Supplementary Material

Study on the occurrence of artificial sweeteners, parabens and other emerging contaminants in hospital wastewater using LC-QToF-MS target screening approach

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Number of supplementary figures: 2

Section A: Quality Assurance and Quality Control (QA/QC) protocol followed during the sample preparation and instrumental analysis

Table S1. CAS number, chemical formula, molecular weight, pKa, and log K_{ow} values of 27 detected and quantified emerging contaminants.

Compounds	CAS number	Chemical formula	M.W.	pKa	log K_{ow}
Artficial sweeteners					
Acesulfame	33665-90-6	$C_4H_5NO_4S$	163.15	2.0	-1.33
Cyclamic acid	100-88-9	$C_6H_{13}NO_3S$	179.24	1.71	-1.61
Saccharine	128-44-9	$C_7H_5NO_3S$	183.19	1.31	0.91
Sucralose	56038-13-2	$C_{12}H_{19}Cl_3O_8$	397.6	11.9	-1.00
Personal care products					
Benzophenon 3	131-57-7	$C_{14}H_{12}O_3$	228.24	7.1	3.79
Galaxolide	1222-05-5	$C_{18}H_{26}O$	258.4	8.24	5.90
Galaxolidone	507442-49-1	$C_{18}H_{24}O_2$	272.4	-6.9	5.34
Ethylparaben	120-47-8	$C_9H_{10}O_3$	166.17	8.34	2.47
Methylparaben	99-76-3	$C_8H_8O_3$	152.15	8.5	1.96
Propylparaben	94-13-3	$C_{10}H_{12}O_3$	180.20	8.5	3.04
Coffee and tobacco-related compounds					
Caffeine	95789-13-2	$C_8H_{10}N_4O_2$	194.19	14.0	-0.07
Theobromine	83-67-0	$C_7H_8N_4O_2$	180.16	9.9	-0.78
Nicotine	54-11-5	$C_{10}H_{14}N_2$	162.23	8.11	1.17
Cotinine	486-56-6	$C_{10}H_{12}N_2O$	176.21	4.79	0.39
Hydroxycotinine	803-421-5	$C_{10}H_{12}N_2O_2$	192.21	13.1	-0.32
Industrial chemicals					
Benzododecinium	10328-35-5	$C_{21}H_{38}N^+$	304.5	18.1	2.63
2-Hydroxybenzothiazole (2-OH-BTH)	934-34-9	C_7H_5NOS	151.19	8.44	2.49
Benzotriazole (BTR)	273-02-9	$C_6H_5N_3$	119.12	8.37	1.44
Lauryl diethanolamide (Lauryl-DEA)	120-40-1	$C_{16}H_{33}NO_3$	287.44	14.1	3.48

Compounds	**CAS number**	**Chemical formula**	**M.W.**	**pKa**	**log K_{ow}**
N,N-Dimethyldecylamine (N,N-diMe-DA)	1120-24-7	$C_{12}H_{27}N$	185.35	9.78	4.84
N,N-Dimethyldodecylamine (N,N-diMe-DDA)	112-18-5	$C_{14}H_{31}N$	213.40	9.97	5.4
N,N-Dimethyldodecylamine N-oxide (N,N-diMe-DDA-N-oxide)	1643-20-5	$C_{14}H_{31}NO$	229.40	4.48	2.23
N,N-Dimethyltetradecylamine (N,N-diMe-TDA)	112-75-4	$C_{16}H_{35}N$	241.46	9.78	5.25
N,N-Dimethyltetradecylamine-N-oxide (N,N-diMe-TDA-N-oxide)	3332-27-2	$C_{16}H_{35}NO$	257.45	4.01	5.28
N-Methyldodecylamine (N-Me-DDA)	7311-30-0	$C_{13}H_{29}N$	199.38	10.8	5.41
Triethylphosphate	78-40-0	$C_6H_{15}O_4P$	182.15	-9.1	0.80
Triethylcitrate	77-93-0	$C_{12}H_{20}O_7$	276.28	11.6	0.33

Table S2. Information on the samples collected in this study (dates, sampling points)

