	9.6	116.8	0.007	0.022	7.3	92.5
Metformin	-3.36	pos	$[M+H]^+$	130.10872	71.06037	8.60	0.004	0.011	8.4	104.0	0.24	0.71	7.5	92.8
Methamidophos	-0.56	pos	$[M+H]^+$	142.00862	112.01581	2.13	0.072	0.22	7.5	114.2	0.098	0.29	8.0	99.0
Niacin	-2.60	pos	$[M+H]^+$	124.03930	80.04948	5.67	0.009	0.027	6.5	101.8	0.006	0.019	6.9	104.2
N-Methyldiethanolamine	-1.79	pos	$[M+H]^+$	120.10191	102.09134	9.50	0.027	0.080	9.7	95.2	0.020	0.059	8.0	97.9
Omethoate	-0.84	pos	$[M+H]^+$	214.02974	142.99263	2.17	0.013	0.040	8.4	91.9	0.010	0.029	8.3	101.4
Sotalol-d_7 (ISTD)	-	pos	$[M+H]^+$	280.17068	-	7.50	-	-	-	-	-	-	-	-
Tetrapropylammonium (TPA)	-1.77	pos	$[M+H]^+$	186.22218	114.12773	7.19	0.006	0.018	10.1	100.9	0.008	0.023	2.2	107.4
Urotropin (Hexamine)	0.99	pos	$[M+H]^+$	141.11347	112.08692	11.30	0.04	0.12	11.0	116.8	0.45	1.3	3.4	141.8
5-Fluorouracil	-1.57	neg	$[M-H]^-$	129.01058	58.99387	1.81	0.008	0.025	4.0	110.7	0.014	0.041	6.3	108.4

Cyanuric acid	-5.61	neg	$[M-H]^-$	128.01016	85.00435	1.77	0.051	0.15	12.8	109.7	0.093	0.28	7.6	94.9
Dichloroacetic acid	-2.86	neg	$[M-H]^-$	126.93591	59.01385	2.10	0.028	0.085	5.4	84.7	0.036	0.11	9.8	86.9
Naphthalene-1,5-disulfonic acid	-5.39	neg	$[M-H]^-$	286.96896	207.01214	1.69	0.14	0.41	16.9	104.1	0.037	0.11	6.1	37.9
Sotalol-d_7 (ISTD)	-	neg	$[M-H]^-$	278.15612	-	7.50	-	-	-	-	-	-	-	-

[a] Log D predicted by ACD/Labs platform (taken from ChemSpider)

FIgure S1. Chromatograms of the standards used for target analysis

Extracted ion chromatogram of a standard (1 µg/L) containing all target compounds in positive mode.



Extracted ion chromatogram of a standard (1 µg/L) containing all target compounds in negative mode

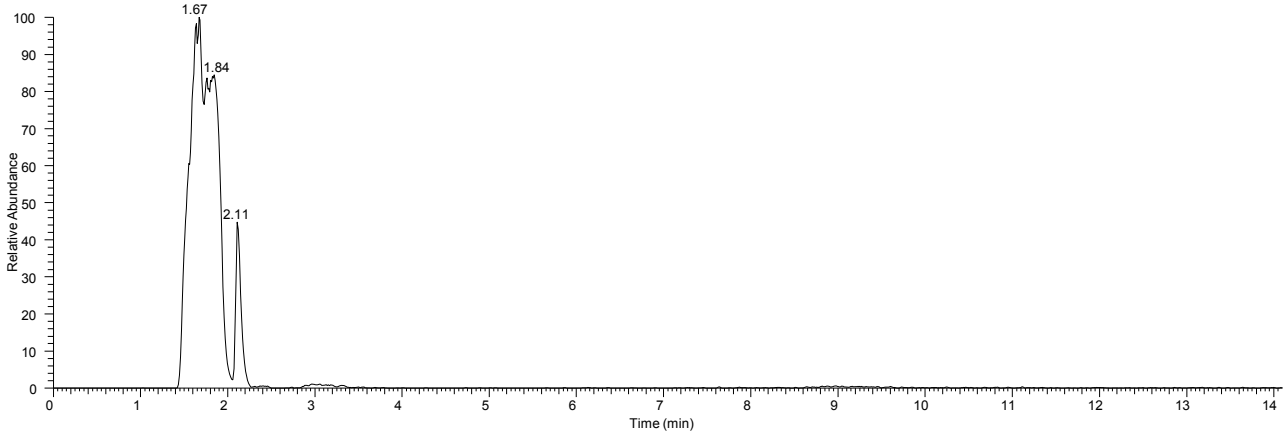


Table S2. Pearson product moment correlation coefficients of concentrations of melamine, melem, melam and cyanuric acid observed in water samples (n=24)

(when values below LOD or between LOD and LOQ were measured, a value of 0.5xLOD or 0.5xLOQ, respectively, was used)

Relationship		Pearson r	p value
Melamine	Melem	0.48	0.017
Melamine	Melam	0.45	0.027
Melamine	Cyanuric acid	0.74	<0.01
Melem	Melam	0.98	<0.01
Melem	Cyanuric acid	0.83	<0.01
Melam	Cyanuric acid	0.78	<0.01

Concentration data (µg/L) used for calculation of Pearson product moment correlation coefficients
(for compounds detected between LOQ and LOD a value of 0.5x LOQ was used (indicated by **), for compounds not detected in a sample, a value of 0.5xLOD was used, indicated by*). Note that LOD/LOQs may differ between surface water and tapwater (for LODs/LOQs see Table S1)

Melamine	Melem	Melam	Cyanuric acid
3.49	0.010*	0.002*	0.14**
2.79	0.127	0.020	0.48
2.18	0.081	0.011	0.33
1.93	0.010*	0.002*	0.14**
1.91	0.010*	0.002*	0.14**
1.88	0.012*	0.001*	0.24
1.49	0.146	0.030*	0.30
0.70	0.012*	0.001*	0.03*
0.10*	0.012*	0.001*	0.14**
0.10*	0.010*	0.002*	0.08*
0.10*	0.012*	0.001*	0.03*
0.10*	0.012*	0.001*	0.03*
0.10*	0.012*	0.001*	0.03*
0.10*	0.012*	0.001*	0.03*
0.10*	0.010*	0.002*	0.08*
0.10*	0.012*	0.001*	0.03*
0.10*	0.010*	0.002*	0.08*
0.10*	0.012*	0.001*	0.03*
0.10*	0.012*	0.001*	0.03*
0.10*	0.010*	0.002*	0.08*
0.10*	0.012*	0.001*	0.03*
0.10*	0.010*	0.002*	0.08*
0.10*	0.012*	0.001*	0.03*
0.10*	0.010*	0.002*	0.08*

