Supplementary information

A comparison of one-dimensional and microscale two-dimensional liquid chromatographic approaches coupled to high resolution mass spectrometry for the analysis of complex samples.

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This document contains supplementary tables including explanations (where necessary) in order of appearance in the manuscript.

Wastewater sample preparation.

To investigate the applicability of the comparison, a real wastewater sample (200 mL) was taken after the first sedimentation step of a municipal wastewater treatment plant. Prior to the analysis, the pH was adjusted to 3 by using hydrochloric acid (2.5% solution). Afterwards, the sample was extracted with a 200 mg Oasis HLB (6 mL) solid phase cartridge (Waters, Eschborn, Germany). The solid-phase extraction was based on the following steps: conditioning: 5 mL methanol (5 min residence time); equilibration: 5 mL water at pH 3; sample application; washing: 5 mL of a water – methanol mixture (95:5, v:v); drying for 5 min; elution: 3 x 3 mL methanol; evaporation of the eluent in a gentle nitrogen stream at 40 °C; dissolving in 2 mL acidified (0.1% FA) water – acetonitrile (95:5, v:v). The wastewater sample was not spiked with the reference compounds.

Column i.d. comparison.

The main reason to use a 0.1 mm i.d. column for the 1st dimension is the maximum available injection volume. Theoretically, the maximum injection volume should be less than 10% of the column void volume. For example, the column void volume of a 50 mm long column with an i.d. of 0.1 mm is approximately 250 nL and the resulting maximum injection volume (10%) is 25 nL. If we reduce the i.d. of the same column to 0.05 mm (which is the smallest commercially available i.d.) the maximum injection volume is reduced to approximately 7 nL. The reduced injection volume has a negative effect on the limit of detection. In combination with the constant dilution by the modulation process, the signal intensity could be decreased dramatically.

Now the question arises, why we did not use an i.d. of 0.3 mm in the 1st dimension. It is also possible to use columns with larger i.d. on the 1st dimension, but the flowrate must be increased to avoid band broadening. A higher flowrate for example of 3 µL/min on the 1st dimension column drastically reduces the fill time of the 300 nL modulation loop. As a result of that, the cycle time of the 2nd dimension must be decreased dramatically to 0.1 minute. Otherwise, the effluent of the 1st dimension would be lost.

For this work we used a cycle time of 1 minute, which corresponds to the optimum of the developed 2D-LC system. Due to general technical limitations, it is not possible to reduce the cycle time to 0.1 minutes for LC x LC applications.

Therefore, the strategy to minimize problems in terms of decreased signal intensity is to use a slightly smaller column i.d. as of the 2nd dimension and the i.d. of 0.1 mm is a very good compromise between maximum injection volume and loop fill time (and the corresponding cycle time of the 2nd dimension).

Furthermore, the inner diameters of the miniaturized system of both dimensions could be compared to conventional online LC x LC setups by the following equation:

$$\Delta\, i.d. = \frac{d_{c,2} - d_{c,1}}{d_{c,2}}$$

$\Delta i.d.$ = relative difference of the column inner diameters of the two coupled LC dimensions

$d_{c,1}$ = i.d. of the 1st dimension

$d_{c,2}$ = i.d. of the 1st dimension

For the 1st dimension of a non-miniaturized LC x LC typically inner diameters of 1.0 mm and 2.1 mm were used and for the 2nd dimension it is mainly 4.6 mm.

	D1 column i.d.	D2 column i.d.	Δ i.d.
Conventional setup 1	1.0	4.6	0.78
Conventional setup 2	2.1	4.6	0.54
Miniaturized setup	0.1	0.3	0.66

As shown in the Table, the Δi.d. of the miniaturized set-up is comparable to the non-miniaturized approaches.

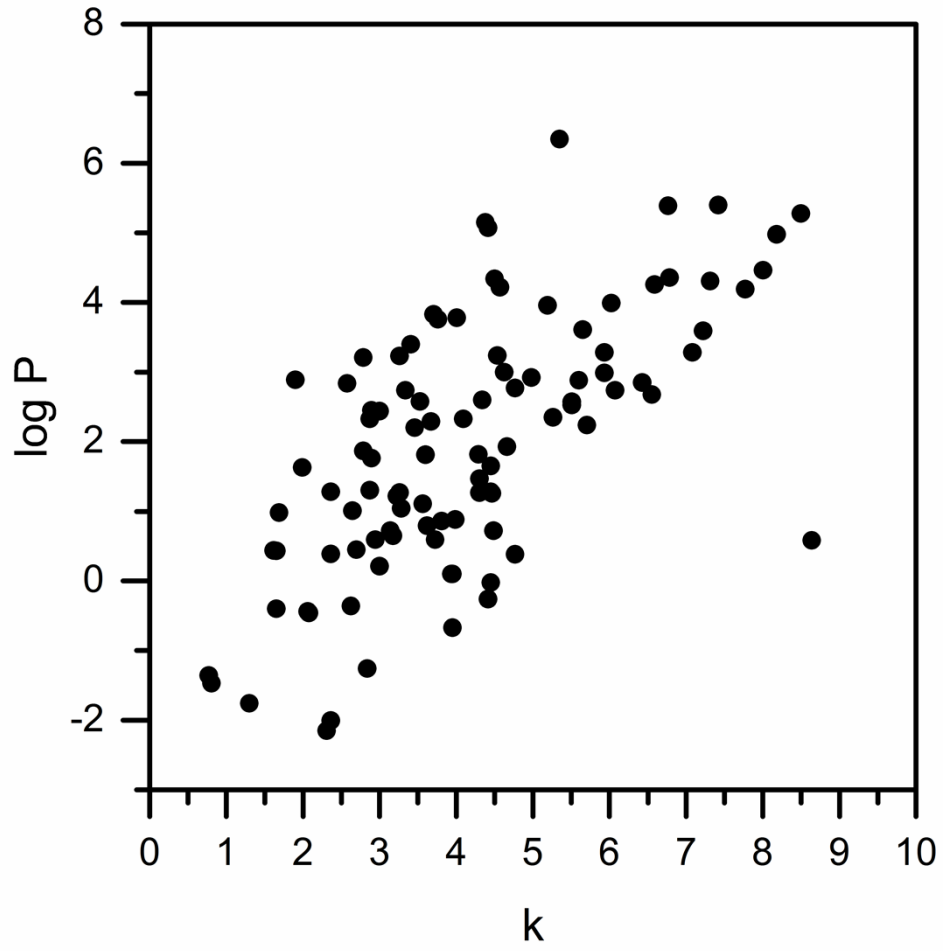


Figure S-1 Correlation plot. The retention factor is plotted against the octanol-water partition coefficient.