Code of HWW	Date of sampling (DD-MM-YYYY)	Sampling point
Period 1		
HWW 1	2/11/2020	A
HWW 2		B
HWW 3	3/11/2020	A
HWW 4		B
HWW 5	4/11/2020	A
HWW 6		B
HWW 7	5/11/2020	A
HWW 8		B
HWW 9	6/11/2020	A
HWW 10		B
Period 2		
HWW 11	15/2/2021	A
HWW 12		B
HWW 13	16/2/2021	A
HWW 14		B
HWW 15	17/2/2021	A
HWW 16		B
HWW 17	18/2/2021	A
HWW 18		B
HWW 19	19/2/2021	A
HWW 20		B

Section A

Quality Assurance and Quality Control (QA/QC) protocol followed during the sample preparation and instrumental analysis

A mixture of isotopically labeled compounds was spiked in every sample before extraction to correct reproducibility issues among the samples of the same or different batches and variabilities in instrumental parameters such as injection volume and MS sensitivity and ensure sufficient recovery of the contaminants from the analysed matrix. Method blanks (reagent blanks) were prepared in every batch of samples to assess any external contamination which might have been introduced during the sample preparation of the final extracts for analysis, whereas spiked and matrix-matched samples with a mixture of environmental contaminants were also prepared and examined with the samples to monitor the recovery and matrix effect for the tested compounds.

Apart from the regular system maintenance, a QA/QC protocol was followed during every instrumental analysis to assure the separation efficiency of the analytes of interest and the good operation of the HRMS system. For a reliable quantitative analysis, blank solutions (instrument blanks) were measured after a sample analysis to monitor and reduce possible memory effect phenomena (or carry-over of analytes). A mix of known analytes (Retention Time Index (RTI) calibrant substances) was used to assess the stability of retention time during instrumental analysis and evaluate potential retention time drift across different dates of analysis (**Aalizadeh et al., 2021- https://doi.org/10.1021/acs.analchem.1c02348**). A QC sample ran every 10-15 injections to ensure the good operation and high sensitivity of the system. Before starting the screening of the chromatograms, the sensitivity of internal standards in each sample was tested to assure satisfactory recovery and proper injection of the extracts into the chromatographic system. Relative areas (peak area of the analyte divided by the peak area of internal standard) were used for quantifying the detected analytes, therefore potential variations in instrumental sensitivity were accounted in the different batches of analysis.

Instrumental analysis

The samples were analysed by two chromatographic systems, for positively and negatively ionized compounds. For positive ionization (PI) mode the mobile phase consisted of (A) Milli-Q H_2O:MeOH (90:10, v/v) and (B) MeOH, both containing 5 mM ammonium formate and 0.01% formic acid, whereas in negative ionization (NI) mode mobile phase was composed of (A) Milli-Q H_2O:MeOH (90:10, v/v) and (B) MeOH, both containing 5 mM ammonium acetate. The LC elution program for both analyses is presented in **Table S2.** The injection volume was fixed at 5 μL. The QToF-MS system was equipped with an electrospray ionization (ESI) interface. The ESI parameters used were the following: capillary voltage, 2500 V (for PI) and 3500 V (for NI); end plate offset, 500 V; nebulizer pressure, 2 bar (N_2); drying gas, 8 L/min (N_2); and drying temperature, 200 °C.

Table S3. The gradient elution program of LC-HRMS analysis.

Time (min)	Flow rate (mL/min)	%A	%B
0	0.2	99	1
1.0	0.2	99	1
3.0	0.2	61	39
14.0	0.4	0.1	99.9
16.0	0.48	0.1	99.9
16.1	0.48	99	1
19.0	0.48	99	1
19.1	0.2	99	1
20.0	0.2	99	1

A QToF-MS external calibration was performed daily before analysis, with a sodium formate solution (10 mM). For internal calibration, a segment in every chromatogram (0.1-0.25 min) was used, where the calibration solution was injected at the beginning of each run. The theoretical exact masses of calibration ions with formulas $Na(NaCOOH)_{1-14}$ in the range of 40−800 Da, were used for calibration. The instrument provided a typical resolving power of 36,000−40,000 during calibration. The QToF-MS system operated in two different acquisition modes. In Data Independent Acquisition (DIA) mode, or broadband collision-induced dissociation (bbCID), two sequential full scan events were acquired. In the first scan, a low collision energy (4 eV) was applied to record MS full data, whereas in the second scan a higher collision energy (25 eV) resulted in a MS/MS all-ion-fragmentation, both in the range m/z 50–1000. In Data Dependent Acquisition (DDA) mode, after acquiring MS full scan spectrum (4

eV, m/z 50–1000), the fragmentation of the 5 most abundant ions was triggered at collision energies dependent on their ion mass and charge state. The scan rate was 2 Hz.