Figure S2. Workflow used in non-target mass spectrometry screening

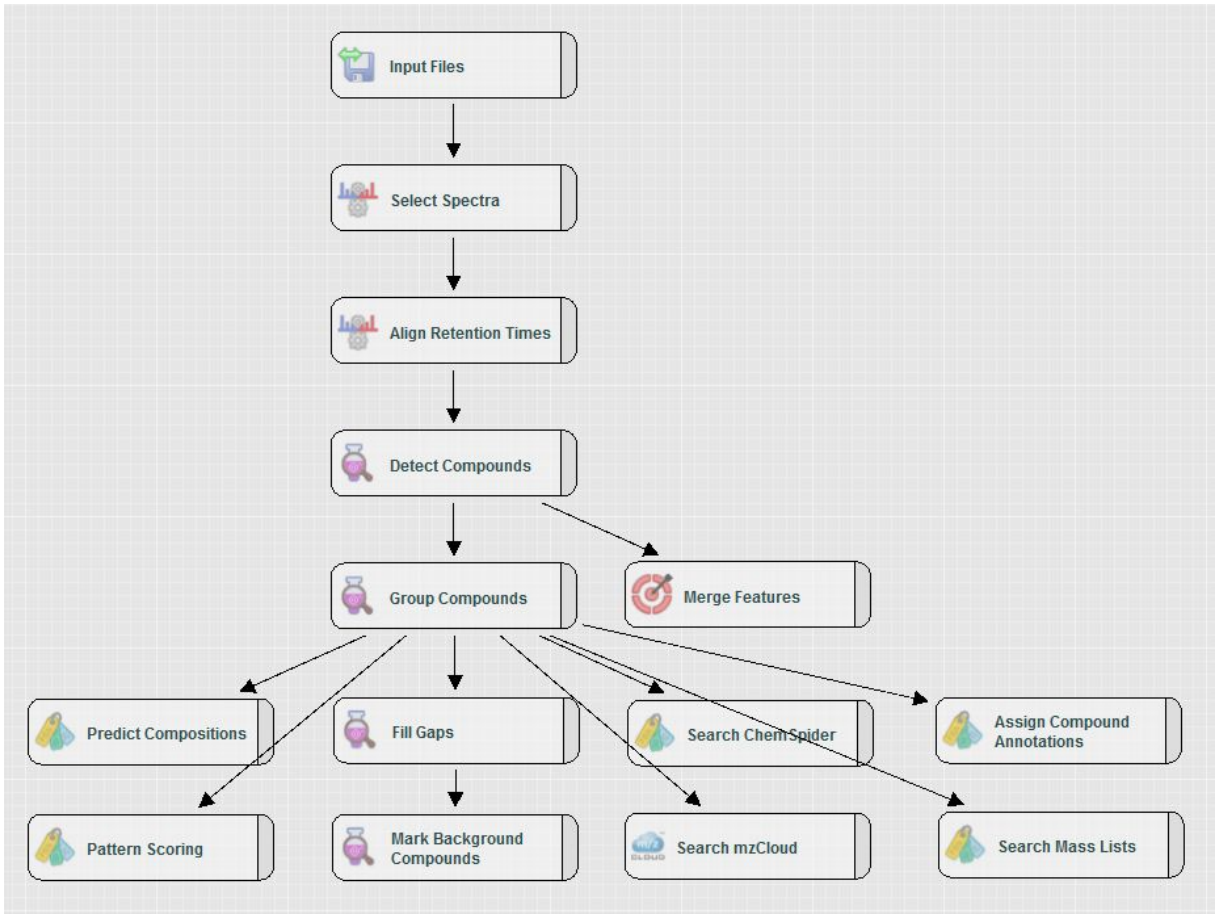


Table S3. Characteristics of the compounds identified in non-target screening

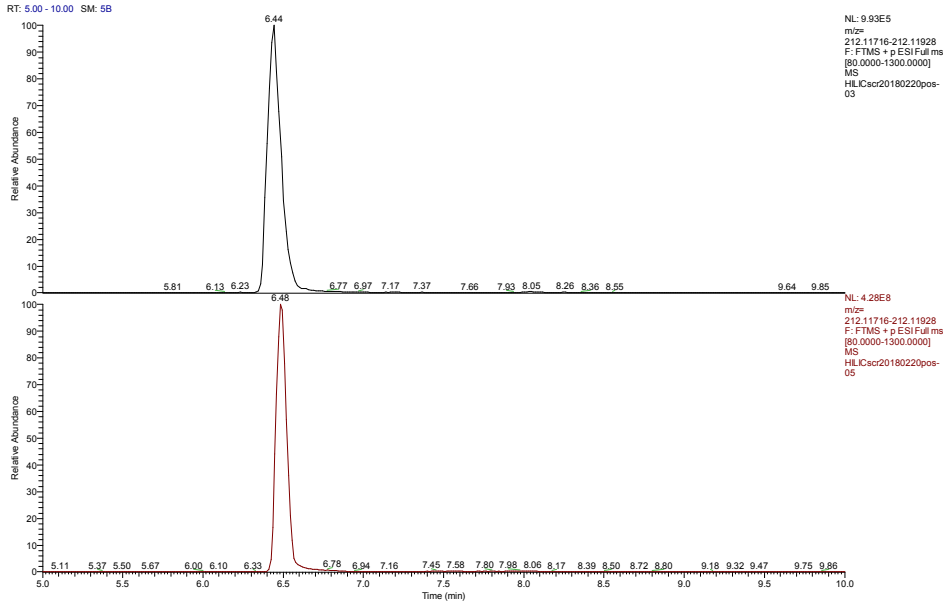
Compound	Log D at pH7.4[a]	Ionisation mode	Ion	Accurate mass	t_R (min)
N,N-diphenylguanidine	-0.41	pos	$[M+H]^+$	212.11822	6.48
Metoprolol	-0.25	pos	$[M+H]^+$	268.19072	7.24
(-)-Nicotine	-0.37	pos	$[M+H]^+$	163.12298	10.07
Guanine	-0.68	pos	$[M+H]^+$	152.05669	5.87
Choline	-3.79	pos	$[M+H]^+$	104.10699	9.94
Tramadol	0.52	pos	$[M+H]^+$	264.19581	7.21
Triisopropanolamine	-1.19	pos	$[M+H]^+$	192.15942	8.34
Phenazone	0.72	pos	$[M+H]^+$	189.10224	2.68
1,3-di-o-tolylguanidine	2.31	pos	$[M+H]^+$	240.14952	6.40
O-desmethylvenlafaxine	0.89	pos	$[M+H]^+$	264.19581	7.64
Triethanolamine	-1.65	pos	$[M+H]^+$	150.11247	9.40
2,2,6,6-tetramethyl-4-piperidinol	-1.11	pos	$[M+H]^+$	158.15394	8.27

[a] Log D predicted by ACD/Labs platform (taken from ChemSpider)

Figures S3-S14: Chromatographic and mass spectrometric confirmation of identity of unknown compounds with a reference standard

Figure S3: Confirmation of the identity of N,N'-diphenylguanidine

Extracted ion chromatogram (m/z 212.1182) of sample (upper panel) and reference standard (lower panel) of N,N'-diphenylguanidine:



Accurate MS2-spectrum of m/z 212.1182 of the sample (upper panel) and the reference standard of N,N'-diphenylguanidine (resolution 15,000, CID CE 35 %):

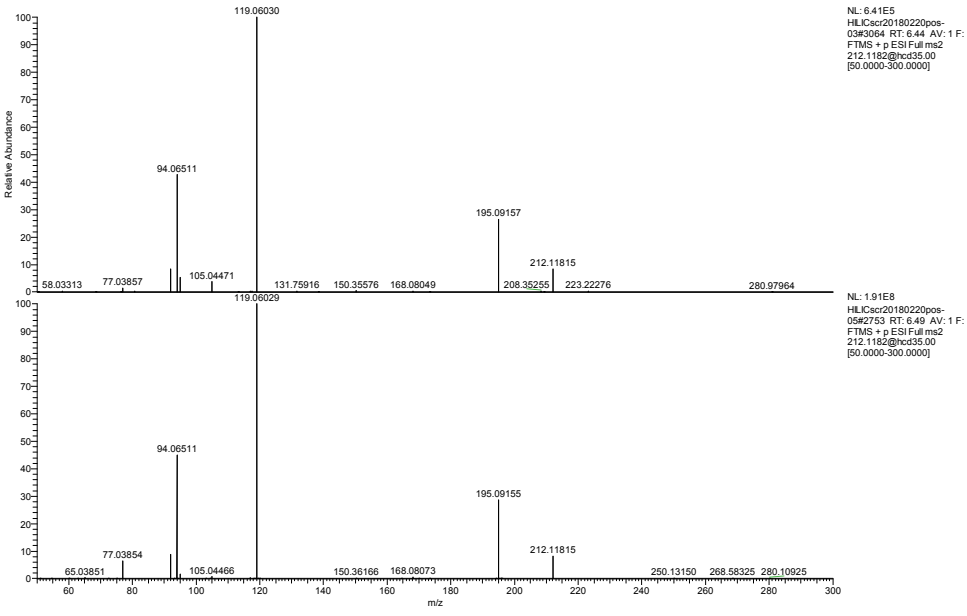
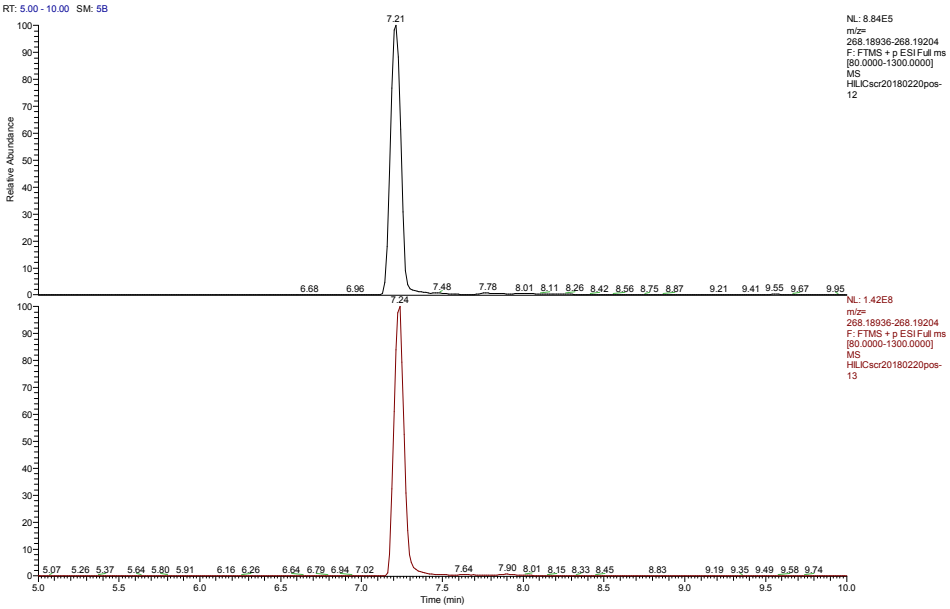