It can be seen from the plot in Figure S-1 that there is no correlation when the retention factor is plotted against the log P and thus, the proposed criterion is neither applicable for a suspected-target nor non-target screening approach.

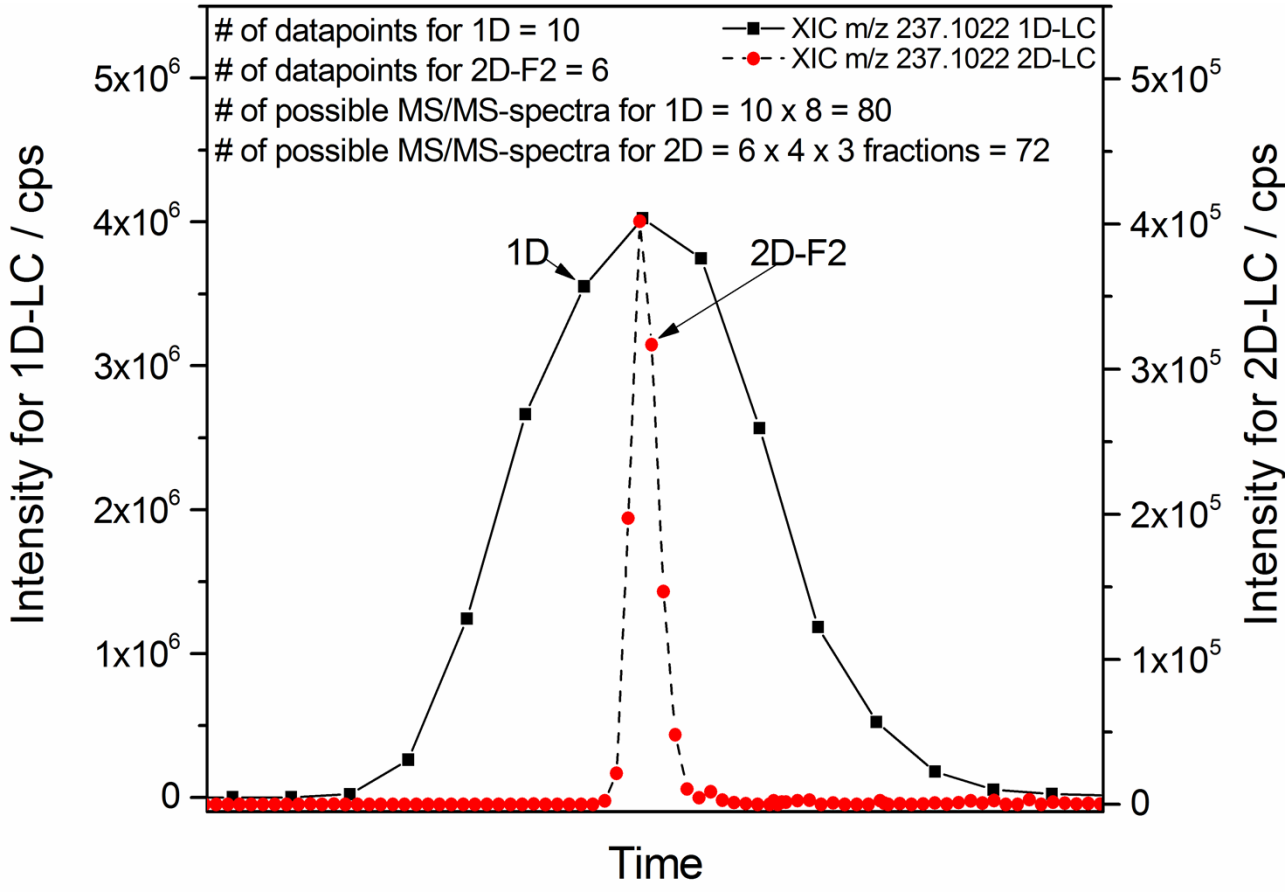
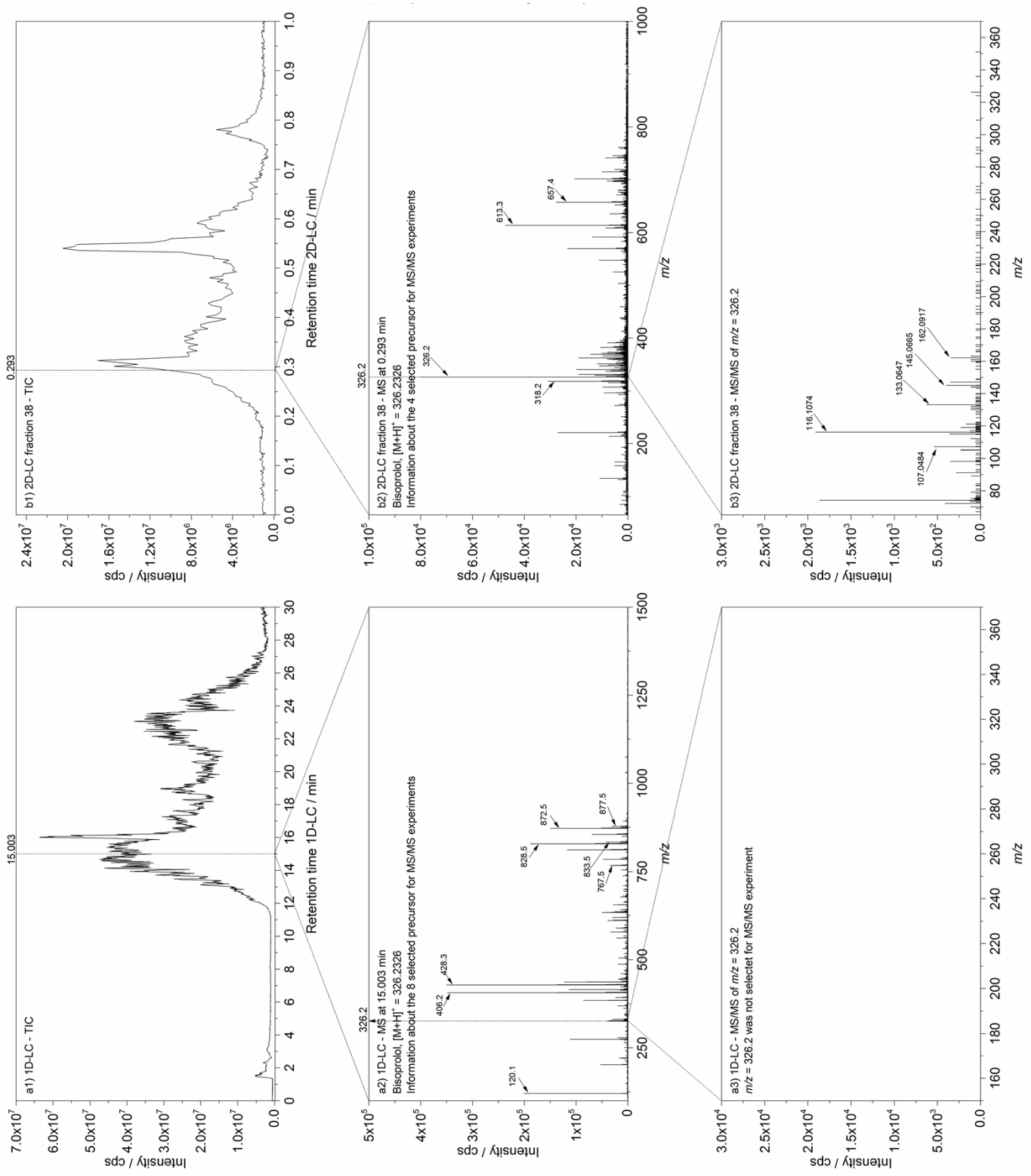