Table S4. UHPLC-ESI-QToF MS identification data for the detected compounds.

Compounds	Retention time (min)	Ion type	Precursor Ion (m/z)	Main fragment ions in bbCID specta (m/z)
Artificial Sweeteners				
Acesulfame	2.3	$[M-H]^-$	161.9867	161.9867, 82.0298, 98.0248, 77.9655
Cyclamic acid	4.1	$[M-H]^-$	178.0543	79.9574, 178.0543, 80.9652, 95.9761
Saccharine	3.1	$[M-H]^-$	181.9917	181.9917, 105.9602, 61.97.7
Sucralose	4.7	$[M-H]^-$	395.0073	395.0073, 359.0299, 59.0136
Personal Care Products				
Benzophenon 3	10.8	$[M+H]^+$	229.0859	105.0334, 183.0805, 77.0386
Galaxolide	13.9	$[M+H]^+$	259.2056	175.1104, 147.0791,161.0944
Galaxolidone	12.0	$[M+H]^+$	273.1849	138.0662, 83.0604, 110.0713, 69.0447
Ethylparaben	7.1	$[M-H]^-$	165.0557	136.0158, 137.0236, 165.0549
Methylparaben	6.0	$[M-H]^-$	151.0401	136.0163, 151.0401
Propylparaben	8.3	$[M-H]^-$	179.0714	107.0315, 136.0160
Coffee and tobacco-related compounds				
Caffeine	4.2	$[M+H]^+$	195.0877	67.0291, 138.0662, 69.0447, 110.0713
Theobromine	3.3	$[M+H]^+$	181.0720	84.0808, 80.0495, 117.0573, 130.0651
Nicotine	2.4	$[M+H]^+$	163.1230	84.0808, 80.0495, 117.0573, 130.0651
Cotinine	3.8	$[M+H]^+$	177.1022	80.0495, 98.0600, 70.0651, 177.1014
Hydroxycotinine	3.1	$[M+H]^+$	193.0972	80.0495, 134.0600
Industrial chemicals				
Benzododecinium	11.2	$[M]^+$	304.2999	91.0542, 58.0651, 212.2373