Figure S4: Confirmation of the identity of metoprolol

Extracted ion chromatogram (m/z 268.1907) of sample (upper panel) and reference standard (lower panel) of metoprolol:



Accurate MS2-spectrum of m/z 268.1907 in the water sample (upper panel) and of the reference standard of metoprolol (resolution 15,000, CID CE 35 %):

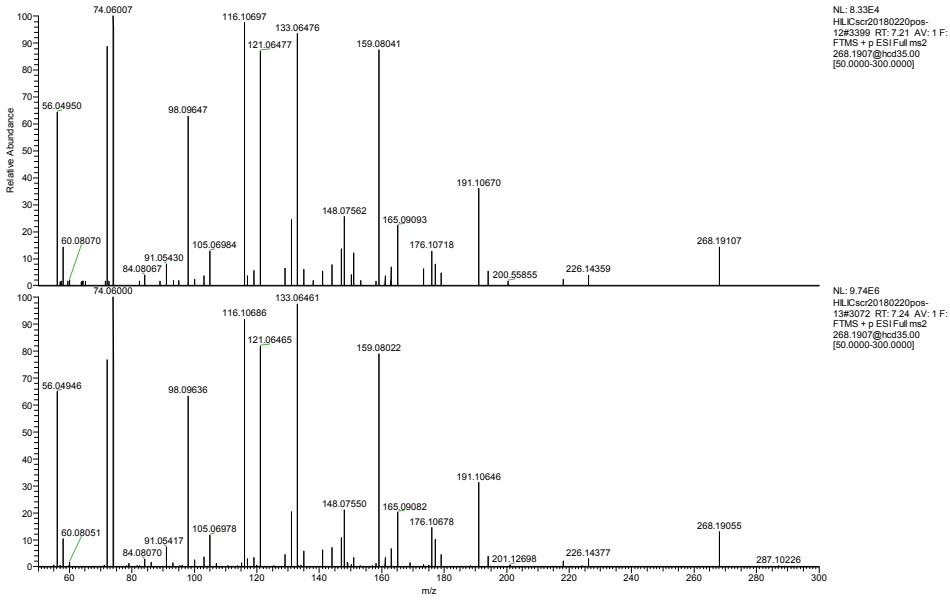
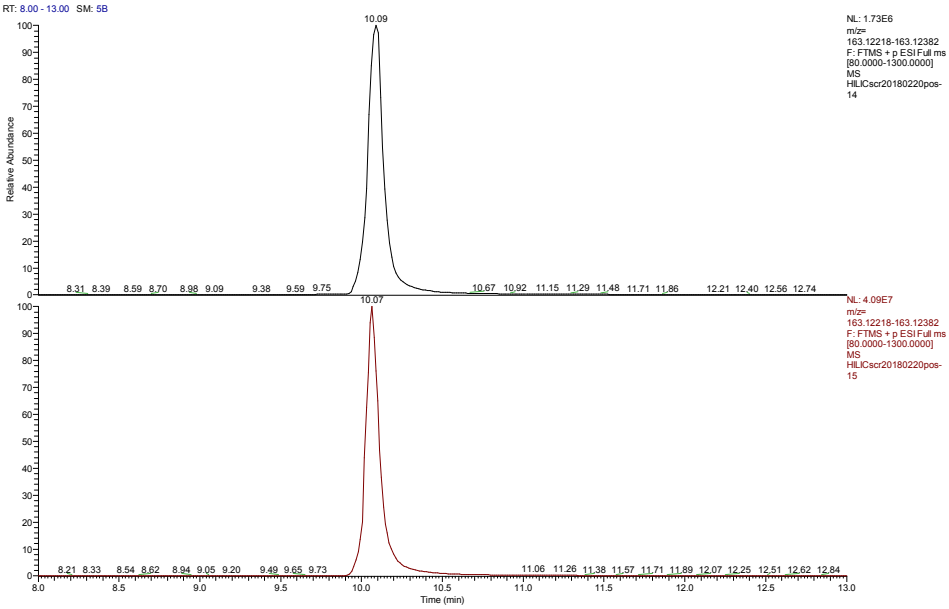


Figure S5: Confirmation of the identity of (-)-nicotine

Extracted ion chromatogram (m/z 163.1230)) of sample (upper panel) and reference standard (lower panel) of (-)-nicotine:



Accurate MS2-spectrum of m/z 163.1230) in the water sample (upper panel) and of the reference standard of (-)-nicotine (resolution 15,000, CID CE 35 %):

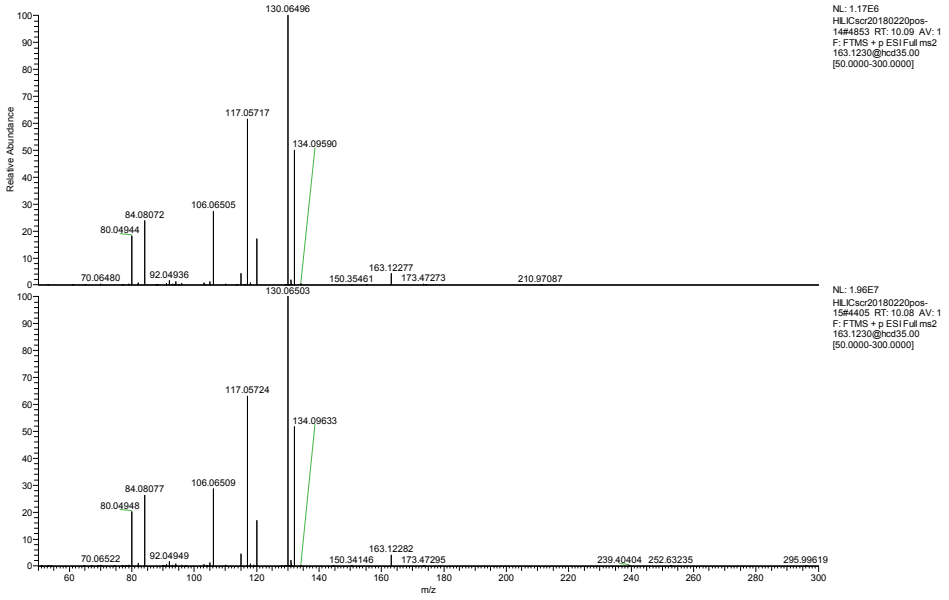
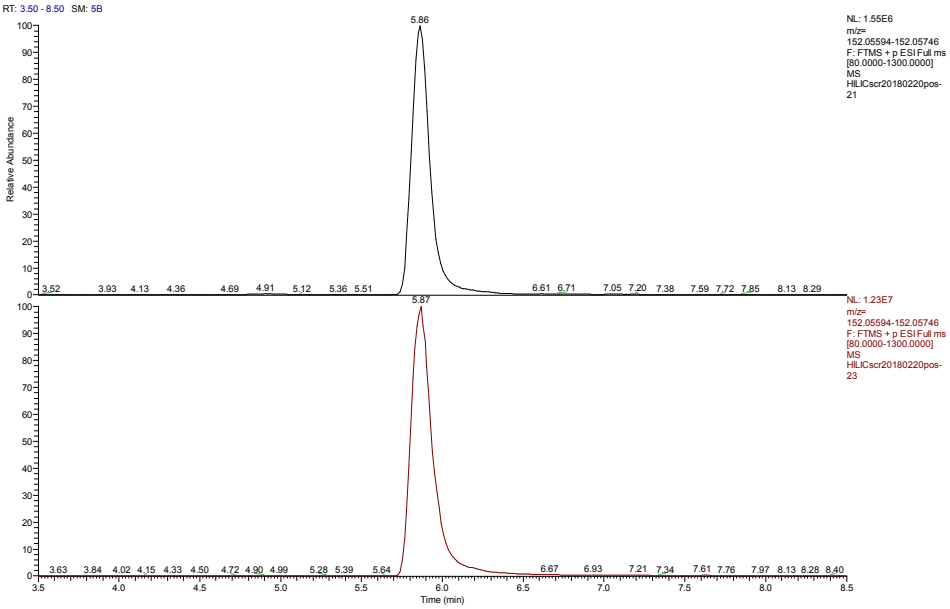