Figure S-2 Comparison of 1D-LC and 2D-LC signal of $[M+H]^+$ 237.1022 with a view to the number of data points and the possible number of MS/MS spectra.

For the 1D-LC signal 10 data points could be observed, each with 8 MS/MS spectra. In total it could be possible to get 80 MS/MS spectra over the 1D-LC signal. For the 2D-LC signal in the second fraction, 6 data points could be observed, each with 4 MS/MS spectra, giving up to 24 MS/MS spectra. However, the total number of possible MS/MS spectra consists of the sum of all spectra given in each fraction of the 2D-LC signal. For example the total number of MS/MS spectra for 3 fractions results in 72 and is nearly equal to the 1D-LC approach.


a1) 1D-LC - TIC
15.003
Intensity / cps
Retention time 1D-LC / min
a2) 1D-LC - MS at 15.003 min
Bisoprolol, [M+H]+ = 326.2326
Information about the 8 selected precursor for MS/MS experiments
326.2
406.2
428.3
120.1
828.5
872.5
877.5
833.5
767.5
m/z
a3) 1D-LC - MS/MS of m/z = 326.2
m/z = 326.2 was not selectet for MS/MS experiment
b1) 2D-LC fraction 38 - TIC
0.293
Retention time 2D-LC / min
b2) 2D-LC fraction 38 - MS at 0.293 min
Bisoprolol, [M+H]+ = 326.2326
Information about the 4 selected precursor for MS/MS experiments
326.2
318.2
613.3
657.4
b3) 2D-LC fraction 38 - MS/MS of m/z = 326.2
116.1074
107.0484
133.0647
145.0665
162.0917

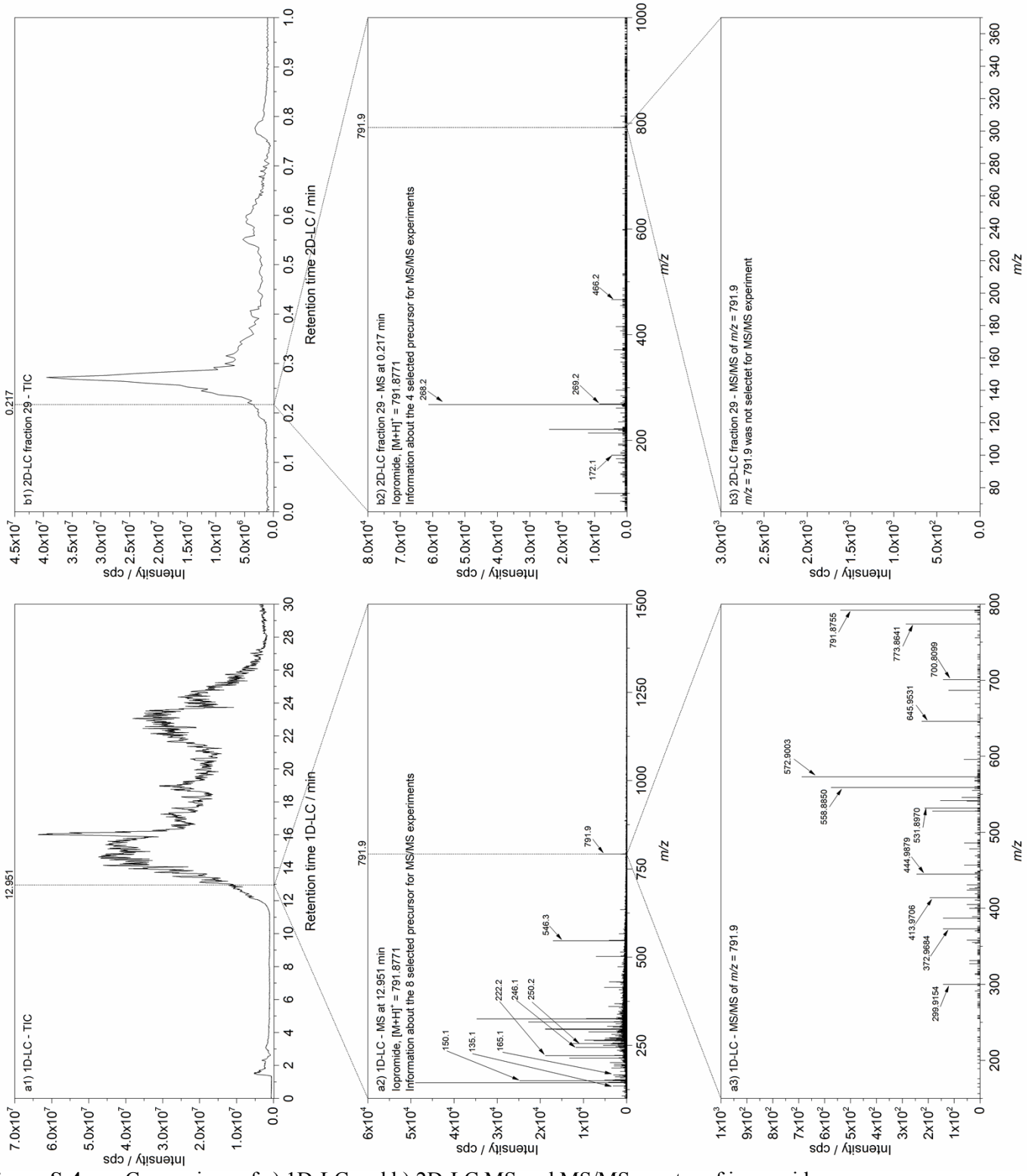


Figure S-4 Comparison of a) 1D-LC and b) 2D-LC MS and MS/MS spectra of iopromide $[M+H]^+$ 791.8771.