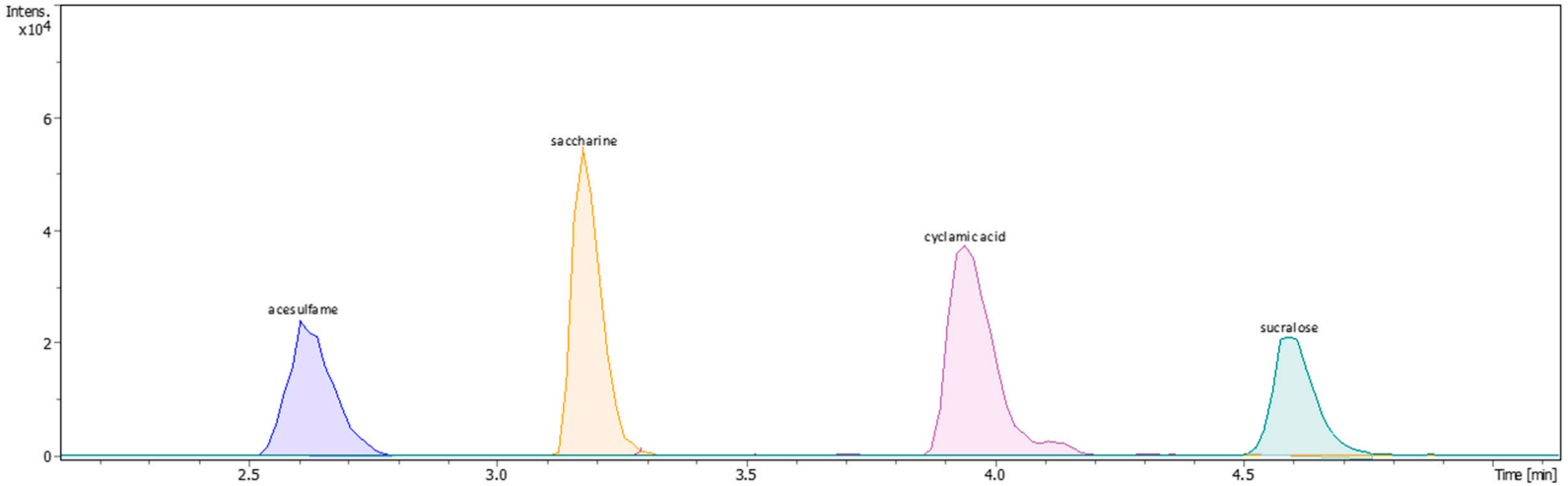
Compounds	Retention time (min)	Ion type	Precursor Ion (m/z)	Main fragment ions in bbCID specta (m/z)
2-Hydroxybenzothiazole (2-OH-BTH)	6.5	$[M+H]^+$	152.0165	124.0226, 119.0364, 152.0165, 134.0049
Benzotriazole (BTR)	4.8	$[M+H]^+$	120.0556	65.0380, 120.0556, 92.0486, 121.0582
Lauryl diethanolamide (Lauryl-DEA)	11.7	$[M+H]^+$	288.2533	88.0758, 97.0820, 124.0868, 226.2136
N,N-Dimethyldecylamine (N,N-diMe-DA)	8.6	$[M+H]^+$	186.2216	186.2216, 187.2234, 188.2258
N,N-Dimethyldodecylamine (N,N-diMe-DDA)	10.4	$[M+H]^+$	214.2529	214.2529, 215.2556, 216.2586
N,N-Dimethyldodecylamine N-oxide (N,N-diMe-DDA-N-oxide)	11.2	$[M+H]^+$	230.2478	128.1419, 205.2094, 212.2362
N,N-Dimethyltetradecylamine (N,N-diMe-TDA)	11.8	$[M+H]^+$	242.2842	242.2848, 243.2868, 244.2901
N,N-Dimethyltetradecylamine-N-oxide (N,N-diMe-TDA-N-oxide)	12.6	$[M+H]^+$	258.2791	113.1262, 226.6465, 240.2678
N-Methyldodecylamine (N-Me-DDA)	10.5	$[M+H]^+$	200.2373	200.2373, 201.2397, 202.2424
Triethyl Phosphate	6.2	$[M+H]^+$	183.0781	127.0164, 155.0480, 183.0798
Triethyl Citrate	6.9	$[M+H]^+$	277.1282	129.0170, 131.0334, 139.0011

Table S5. Performance of method applied for the analyses of emerging contaminants.

Compounds	***LOD (µg/L)***	***LOQ (µg/L)***	***% Recovery***	***%RSD (n=3)***	***%Matrix effect***
			C: 0.5 µg/L		
Artificial Sweeteners					
Acesulfame	0.720	2.16	89	9.7	-58
Cyclamic acid	0.345	1.04	107	11	-15
Saccharine	0.0888	0.267	104	5.9	-12
Sucralose	0.807	2.42	114	5.3	-22
Personal Care Products					
Benzophenon 3	0.0947	0.284	84	4.6	24
Galaxolide	0.0113	0.0339	60	8.2	-35
Galaxolidone	0.0000517	0.000155	62	10	-37
Ethylparaben	0.0264	0.0793	78	8.6	-45
Methylparaben	0.0412	0.124	80	9.2	-30
Propylparaben	0.809	2.43	75	8.5	-29
Coffee and tobacco-related compounds					
Caffeine	0.138	0.413	101	10	-41
Theobromine	0.0403	0.121	77	6.3	8
Nicotine	0.0690	0.207	83	3.0	-56
Cotinine	0.0169	0.0508	81	6.2	-20
Hydroxycotinine	0.0438	0.131	96	5.6	-35
Industrial chemicals					
Benzododecinium	0.832	2.50	103	10	-21
2-hydroxybenzothiazole (2-OH-BTH)	0.172	0.517	105	13	-41
Benzotriazole (BTR)	0.0196	0.0587	85	13	-56