Figure S6: Confirmation of the identity of guanine

Extracted ion chromatogram (m/z 152.0567) of sample (upper panel) and reference standard (lower panel) of guanine:



Accurate MS2-spectrum of m/z 152.0567 in the water sample (upper panel) and of the reference standard of guanine (resolution 15,000, CID CE 35 %):

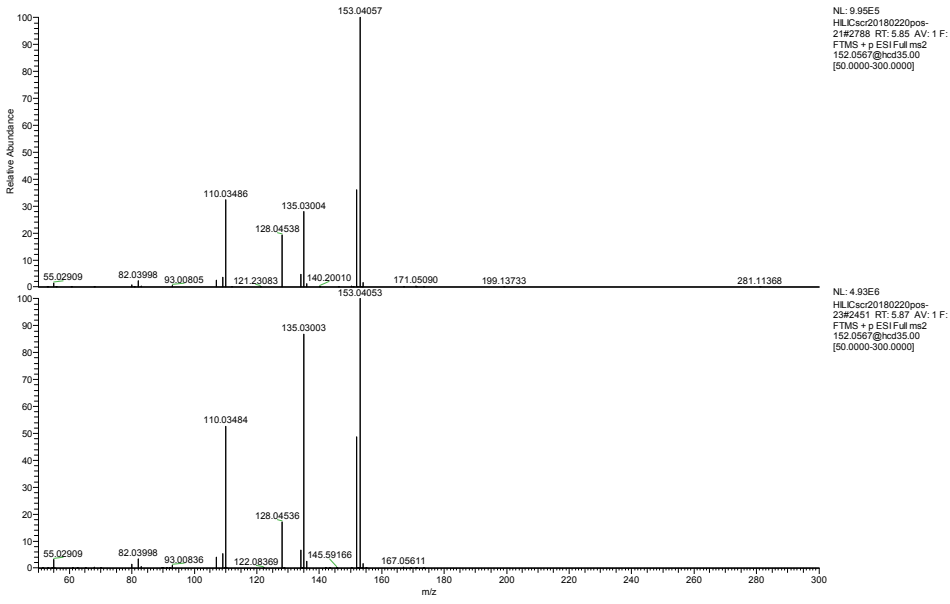
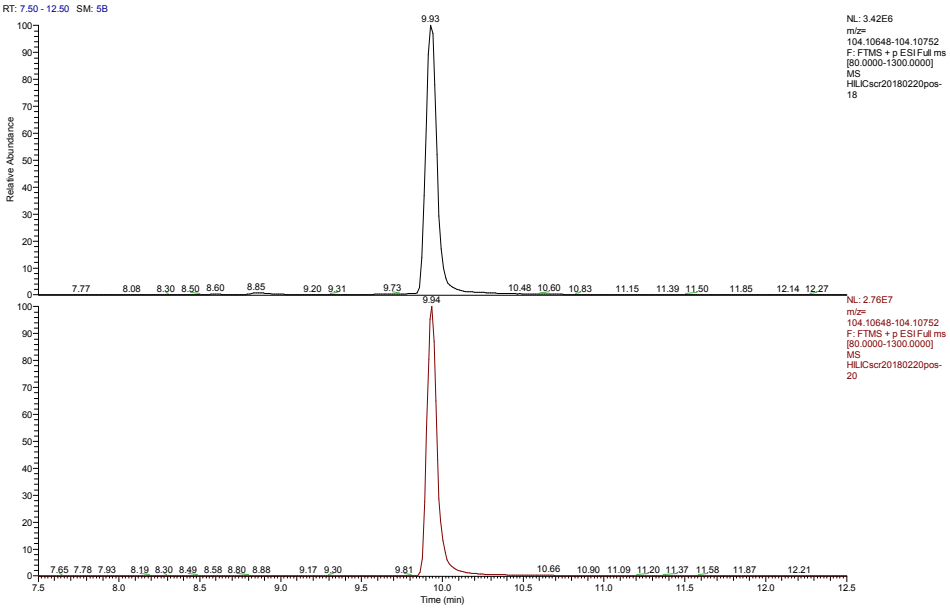


Figure S7: Confirmation of the identity of choline

Extracted ion chromatogram (m/z 104.1070) of sample (upper panel) and reference standard (lower panel) of choline:



Accurate MS2-spectrum of m/z 104.1070 in the water sample (upper panel) and of the reference standard of choline (resolution 15,000, CID CE 35 %):

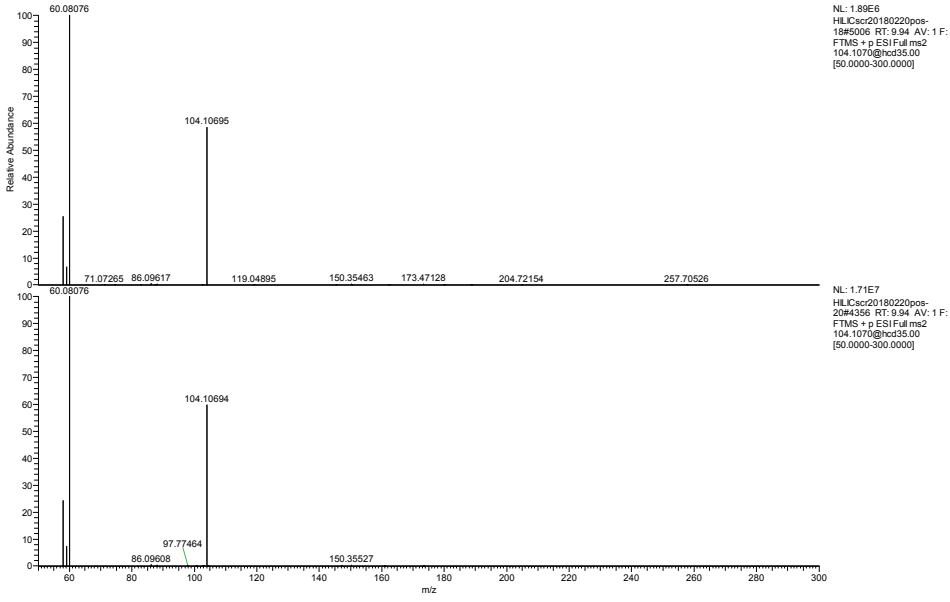
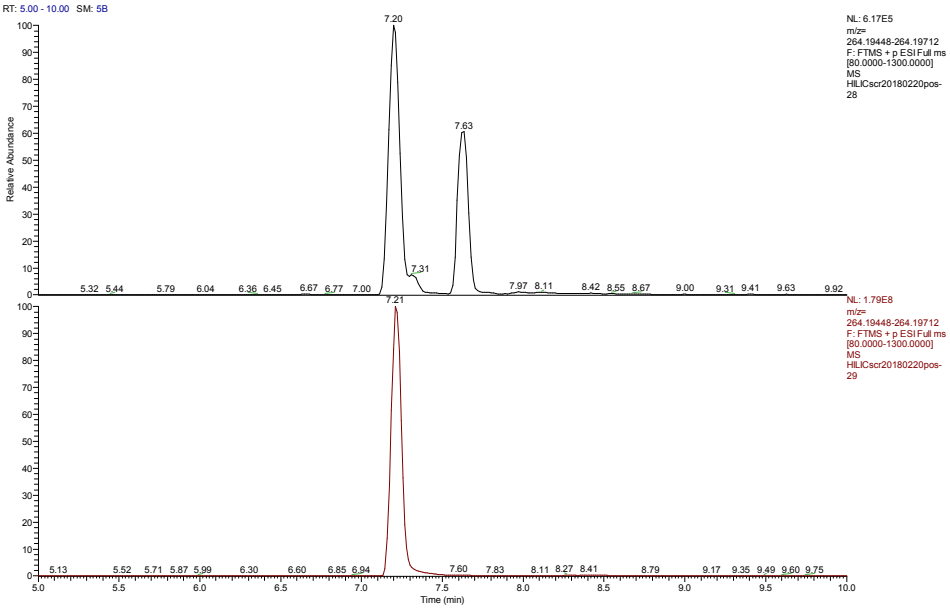