The retention time of bisoprolol is located at the local maximum inside the TIC chromatogram of both approaches (see Fig. S-3). As discussed before it could be seen that the matrix impact at this region is very high and the bisoprolol precursor ion could not be selected for an MS/MS experiment due to the relative low signal intensity for the 1D-LC approach. With the 2D-LC approach bisoprolol could be separated chromatographically from the interfering matrix. Therefore bisoprolol precursor could be selected as a precursor with the highest intensity for an MS/MS experiment.

The retention time of iopromide is located at the rising edge of the TIC chromatogram of both approaches (see Fig. S-4). The matrix impact at this region is not as pronounced as for bisoprolol. The precursor intensity of iopromide for the 1D-LC approach is high enough for a selection for an MS/MS experiment. For the 2D-LC approach the absolute precursor intensity was not high enough, therefore iopromide was excluded from the MS/MS experiment.

21 pages in total

Table S-1. Detailed list of the compounds used for the 99 component reference standard.

Analyte	purchased as	CAS No.	Purity*	Source	log P**
5,6-Dimethyl-1H-benzotriazole	5,6-Dimethyl-1H-benzotriazole monohydrate	4184-79-6	99%	Aldrich	2.33
5-Methyl-1H-benzotriazole	5-Methyl-1H-benzotriazole	136-85-6	≥ 98%	Fluka	1.81
Acetylsulfadiazine	N^4-Acetylsulfadiazine	127-74-2	99%	IUTA***	0.45
Acetylsulfadimidine	N^4-Acetylsulfamethazine	100-90-3	99%	IUTA***	0.72
Acetylsulfamerazine	N^4-Acetylsulfamerazine	127-73-1	99%	IUTA***	0.59
Acetylsulfamethoxazole	N^4-Acetylsulfamethoxazole	21312-10-7		LGC Standards	0.86
Allopurinol	Allopurinol	315-30-0		Sigma	-1.76
Ampicillin	Ampicillin	69-53-4		Sigma	-2.01
Atenolol	Atenolol	29122-68-7	≥ 98%	Sigma	0.43
Atorvastatin	Atorvastatin calcium salt	134523-03-8		Dr. Ehrenstorfer	5.39
Azithromycin	Azithromycin	83905-01-5	≥ 95%	Sigma	2.44
Benzotriazole	Benzotriazole	95-14-7	99%	Sigma-Aldrich	1.30
Bezafibrate	Bezafibrate	41859-67-0	≥ 98%	Sigma	3.99
Bisoprolol	Bisoprolol fumarate	104344-23-2		LGC Standards	2.20
Carbamazepine	Carbamazepine	298-46-4		Sigma	2.77
Carbetamide	Carbetamide	16118-49-3	Pestanal	Fluka	1.65
Cefalexin	Cephalexin hydrate	15686-71-2		Sigma	-2.15
Chloramphenicol	Chloramphenicol	56-75-7	≥ 98%	Sigma	0.88
Chlorbromuron	Chlorobromouron	13360-45-7	Pestanal	Fluka	2.85
Chloridazon	Chloridazon	1698-60-8	Pestanal	Fluka	1.11
Chlorprothixene	Chlorprothixene hydrochloride	6469-93-8		Sigma	5.07
Cilastatin	Cilastatin sodium salt	81129-83-1	≥ 98%	Sigma	-1.26
Citalopram	Citalopram hydrobromide	59729-32-7		Sigma	3.76
Clarithromycin	Clarithromycin	81103-11-9		LGC Standards	3.24
Clenbuterol	Clenbuterol hydrochloride	21898-19-1	≥ 95%	Sigma	2.33
Climbazole	Climbazole	38083-17-9	Pestanal	Fluka	4.34
Clindamycin	Clindamycin hydrochloride	21462-39-5		Sigma	1.04
Clozapine	Clozapine	5786-21-0		Sigma	3.40
Cyclophosphamide	Cyclophosphamide monohydrate	6055-19-2	≥ 97%	Sigma	0.10
Dapsone	Dapsone	80-08-0		Campro Scientific	1.27
Dehydrato-Erythromycin	Erythromycin	114-07-8		Sigma-Aldrich	2.60
Diacetoxyscirpenol	Diacetoxyscirpenol	2270-40-8		Sigma	0.38
Diatrizoate	Amidotrizoic acid dihydrate	50978-11-5		LGC Standards	2.89
Diclofenac	Diclofenac sodium salt	15307-79-6		Sigma	4.26
Dimpylate	Diazinon	333-41-5	Pestanal	Fluka	4.19
Diuron	Diuron	330-54-1	Pestanal	Fluka	2.53
Enalapril	Enalapril maleate	76095-16-4		Campro Scientific	0.59
Fenofibrate	Fenofibrate	49562-28-9	≥ 99%	Sigma	5.28
Fenofibric acid	Fenofibric acid	42017-89-0		Chemos	4.36
Fumonisin B1	Fuminosin B1	116355-83-0	95%	AppliChem	-0.67
Fumonisin B2	Fuminosin B2	116355-84-1		Iris Biotech	0.72
Gemcitabine	Gemcitabine hydrochloride	122111-03-9		LGC Standards	-1.47
Gliotoxin	Gliotoxin	67-99-2	≥ 98%	Fluka	-0.02
HT-2 Toxin	HT2 Toxin	26934-87-2	98%	AppliChem	0.58
Hydrocortisone	Hydrocortisone	50-23-7		Dr. Ehrenstorfer	1.28
Ifosfamide	Ifosfamide	3778-73-2		Sigma	0.10