Compounds	***LOD (µg/L)***	***LOQ (µg/L)***	***% Recovery***	***%RSD (n=3)***	***%Matrix effect***
			C: 0.5 µg/L		
Lauryl diethanolamide (Lauryl-DEA)	0.00453	0.0136	80	9.5	-32
N,N-Dimethyldecylamine (N,N-diMe-DA)	0.00739	0.0222	75	12	-22
N,N-Dimethyldodecylamine (N,N-diMe-DDA)	0.00812	0.0243	68	11	-30
N,N-Dimethyldodecylamine N-oxide (N,N-diMe-DDA-N-oxide)	0.0110	0.0329	75	13	-28
N,N-Dimethyltetradecylamine (N,N-diMe-TDA)	0.00620	0.0186	80	14	-39
N,N-Dimethyltetradecylamine-N-oxide (N,N-diMe-TDA-N-oxide)	0.0105	0.0314	86	9.4	-37
N-Methyldodecylamine (N-Me-DDA)	0.00406	0.0122	104	12	-40
Triethyl Phosphate	0.0320	0.0960	94	5.0	-42
Triethyl Citrate	0.0191	0.0574	76	6.5	-50

(a)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4
2
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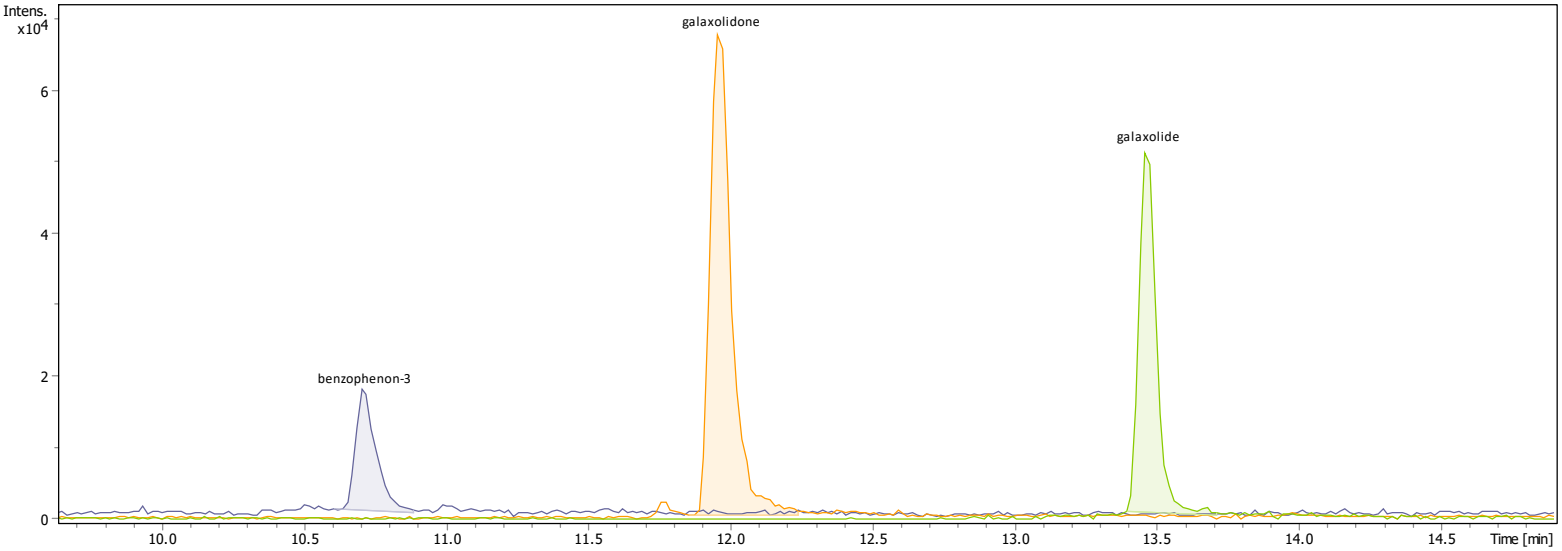
cyclamic acid
acesulfame
sucralose
2.5
3.0
3.5
4.0
4.5
Time [min]