Figure S8: Confirmation of the identity of tramadol

Extracted ion chromatogram (m/z 264.1958) of sample (upper panel) and reference standard (lower panel) of tramadol:



Accurate MS2-spectrum of m/z 264.1958 (rt=7.2 min) in the water sample (upper panel) and of the reference standard of tramadol (resolution 15,000, CID CE 35 %):

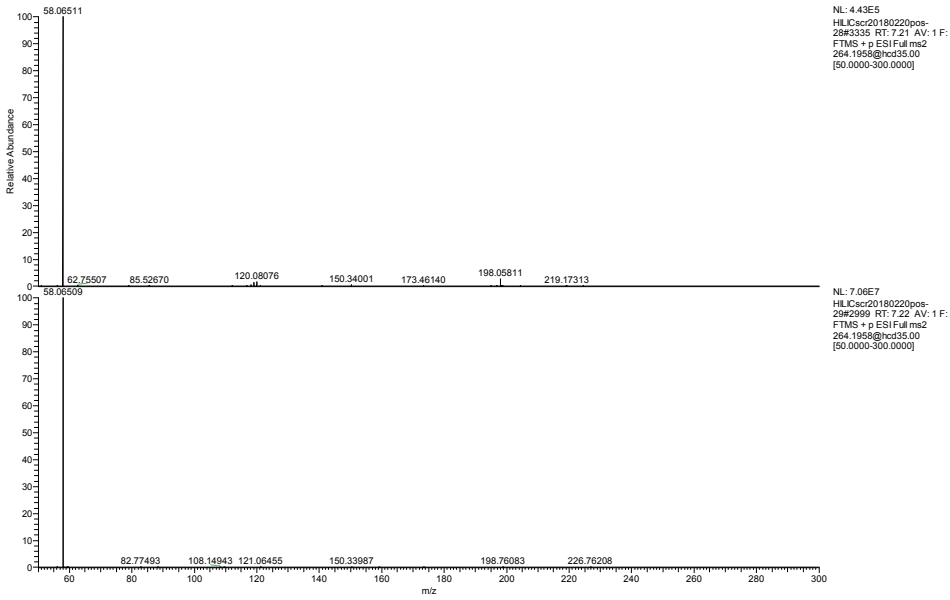
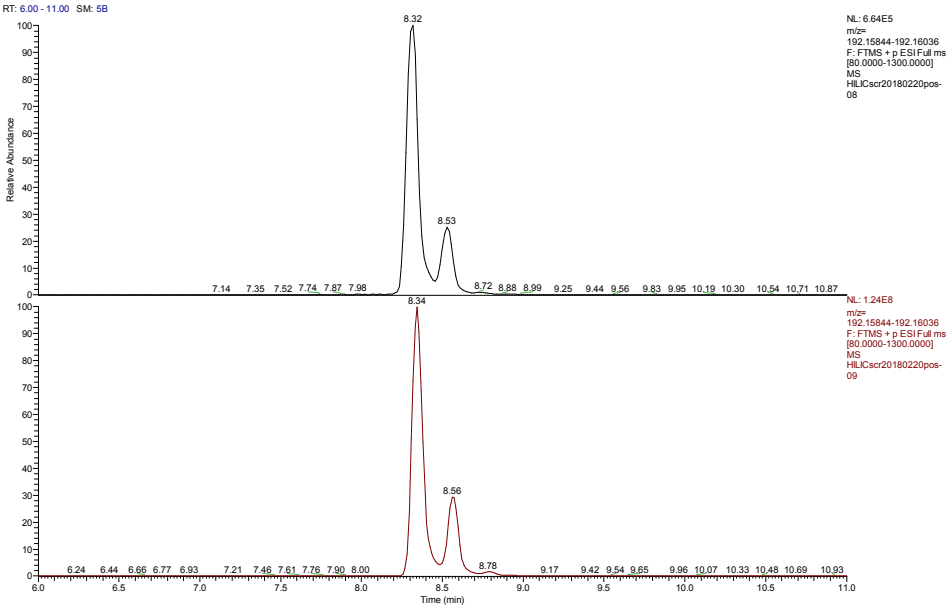


Figure S9: Confirmation of the identity of triisopropanolamine

Extracted ion chromatogram (m/z 192.1594) of sample (upper panel) and reference standard (lower panel) of triisopropanolamine:



Accurate MS2-spectrum of m/z 192.1594 (rt = 8.3 min) in the water sample (upper panel) and of the reference standard of triisopropanolamine (resolution 15,000, CID CE 35 %):

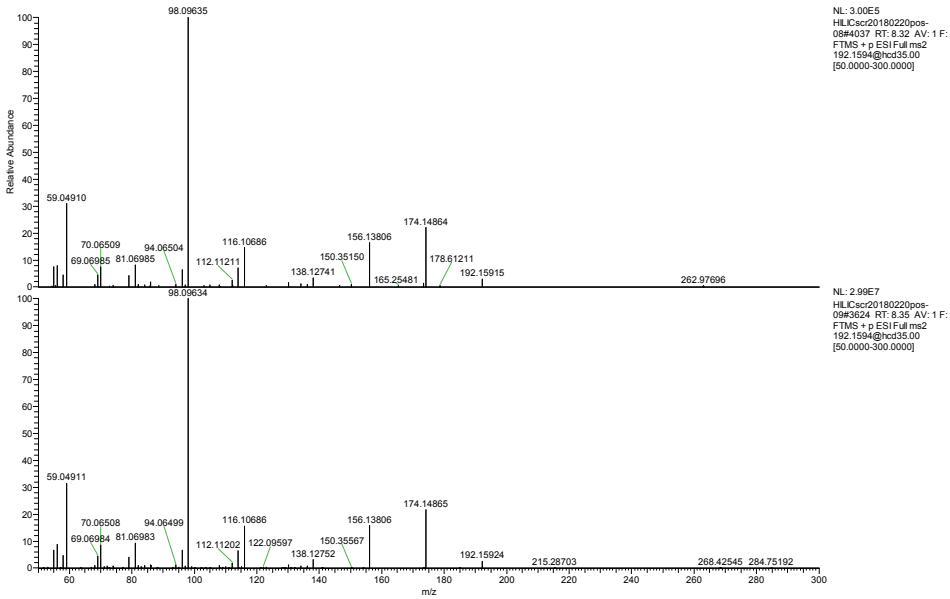
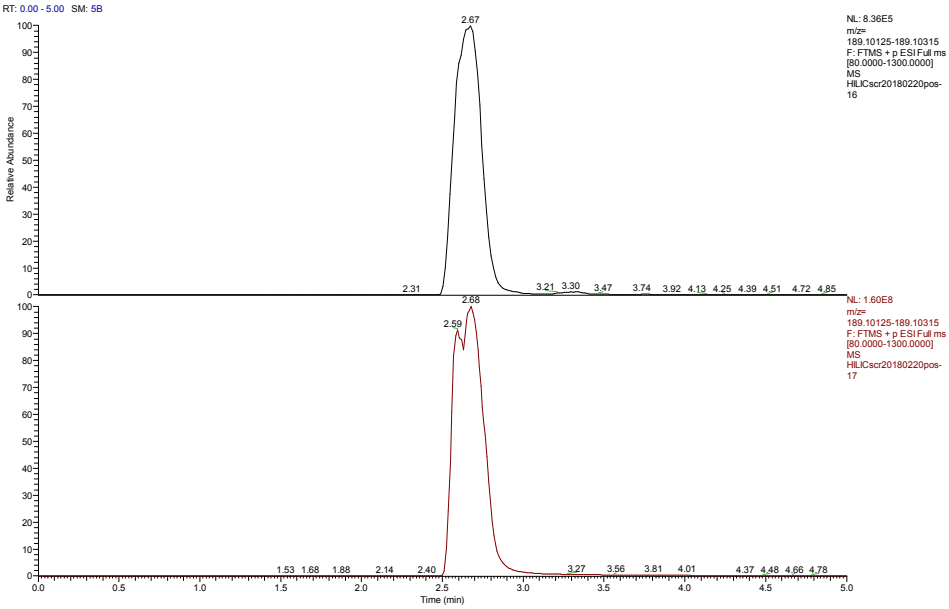