Analyte	purchased as	CAS No.	Purity*	Source	log P**
Iopromide	Iopromide	73334-07-3	≥ 99%	LGC Standards	-0.44
Isoproturon	Isoproturon	34123-59-6	Pestanal	Fluka	2.57
Ketoprofen	Ketoprofen	22071-15-4	≥ 98%	Sigma	3.61
Lidocaine	Lidocaine	137-58-6		Sigma	2.84
Linuron	Linuron	330-55-2		Campro Scientific	2.68
Losartan	Losartan potassium salt	124750-99-8		Campro Scientific	3.96
Mefenamic acid	Mefenamic acid	61-68-7		Sigma	5.40
Megestrol	Megestrol acetate	595-33-5	Vetranal	Fluka	3.28
Melperone	Melperone hydrochloride	1622-79-3		Chemos	3.23
Metconazole	Metconazole	125116-23-6	Pestanal	Fluka	3.59
Metformin	1,1-Dimethylbiguanide hydrochloride	1115-70-4	97%	Aldrich	-1.36
Metobromuron	Metobromuron	3060-89-7	Pestanal	Fluka	2.24
Metoprolol	(±)-Metoprolol (+)-tartrate	56392-17-7	≥ 98%	Sigma	1.76
Metronidazole	Metronidazole	443-48-1		Sigma	-0.46
Mianserin	Mianserin hydrochloride	21535-47-7		Sigma	3.83
Mirtazapine	Mirtazapine	85650-52-8	≥ 98%	Sigma	3.21
Monuron	Monuron	150-68-5	Pestanal	Fluka	1.93
Nafcillin	Nafcillin sodium salt	985-16-0		Sigma	2.29
Naproxen	Naproxen	22204-53-1		Sigma-Aldrich	2.99
Oxazepam	Oxazepam	604-75-1		Sigma	2.92
Oxcarbazepine	Oxcarbazepine	28721-07-5	≥ 98%	Sigma	1.82
Paracetamol	Paracetamol	103-90-2		Fluka	1.63
Phenazone	Antipyrine	60-80-0		Fluka	1.22
Picoxystrobin	Picoxystrobin	117428-22-5		Campro Scientific	4.31
Pipamperone	Pipamperone dihydrochloride	2448-68-2	99%	Sigma	1.87
Piperacillin	Piperacillin sodium salt	59703-84-3		Sigma	-0.26
Prednisolone	Prednisolone	50-24-8	≥ 99%	Sigma	1.27
Propranolol	(±)-Propranolol hydrochloride	318-98-9	≥ 98%	Sigma	2.58
Propyphenazone	Propyphenazone	479-92-5		LGC Standards	2.35
Quinoxyfen	Quinoxyfen	124495-18-7	Pestanal	Fluka	4.98
Ramipril	Ramipril	87333-19-5		Campro Scientific	1.47
Ranitidine	Ranitidine hydrochloride	66357-59-3		Campro Scientific	0.98
Ritalinic acid	Ritalinic acid	19395-41-6	99%	Aldrich	-0.36
Roxithromycin	Roxithromycin	80214-83-1	≥ 90%	Sigma	3.00
Sertraline	Sertraline hydrochloride	79559-97-0		Campro Scientific	5.15
Simvastatin	Simvastatin	79902-63-9	≥ 97%	Sigma	4.46
Sotalol	Sotalol hydrochloride	959-24-0		Dr. Ehrenstorfer	-0.40
Stachybotrylactam	Stachybotrylactam	163391-76-2	≥ 95%	Bioaustralis	3.28
Sulfadiazine	Sulfadiazine	68-35-9	≥ 99%	Sigma	0.39
Sulfadimethoxine	Sulfadimethoxine	122-11-2	≥ 98.5%	Fluka	1.26
Sulfadimidine	Sulfamethazine	57-68-1	≥ 99%	Sigma	0.65
Sulfamethizole	Sulfamethizole	144-82-1	≥ 99%	Fluka	0.21
Sulfamethoxazole	Sulfamethoxazole	723-46-6		Fluka	0.79
Sulfapyridine	Sulfapyridine	144-83-2	≥ 99%	Fluka	1.01
Tamoxifen	Tamoxifen citrate	54965-24-1		Fluka	6.35
Terbutaline	Terbutaline hemisulfate	23031-32-5		Sigma	0.44
Terbutryn	Terbutryn	886-50-0	Pestanal	Fluka	2.88
Tramadol	Tramadol hydrochloride	36282-47-0	≥ 99%	Sigma	2.45

Analyte	purchased as	CAS No.	Purity*	Source	log P**
Trimethoprim	Trimethoprim	738-70-5	≥ 98%	Sigma	1.28
Venlafaxine	Venlafaxine hydrochloride	99300-78-4	≥ 98%	Sigma	2.74
Warfarin	Warfarin	81-81-2		Campro Scientific	2.74
Xylometazoline	Xylometazoline hydrochloride	1218-35-5		Sigma	3.78
Zuclopenthixol	Zuclopenthixol	53772-83-1		Chemos	4.22

Legend to Table S-1:

***** Purity was at least pro analysi (p. a.) unless otherwise noted.

****** log P values are predicted by ChemAxon – www.chemicalize.org

******* These standards have been prepared in-house according to the procedure described in the following article: Pfeifer T, Tuerk J, Bester K, Spiteller M (2002) Rapid Commun Mass Sp 16(7):663–669.

Manufacturer information:

- Products from Aldrich, Fluka, Sigma and Supelco were purchased from Sigma-Aldrich, Schnelldorf, Germany.
- AppliChem, Darmstadt, Germany.
- Bioaustralis products via tebu-bio, Offenbach, Germany.
- Campro Scientific, Berlin, Germany.
- Chemos, Regenstauf, Germany.
- Dr. Ehrenstorfer, Augsburg, Germany.
- Iris Biotech, Marktredwitz, Germany.
- LGC Standards, Wesel, Germany.

Table S-2. MS Parameters for 1D-LC and 2D-LC approaches.

Parameter	1D-HPLC	2D-HPLC
m/z range	100 - 1500	65 - 1000
m/z range for IDA mode	150 - 1500	130 - 1000
# of MS/MS experiments / spectra	8	4
Dwell time fullscan / ms	250	20
Dwell time MS/MS experiment / ms	100	20
Period cycle time / ms	1101	150
MS/MS for ions greater than / Da	150	130
MS/MS mass tolerance / ppm	5	5
MS/MS exclusion after / occurrences	6	4
MS/MS exclusion time / s	30	6
Curtain gas / psi	35	10
Ion source gas 1 / psi	35	25
Ion source gas 2 / psi	45	25
IonSpray voltage floating / V	5500	5500
Temperature / °C	550	500
Collision energy / eV	10	10
Collision energy for MS/MS / eV	40	40
Declustering potential / V	60	80

Table S-3. Detailed list of the 1D-LC separation of the 99 detected reference compounds with $\Delta\ m/z = \pm 5$ ppm.