(b)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x10^4
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4
2
0
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galaxolide
benzophenon-3
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10.5
11.0
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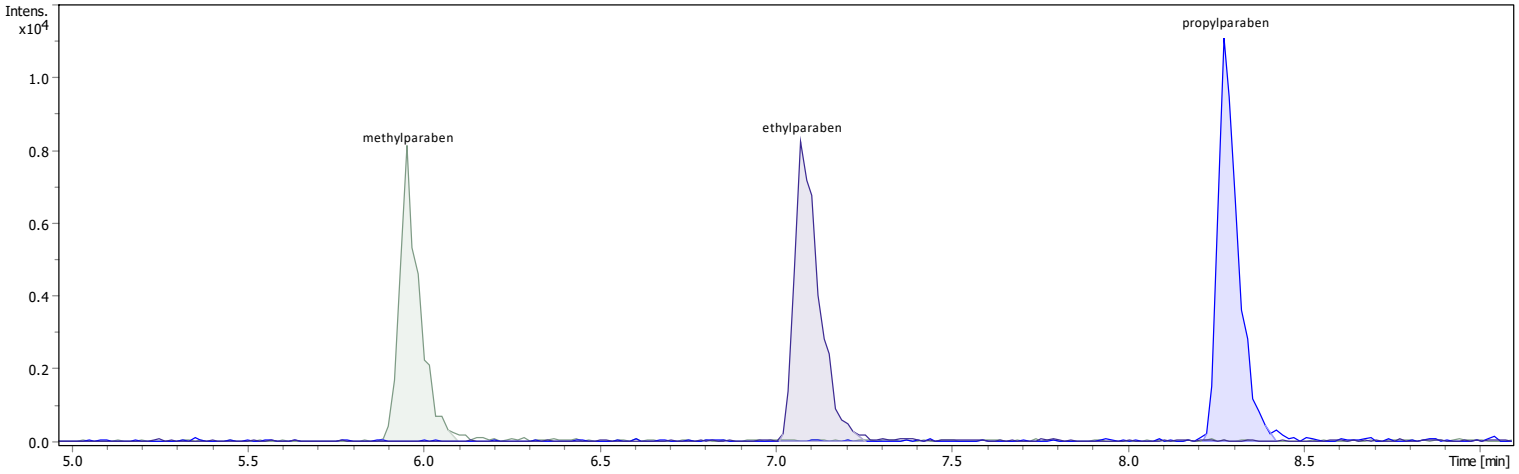
13.0
13.5
14.0
14.5
Time [min]

(c)


Intens.
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0.6
0.4
0.2
0.0
methylparaben
ethylparaben
propylparaben
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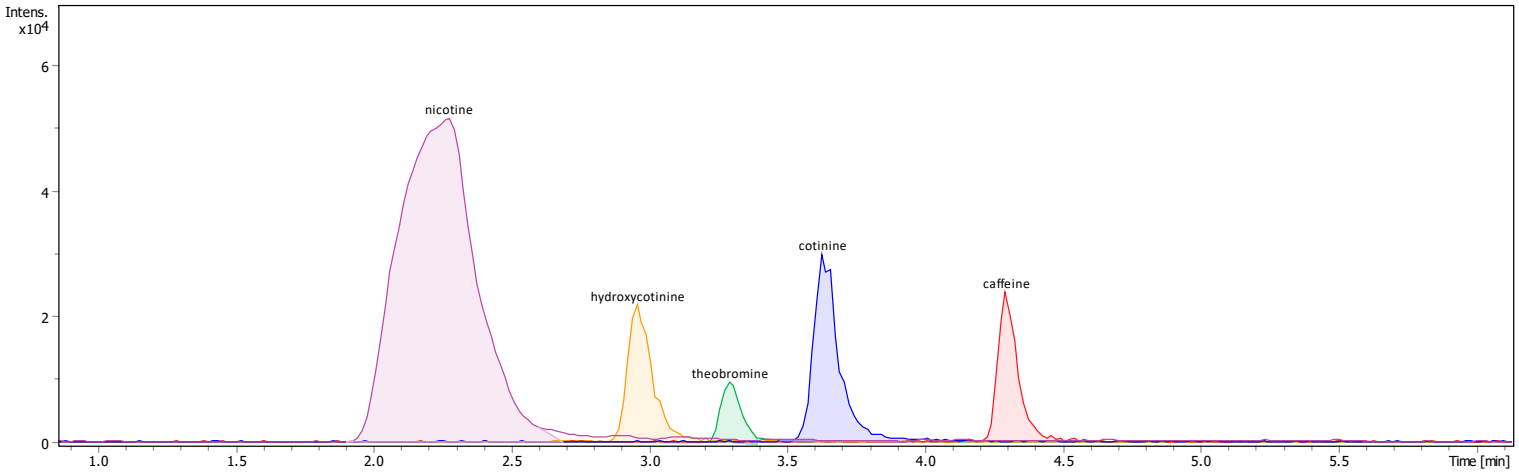
5.5
6.0
6.5
7.0
7.5
8.0
8.5
Time [min]