Figure S10: Confirmation of the identity of phenazone

Extracted ion chromatogram (m/z 189.1022) of sample (upper panel) and reference standard (lower panel) of phenazone:



Accurate MS2-spectrum of m/z 189.1022 in the water sample (upper panel) and of the reference standard of phenazone (resolution 15,000, CID CE 35 %):

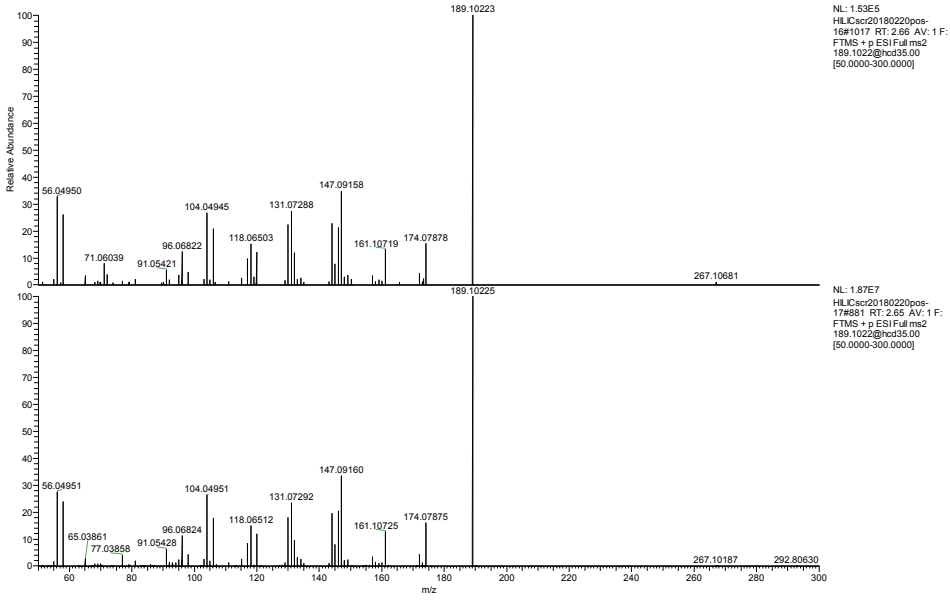
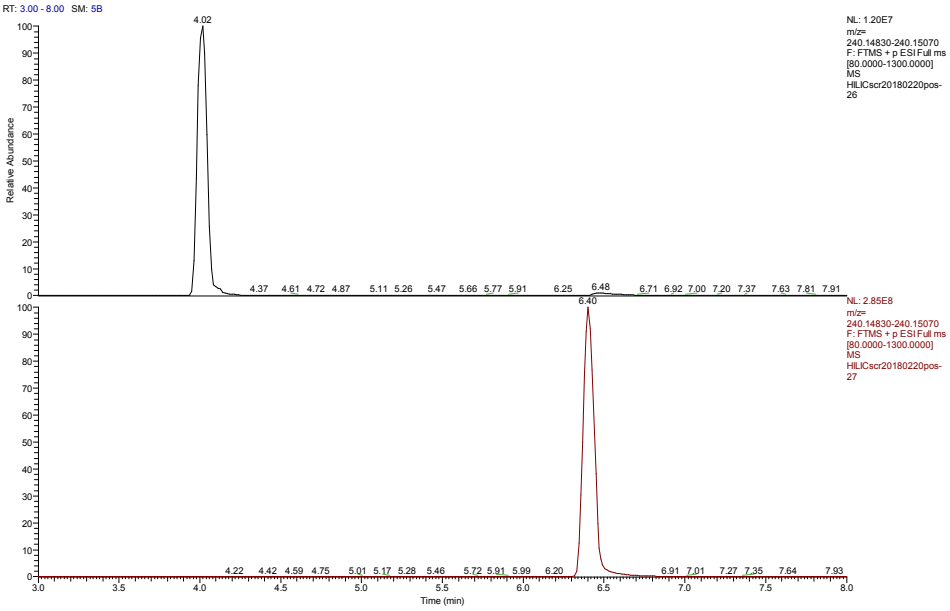


Figure S11: No confirmation of the identity of 1,3-di-o-tolylguanidine

Extracted ion chromatogram (m/z 240.1493) of sample (upper panel) and reference standard (lower panel) of 1,3-di-o-tolylguanidine:



Accurate MS2-spectrum of m/z 240.1493 in the water sample (upper panel) and of the reference standard of 1,3-di-o-tolylguanidine (resolution 15,000, CID CE 35 %):

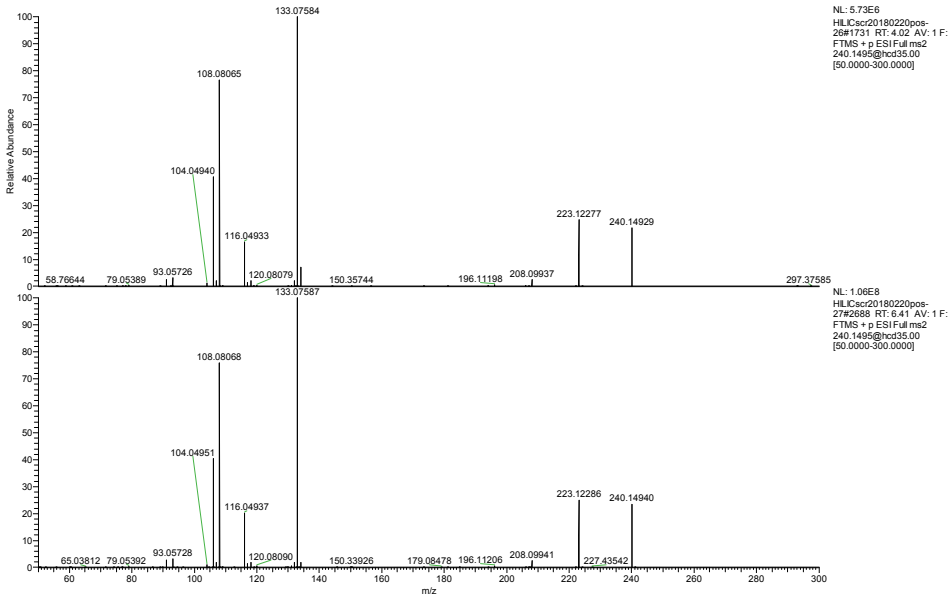
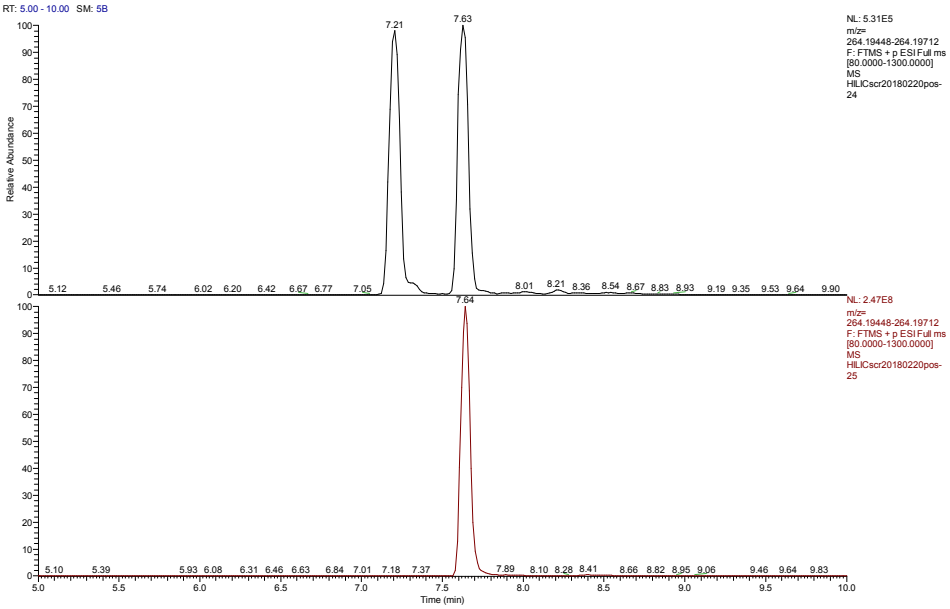