Analyte	Molecular formula of [M]	Monoisotopic mass* of $[M+H]^+$	$\Delta\ m/z$ (ppm)	Retention time of 1 µg mL^{-1} ref. standard (min)	Average retention time of 0.45 ng mL^{-1} - 1 µg mL^{-1} ref. standard (min)
5,6-Dimethyl-1H-benzotriazole	$C_8H_9N_3$	148.0869	2.0	16.93	16.92
5-Methyl-1H-benzotriazole	$C_7H_7N_3$	134.0713	1.6	16.15	16.16
Acetylsulfadiazine	$C_{12}H_{12}N_4O_3S$	293.0703	0.6	14.66	14.67
Acetylsulfadimidine	$C_{14}H_{16}N_4O_3S$	321.1016	0.8	15.07	15.08
Acetylsulfamerazine	$C_{13}H_{14}N_4O_3S$	307.0859	0.6	14.90	14.91
Acetylsulfamethoxazole	$C_{12}H_{13}N_3O_4S$	296.0700	0.8	16.64	16.64
Allopurinol	$C_5H_4N_4O$	137.0458	-0.8	4.87	4.94
Ampicillin	$C_{16}H_{19}N_3O_4S$	350.1169	-0.4	13.44	13.46
Atenolol	$C_{14}H_{22}N_2O_3$	267.1703	0.3	12.02	12.04
Atorvastatin	$C_{33}H_{35}FN_2O_5$	559.2603	-2.7	20.66	20.65
Azithromycin	$C_{38}H_{72}N_2O_{12}$	749.5158	-0.6	13.97	14.01
Benzotriazole	$C_6H_5N_3$	120.0556	0.2	14.99	15.00
Bezafibrate	$C_{19}H_{20}ClNO_4$	362.1154	-1.0	19.63	19.62
Bisoprolol	$C_{18}H_{31}NO_4$	326.2326	0.7	14.64	14.67
Carbamazepine	$C_{15}H_{12}N_2O$	237.1022	0.7	17.80	17.79
Carbetamide	$C_{12}H_{16}N_2O_3$	237.1234	0.8	17.31	17.31
Cefalexin	$C_{16}H_{17}N_3O_4S$	348.1013	0.7	13.49	13.51
Chloramphenicol	$C_{11}H_{12}Cl_2N_2O_5$	323.0196	-1.0	16.81	16.81
Chlorbromuron	$C_9H_{10}BrClN_2O_2$	292.9687	0.4	21.15	21.15
Chloridazon	$C_{10}H_8ClN_3O$	222.0429	0.3	16.09	16.10
Chlorprothixene	$C_{18}H_{18}ClNS$	316.0921	0.8	16.14	16.17
Cilastatin	$C_{16}H_{26}N_2O_5S$	359.1635	1.1	14.32	14.33
Citalopram	$C_{20}H_{21}FN_2O$	325.1711	0.9	15.36	15.38
Clarithromycin	$C_{38}H_{69}NO_{13}$	748.4842	-1.8	15.82	15.84
Clenbuterol	$C_{12}H_{18}Cl_2N_2O$	277.0869	0.5	14.09	14.12
Climbazole	$C_{15}H_{17}ClN_2O_2$	293.1051	0.0	16.35	16.36
Clindamycin	$C_{18}H_{33}ClN_2O_5S$	425.1872	0.3	14.54	14.56
Clozapine	$C_{18}H_{19}ClN_4$	327.1371	0.9	14.56	14.59
Cyclophosphamide	$C_7H_{15}Cl_2N_2O_2P$	261.0321	0.7	16.51	16.52
Dapsone	$C_{12}H_{12}N_2O_2S$	249.0692	0.6	16.23	16.24
Dehydrato-Erythromycin	$C_{37}H_{65}NO_{12}$	716.4580	-2.6	15.61	15.21
Diacetoxyscirpenol	$C_{19}H_{26}O_7$	367.1751	-0.6	17.62	17.62
Diatrizoate	$C_{11}H_9I_3N_2O_4$	614.7769	-0.8	13.08	13.08
Diclofenac	$C_{14}H_{11}Cl_2NO_2$	296.0240	0.6	21.35	21.35
Dimpylate	$C_{12}H_{21}N_2O_3PS$	305.1083	0.7	23.37	23.37
Diuron	$C_9H_{10}Cl_2N_2O$	233.0243	-0.1	19.38	19.37
Enalapril	$C_{20}H_{28}N_2O_5$	377.2071	0.9	15.19	15.19
Fenofibrate	$C_{20}H_{21}O_4Cl$	361.1201	-1.8	24.92	24.92
Fenofibric acid	$C_{17}H_{15}ClO_4$	319.0732	0.8	21.44	21.43
Fumonisin B1	$C_{34}H_{59}NO_{15}$	722.3958	-2.4	15.49	15.51
Fumonisin B2	$C_{34}H_{59}NO_{14}$	706.4008	-2.7	16.19	16.18
Gemcitabine	$C_9H_{11}F_2N_3O_4$	264.0790	0.9	2.32	2.86
Gliotoxin	$C_{13}H_{14}N_2O_4S_2$	327.0468	-0.7	17.40	17.39