(d)


Intens.
x10^4
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0
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theobromine
cotinine
caffeine
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1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
Time [min]

(e)

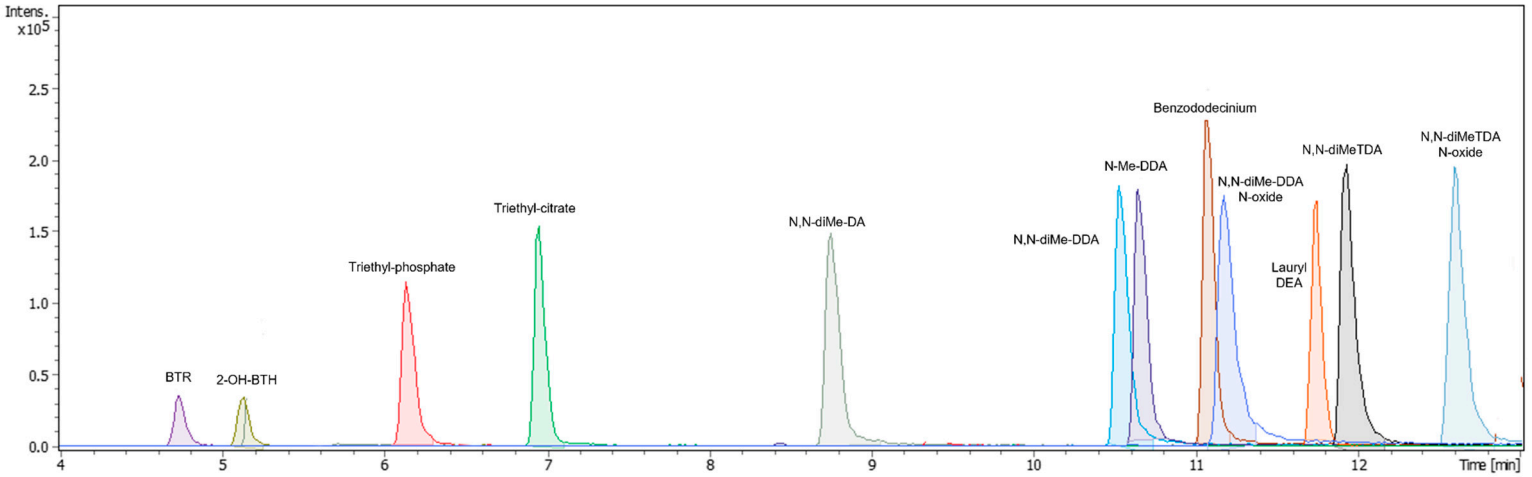

Figure S1. Extracted Ion Chromatograms for the detected compounds; (a) artificial sweeteners by LC-ESI(-)-QToF-MS, (b) personal care products by LC-ESI(+)-QToF-MS, (c) personal care products by LC-ESI(-)-QToF MS, (d) coffee and tobacco-related compounds by LC-ESI(+)-QToF-MS, and (e) industrial chemicals by LC-ESI(+)-QToF-MS.