Figure S12: Confirmation of the identity of o-desmethylvenlafaxine

Extracted ion chromatogram (m/z 264.1958) of sample (upper panel) and reference standard (lower panel) of o-desmethylvenlafaxine:



Accurate MS2-spectrum of m/z 264.1958 in the water sample (upper panel) and of the reference standard of o-desmethylvenlafaxine (resolution 15,000, CID CE 35 %):

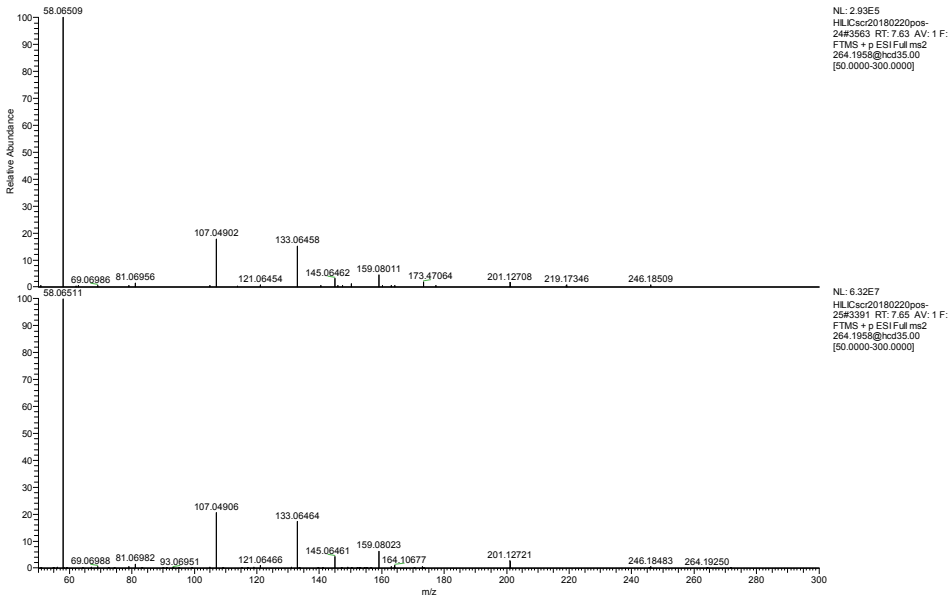
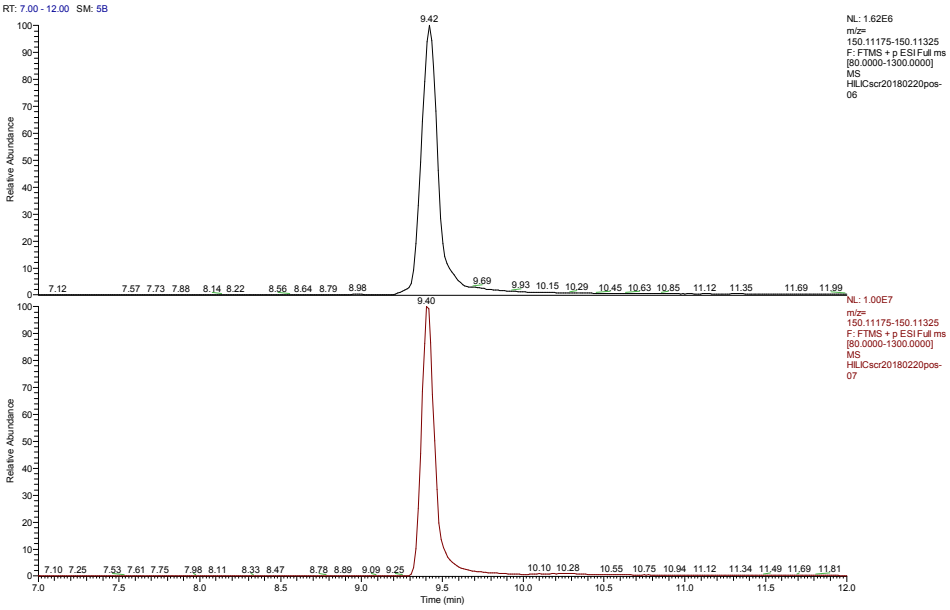


Figure S13: Confirmation of the identity of triethanolamine

Extracted ion chromatogram (m/z 150.1125) of sample (upper panel) and reference standard (lower panel) of triethanolamine:



Accurate MS2-spectrum of m/z 150.1125 in the water sample (upper panel) and of the reference standard of triethanolamine (resolution 15,000, CID CE 35 %):

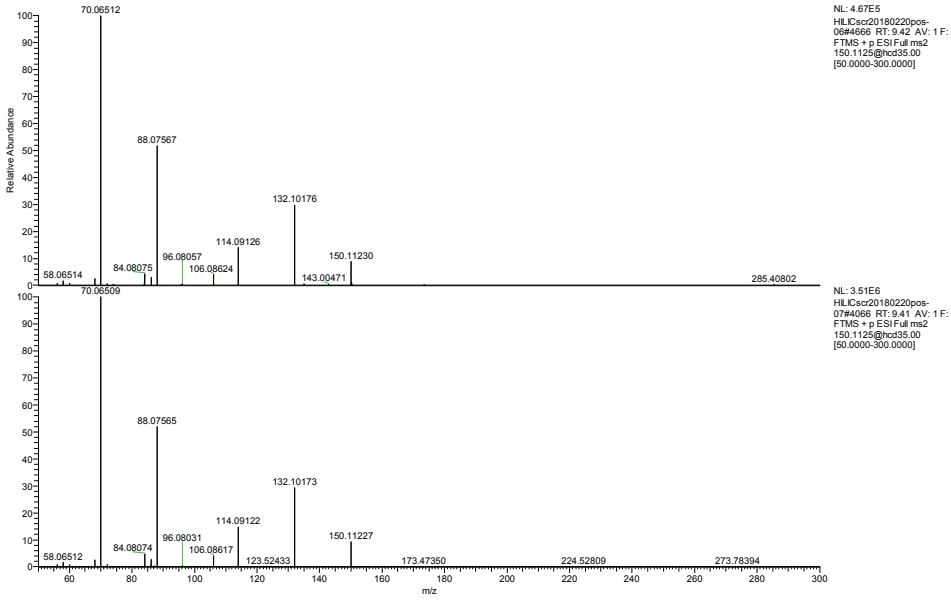
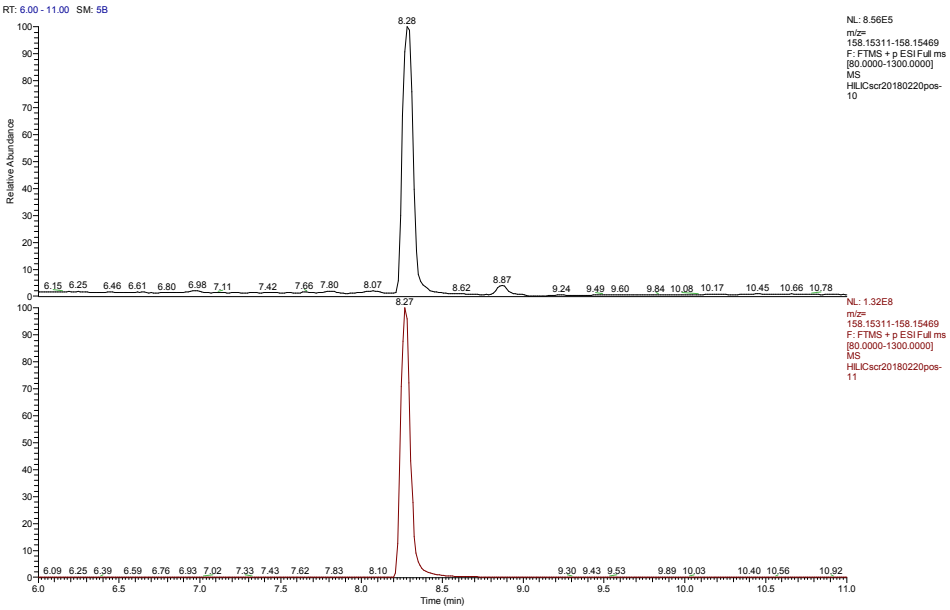
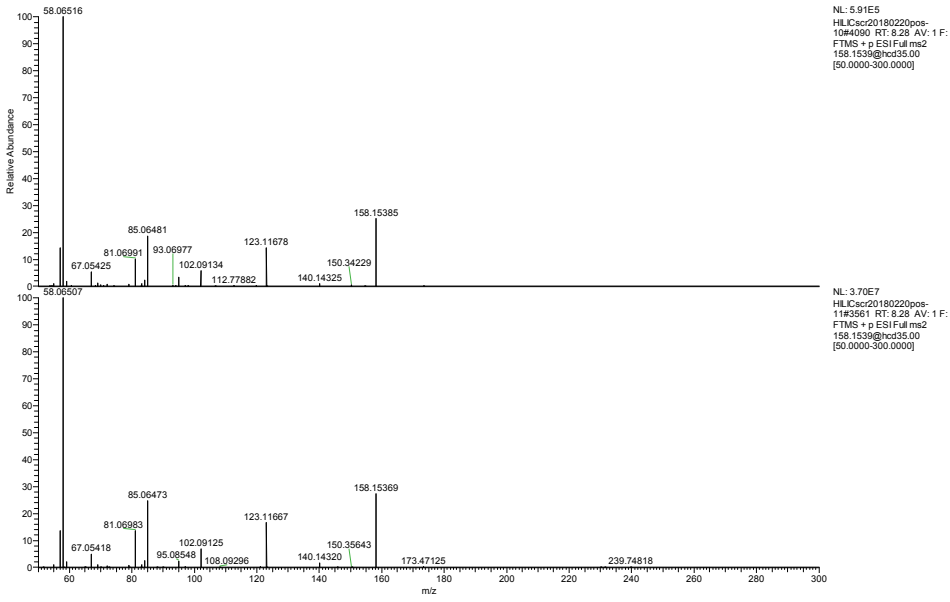