Analyte	Molecular formula of [M]	Monoisotopic mass* of $[M+H]^+$	Δ *m/z* (ppm)	Retention time of 1 µg mL^{-1} ref. standard (min)	Average retention time of 0.45 ng mL^{-1} - 1 µg mL^{-1} ref. standard (min)
HT-2 Toxin	$C_{22}H_{32}O_8$	425.2170	-0.5	17.93	17.93
Hydrocortisone	$C_{21}H_{30}O_5$	363.2166	0.9	16.91	16.91
Ifosfamide	$C_7H_{15}Cl_2N_2O_2P$	261.0321	0.7	16.36	16.37
Iopromide	$C_{18}H_{24}I_3N_3O_8$	791.8771	-0.8	12.98	13.02
Isoproturon	$C_{12}H_{18}N_2O$	207.1492	0.8	19.20	19.20
Ketoprofen	$C_{16}H_{14}O_3$	255.1016	0.3	19.54	19.54
Lidocaine	$C_{14}H_{22}N_2O$	235.1805	-0.9	13.56	13.61
Linuron	$C_9H_{10}Cl_2N_2O_2$	249.0192	0.2	20.92	20.92
Losartan	$C_{22}H_{23}ClN_6O$	423.1695	-1.0	18.23	18.23
Mefenamic acid	$C_{15}H_{15}NO_2$	242.1176	-0.2	22.47	22.47
Megestrol	$C_{24}H_{32}O_4$	385.2373	-0.4	22.42	22.42
Melperone	$C_{16}H_{22}FNO$	264.1758	0.9	14.67	14.72
Metconazole	$C_{17}H_{22}ClN_3O$	320.1524	-0.1	21.85	21.85
Metformin	$C_4H_{11}N_5$	130.1087	1.0	1.74	1.77
Metobromuron	$C_9H_{11}BrN_2O_2$	259.0077	-0.5	19.81	19.81
Metoprolol	$C_{15}H_{25}NO_3$	268.1907	0.2	14.04	14.07
Metronidazole	$C_6H_9N_3O_3$	172.0717	0.3	12.68	12.73
Mianserin	$C_{18}H_{20}N_2$	265.1699	0.4	15.26	15.29
Mirtazapine	$C_{17}H_{19}N_3$	266.1652	0.4	13.75	13.79
Monuron	$C_9H_{11}ClN_2O$	199.0633	0.8	17.87	17.87
Nafcillin	$C_{21}H_{22}N_2O_5S$	415.1322	-0.7	15.81	15.80
Naproxen	$C_{14}H_{14}O_3$	231.1016	-1.0	19.65	19.65
Oxazepam	$C_{15}H_{11}ClN_2O_2$	287.0582	0.3	18.11	18.11
Oxcarbazepine	$C_{15}H_{12}N_2O_2$	253.0972	0.7	16.98	16.98
Paracetamol	$C_8H_9NO_2$	152.0706	-2.7	12.97	13.03
Phenazone	$C_{11}H_{12}N_2O$	189.1022	-0.5	15.11	15.12
Picoxystrobin	$C_{18}H_{16}F_3NO_4$	368.1104	-0.7	22.65	22.64
Pipamperone	$C_{21}H_{30}FN_3O_2$	376.2395	0.9	13.05	13.13
Piperacillin	$C_{23}H_{27}N_5O_7S$	518.1704	-2.3	17.28	17.27
Prednisolone	$C_{21}H_{28}O_5$	361.2010	-0.7	16.79	16.79
Propranolol	$C_{16}H_{21}NO_2$	260.1645	-0.1	15.00	15.03
Propyphenazone	$C_{14}H_{18}N_2O$	231.1492	0.0	18.56	18.56
Quinoxyfen	$C_{15}H_8Cl_2FNO$	308.0040	-0.3	24.28	24.28
Ramipril	$C_{23}H_{32}N_2O_5$	417.2384	0.1	15.73	15.74
Ranitidine	$C_{13}H_{22}N_4O_3S$	315.1485	1.2	12.17	12.22
Ritalinic acid	$C_{13}H_{17}NO_2$	220.1332	-0.6	13.92	13.93
Roxithromycin	$C_{41}H_{76}N_2O_{15}$	837.5319	-1.9	15.90	15.92
Sertraline	$C_{17}H_{17}Cl_2N$	306.0811	1.0	16.05	16.08
Simvastatin	$C_{25}H_{38}O_5$	419.2792	-2.1	24.24	24.26
Sotalol	$C_{12}H_{20}N_2O_3S$	273.1267	-0.4	10.35	10.66
Stachybotrylactam	$C_{23}H_{31}NO_4$	386.2326	-1.2	19.56	19.56
Sulfadiazine	$C_{10}H_{10}N_4O_2S$	251.0597	0.1	13.98	14.00
Sulfadimethoxine	$C_{12}H_{14}N_4O_4S$	311.0809	0.9	17.40	17.39
Sulfadimidine	$C_{12}H_{14}N_4O_2S$	279.0910	0.9	15.29	15.30
Sulfamethizole	$C_9H_{10}N_4O_2S_2$	271.0318	0.7	15.41	15.41
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	254.0594	0.2	16.67	16.68
Sulfapyridine	$C_{11}H_{11}N_3O_2S$	250.0645	0.9	14.47	14.47

Analyte	Molecular formula of [M]	Monoisotopic mass* of $[M+H]^+$	Δ *m/z* (ppm)	Retention time of 1 µg mL^{-1} ref. standard (min)	Average retention time of 0.45 ng mL^{-1} - 1 µg mL^{-1} ref. standard (min)
Tamoxifen	$C_{26}H_{29}NO$	372.2322	-0.4	17.39	17.40
Terbutaline	$C_{12}H_{19}NO_3$	226.1438	-0.5	9.41	9.75
Terbutryn	$C_{10}H_{19}N_5S$	242.1434	-0.7	18.13	18.12
Tramadol	$C_{16}H_{25}NO_2$	264.1958	0.3	14.06	14.09
Trimethoprim	$C_{14}H_{18}N_4O_3$	291.1452	1.2	13.39	13.42
Venlafaxine	$C_{17}H_{27}NO_2$	278.2115	0.4	14.63	14.66
Warfarin	$C_{19}H_{16}O_4$	309.1121	0.1	20.45	20.44
Xylometazoline	$C_{16}H_{24}N_2$	245.2012	0.6	15.52	15.55
Zuclopenthixol	$C_{22}H_{25}ClN_2OS$	401.1449	0.5	15.77	15.81

* The monoisotopic mass was calculated by using the integrated calculator of the PeakView software (Sciex).

Table S-4. Detailed list of the detected targets with 1D-LC and 2D-LC approach in the wastewater sample on the basis of the three criteria (Δ *m/z* = ± 5 ppm, Δ tR = ± 2.5% and MS/MS database hit).

"v" = criteria are fulfilled
"-" = criteria are not fulfilled

Analyte	± 5 ppm		± 5 ppm + 2.5 %		± 5 ppm + 2.5% + MS/MS	
	1D	2D	1D	2D	1D	2D
5,6-Dimethyl-1H-benzotriazole	v	v	v	v	-	v
5-Methyl-1H-benzotriazole	v	v	v	v	-	v
Acetylsulfadiazine	v	-	v	-	-	-
Acetylsulfamerazine	-	v	-	v	-	-
Acetylsulfamethoxazole	v	v	v	v	v	-
Atenolol	v	v	v	v	v	v
Atorvastatin	v	-	v	-	-	-
Benzotriazole	v	v	v	v	-	-
Bezafibrate	v	v	v	v	v	-
Bisoprolol	v	v	v	v	-	v
Carbamazepine	v	v	v	v	v	v
Carbetamide	-	v	-	v	-	-
Chlorbromuron	-	v	-	v	-	-
Chloridazon	-	v	-	v	-	-
Cilastatin	v	-	v	-	v	-
Citalopram	-	v	-	v	-	-
Clarithromycin	v	v	v	v	-	v
Clenbuterol	-	-	-	-	-	-
Climbazole	v	v	v	v	-	-
Clindamycin	-	v	-	v	-	-
Clozapine	-	v	-	v	-	-
Cyclophosphamide	-	v	-	v	-	v
Dehydrato-Erythromycin	-	v	-	v	-	-
Diatrizoate	v	-	v	-	-	-
Diclofenac	v	v	v	v	v	v
Diuron	v	v	v	v	-	-
Enalapril	v	v	v	v	-	-
Fenofibrate	-	v	-	v	-	-
Fenofibric acid	v	v	v	v	v	-
Gemcitabine	-	v	-	-	-	-
Hydrocortisone	v	-	v	-	-	-
Ifosfamide	-	v	-	v	-	-
Iopromide	v	v	v	v	v	-
Isoproturon	-	v	-	v	-	v
Ketoprofen	v	v	v	v	-	-
Lidocaine	v	v	v	v	-	-
Linuron	-	v	-	v	-	-
Losartan	v	v	v	v	v	v
Mefenamic acid	-	v	-	v	-	v
Megestrol	-	v	-	v	-	v
Melperone	-	v	-	v	-	v