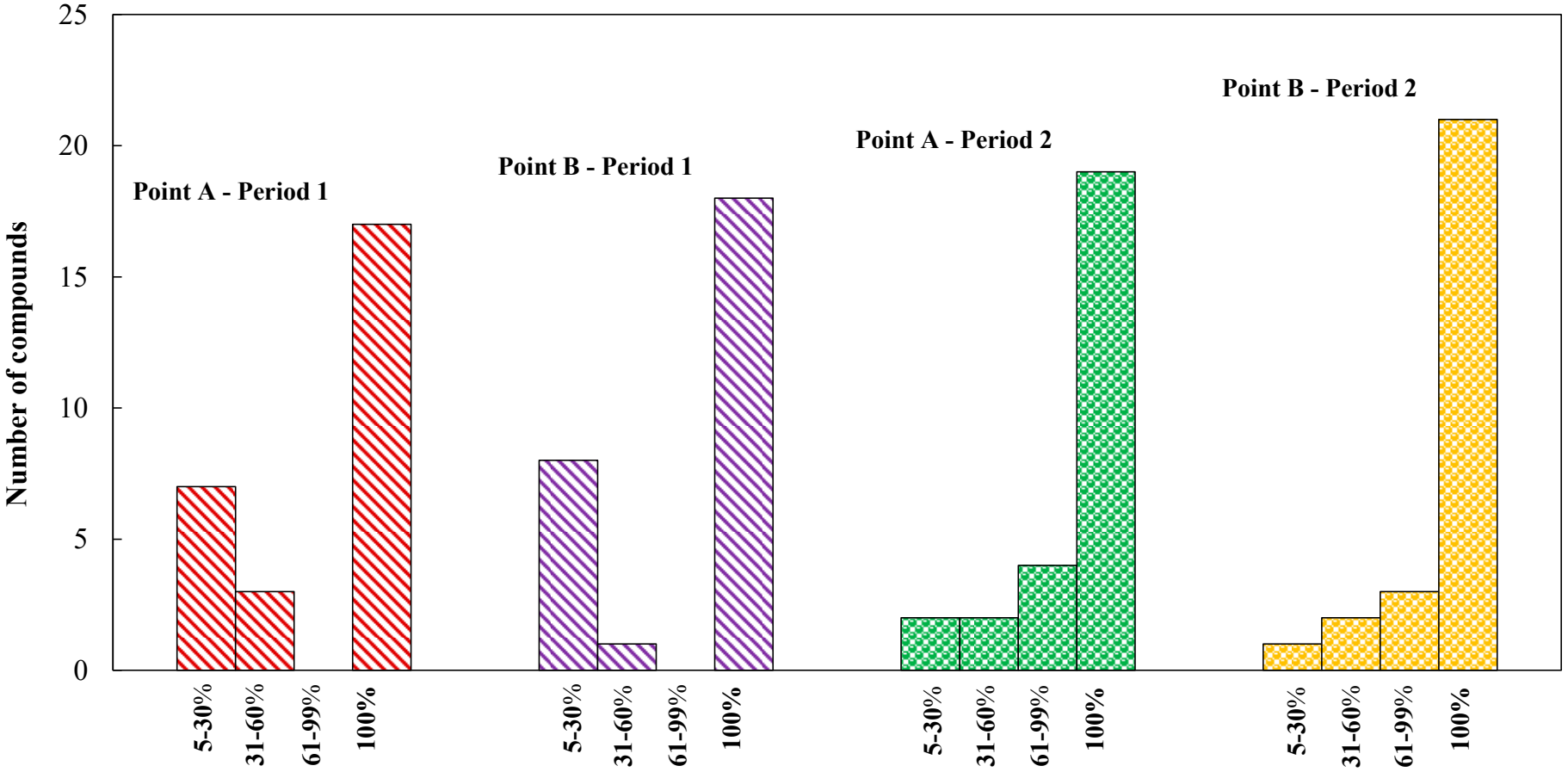

Figure S2. % Frequency of appearance (% FoA) of the detected ECs in Building A (Point A) and entire hospital (Point B) of the two studied periods.