Figure S14: Confirmation of the identity of 2,2,6,6-tetramethyl-4-piperidinol

Extracted ion chromatogram (m/z 158.1539) of sample (upper panel) and reference standard (lower panel) of 2,2,6,6-tetramethyl-4-piperidinol:



Accurate MS2-spectrum of m/z 158.1539 in the water sample (upper panel) and of the reference standard of 2,2,6,6-tetramethyl-4-piperidinol (resolution 15,000, CID CE 35 %):



Summary of data processing parameters and Compound Discoverer 2.1 settings

Processing node 1: Select Spectra

---

1. General Settings:
- Precursor Selection: Use MS(n - 1) Precursor
- Use New Precursor Reevaluation: True
- Use Isotope Pattern in Precursor Reevaluation: True
- Store Chromatograms: False

2. Spectrum Properties Filter:
- Lower RT Limit: 2.5
- Upper RT Limit: 0
- First Scan: 0
- Last Scan: 0
- Ignore Specified Scans: (not specified)
- Lowest Charge State: 0
- Highest Charge State: 0
- Min. Precursor Mass: 80 Da
- Max. Precursor Mass: 5000 Da
- Total Intensity Threshold: 0
- Minimum Peak Count: 1

3. Scan Event Filters:
- Mass Analyzer: (not specified)
- MS Order: Any
- Activation Type: (not specified)
- Min. Collision Energy: 0
- Max. Collision Energy: 1000
- Scan Type: Any
- Polarity Mode: (not specified)

4. Peak Filters:
- S/N Threshold (FT-only): 1.5

5. Replacements for Unrecognized Properties:
- Unrecognized Charge Replacements: 1
- Unrecognized Mass Analyzer Replacements: ITMS
- Unrecognized MS Order Replacements: MS2
- Unrecognized Activation Type Replacements: CID
- Unrecognized Polarity Replacements: +
- Unrecognized MS Resolution@200 Replacements: 60000
- Unrecognized MSn Resolution@200 Replacements: 30000

Processing node 2: Align Retention Times

---

1. General Settings:
- Alignment Model: Adaptive curve
- Alignment Fallback: Use Linear Model
- Maximum Shift [min]: 0.5
- Shift Reference File: True
- Mass Tolerance: 5 ppm
- Remove Outlier: True

Processing node 3: Detect Unknown Compounds
---
1. General Settings:
- Mass Tolerance [ppm]: 5 ppm
- Intensity Tolerance [%]: 30
- S/N Threshold: 10
- Min. Peak Intensity: 1000000
- Ions:
    [2M+H]+1
    [M+2H]+2
    [M+ACN+H]+1
    [M+H]+1
    [M+H-H2O]+1
    [M+K]+1
    [M+Na]+1
    [M+NH4]+1
- Base Ions: [M+H]+1; [M-H]-1
- Min. Element Counts: C H
- Max. Element Counts: C90 H190 Br3 Cl4 K2 N10 Na2 O15 P2 S5

2. Peak Detection:
- Filter Peaks: True
- Max. Peak Width [min]: 0.8
- Remove Singlets: True
- Min. # Scans per Peak: 5
- Min. # Isotopes: 1

Processing node 4: Group Unknown Compounds
---
1. Compound Consolidation:
- Mass Tolerance: 5 ppm
- RT Tolerance [min]: 0.1

2. Fragment Data Selection:
- Preferred Ions: [M+H]+1; [M-H]-1

Processing node 7: Fill Gaps
---
1. General Settings:
- Mass Tolerance: 5 ppm
- S/N Threshold: 1.5
- Use Real Peak Detection: True

Processing node 5: Mark Background Compounds
---
1. General Settings:
- Max. Sample/Blank: 5
- Max. Blank/Sample: 0
- Hide Background: False

Processing node 20: Search mzCloud
---
1. Search Settings:
- Compound Classes: All
- Match Ion Activation Type: True
- Match Ion Activation Energy: Match with Tolerance
- Ion Activation Energy Tolerance: 20
- Apply Intensity Threshold: True
- Precursor Mass Tolerance: 10 ppm
- FT Fragment Mass Tolerance: 0.0025 Da
- IT Fragment Mass Tolerance: 0.4 Da
- Identity Search: HighChem HighRes
- Similarity Search: None
- Library: Reference
- Post Processing: Recalibrated
- Match Factor Threshold: 20
- Max. # Results: 10

Processing node 21: Pattern Scoring
---
1. General Settings:
- Isotope Patterns: Cl; Cl2; Br; Br2; Cl3
- Mass Tolerance: 5 ppm
- Intensity Tolerance [%]: 30
- SN Threshold: 10
- Min. Spectral Fit [%]: 0

Processing node 6: Predict Compositions
---
1. Prediction Settings:
- Mass Tolerance: 5 ppm
- Min. Element Counts: C H
- Max. Element Counts: C90 H190 Br3 Cl4 N10 O15 P2 S5
- Min. RDBE: -1
- Max. RDBE: 40
- Min. H/C: 0.1
- Max. H/C: 3
- Max. # Candidates: 10
- Max. # Internal Candidates: 200

2. Pattern Matching:
- Intensity Tolerance [%]: 30
- Intensity Threshold [%]: 0.1
- S/N Threshold: 3
- Min. Spectral Fit [%]: 10
- Min. Pattern Cov. [%]: 90
- Use Dynamic Recalibration: True

3. Fragments Matching:
- Use Fragments Matching: True
- Mass Tolerance: 5 ppm

- S/N Threshold: 3

Processing node 23: Search Mass Lists
---
1. Search Settings:
- Input file(s): hilic.csv;extracompc18.csv
- Mass Tolerance: 5 ppm
- Show extra Fields as Columns: False
- Consider Retention Time: True
- RT Tolerance : 0.05

Processing node 26: Search ChemSpider
---
1. Search Settings:
- Mass Tolerance: 5 ppm
- Database(s): KEGG
- Max. # of results per compound: 100
- Max. # of Predicted Compositions to be searched per Compound: 3
- Result Order (for Max. # of results per compound): Order By Reference Count (DESC)

2. Predicted Composition Annotation:
- Check All Predicted Compositions: False

Processing node 14: Merge Features
---
1. Peak Consolidation:
- Mass Tolerance: 5 ppm
- RT Tolerance [min]: 0.1

Processing node 15: Differential Analysis
---
1. General Settings:
- Log10 Transform Values: True

Processing node 24: Descriptive Statistics

No parameters

Processing node 25: Assign Compound Annotations
---
1. General Settings:
- Mass Tolerance: 5 ppm

2. Data Sources:
- Data Source #1: mzCloud Search
- Data Source #2: Predicted Compositions
- Data Source #3: MassList Match
- Data Source #4: ChemSpider Search