Analyte	± 5 ppm		± 5 ppm + 2.5 %		± 5 ppm + 2.5% + MS/MS	
	1D	2D	1D	2D	1D	2D
Metconazole	-	v	-	v	-	-
Metformin	v	v	v	v	-	v
Metobromuron	-	v	-	v	-	-
Metoprolol	v	v	v	v	v	v
Metronidazole	-	v	-	v	-	v
Monuron	-	v	-	v	-	v
Nafcillin	v	-	v	-	-	-
Naproxen	v	-	v	-	v	-
Oxazepam	v	v	v	v	v	v
Oxcarbazepine	v	-	v	-	-	-
Paracetamol	v	v	v	v	v	v
Phenazone	-	v	-	v	-	-
Piperacillin	v	-	v	-	-	-
Prednisolone	v	v	v	v	-	-
Propranolol	v	v	v	v	-	v
Propyphenazone	-	v	-	v	-	-
Quinoxyfen	v	v	v	v	-	-
Ramipril	v	v	-	v	-	v
Ranitidine	v	v	v	v	-	v
Ritalinic acid	v	v	v	v	v	-
Roxithromycin	v	v	v	v	-	-
Sertraline	-	v	-	v	-	-
Simvastatin	v	-	-	-	-	-
Sotalol	v	v	-	v	-	v
Sulfadimethoxine	-	v	-	v	-	v
Sulfamethoxazole	v	v	v	v	-	-
Sulfapyridine	v	-	v	-	-	-
Tamoxifen	-	v	-	v	-	v
Terbutaline	-	v	-	v	-	v
Terbutryn	v	v	v	v	-	v
Tramadol	v	v	v	v	v	-
Trimethoprim	v	v	v	v	v	v
Venlafaxine	v	v	v	v	-	v
Warfarin	v	v	v	v	-	v
Xylometazoline	v	v	-	v	-	v
Zuclopenthixol	-	v	-	v	-	-

Table S-5. Detailed list of the detected targets with a) 1D-LC and b) 2D-LC approach in the waste water sample on the basis of the two criteria Δ *m/z* = ± 5 ppm and Δ tR = ± 2.5%. The ranking position shows the priority, based on the signal intensity at the given retention time, for a selection for an IDA experiment.

For 1D-LC all targets with the priority between 1 to 8 are selected for an IDA experiment.
For 2D-LC all targets with the priority between 1 to 4 are selected for an IDA experiment.

a) Analyte 1D-LC	Ranking position 1D-LC	MS/MS found at (min)	b) Analyte 2D-LC	Ranking position 2D-LC	MS/MS found in D1 fraction #	MS/MS found in D2 at (min)
5,6-Dimethyl-1H-benzotriazole	8	16.63	5,6-Dimethyl-1H-benzotriazole	3	46	0.330
5-Methyl-1H-benzotriazole	2	16.15	5-Methyl-1H-benzotriazole	4	37	0.303
Acetylsulfadiazine	57	14.72	Acetylsulfamerazine	36	30	0.345
Acetylsulfamethoxazole	1	16.65	Acetylsulfamethoxazole	46	44	0.317
Atenolol	1	12.09	Atenolol	3	26	0.189
Atorvastatin	156	20.65	Benzotriazole	22	31	0.262
Benzotriazole	3	15.00	Bezafibrate	139	65	0.439
Bezafibrate	1	19.64	Bisoprolol	2	28	0.294
Bisoprolol	31	15.00	Carbamazepine	4	33	0.364
Carbamazepine	1	17.80	Carbetamide	23	35	0.350
Cilastatin	8	14.33	Chlorbromuron	76	69	0.464
Clarithromycin	380	16.08	Chloridazon	4	43	0.302
Climbazole	23	16.67	Citalopram	73	36	0.322
Diatrizoate	150	13.14	Clarithromycin	1	54	0.370
Diclofenac	8	21.35	Climbazole	6	37	0.354
Diuron	675	19.38	Clindamycin	24	28	0.281
Enalapril	91	15.43	Clozapine	41	31	0.288
Fenofibric acid	2	21.43	Cyclophosphamide	4	28	0.324
Hydrocortisone	95	16.89	Dehydrato-Erythromycin	41	50	0.345
Iopromide	3	12.95	Diclofenac	4	63	0.485
Ketoprofen	173	19.56	Diuron	19	68	0.416
Lidocaine	4	13.61	Enalapril	2	33	0.309
Losartan	7	18.25	Fenofibrate	13	63	0.593
Metformin	5	1.76	Fenofibric acid	1	56	0.488
Metoprolol	4	14.07	Ifosfamide	11	26	0.323
Nafcillin	6	15.84	Iopromide	8	29	0.217
Naproxen	7	19.65	Isoproturon	2	36	0.410
Oxazepam	3	18.13	Ketoprofen	14	38	0.413
Oxcarbazepine	56	16.98	Lidocaine	10	25	0.246
Paracetamol	2	13.02	Linuron	24	59	0.468
Piperacillin	637	17.29	Losartan	4	41	0.394
Prednisolone	8	17.10	Mefenamic acid	4	54	0.516
Propranolol	172	14.83	Megestrol	2	46	0.501
Quinoxyfen	572	24.34	Melperone	1	42	0.287
Ranitidine	30	12.24	Metconazole	3	58	0.507
Ritalinic acid	7	13.92	Metformin	1	12	0.135
Roxithromycin	650	16.14	Metobromuron	27	44	0.421
Sulfamethoxazole	9	16.68	Metoprolol	4	30	0.262
Sulfapyridine	20	14.46	Metronidazole	3	29	0.213

a) Analyte 1D-LC	Ranking position 1D-LC	MS/MS found at (min)	b) Analyte 2D-LC	Ranking position 2D-LC	MS/MS found in D1 fraction #	MS/MS found in D2 at (min)
Terbutryn	41	17.84	Monuron	1	43	0.362
Tramadol	3	13.81	Oxazepam	2	33	0.385
Trimethoprim	8	13.43	Paracetamol	1	31	0.209
Venlafaxine	66	14.66	Phenazone	2	26	0.279
Warfarin	631	20.47	Prednisolone	2	28	0.345
			Propranolol	1	51	0.299
			Propyphenazone	1	27	0.395
			Quinoxyfen	112	71	0.557
			Ramipril	2	36	0.341
			Ranitidine	1	30	0.194
			Ritalinic acid	11	22	0.247
			Roxithromycin	38	42	0.359
			Sertraline	74	41	0.349
			Sotalol	1	25	0.191
			Sulfadimethoxine	4	56	0.352
			Sulfamethoxazole	53	34	0.306
			Tamoxifen	2	39	0.405
			Terbutaline	1	21	0.185
			Terbutryn	3	42	0.415
			Tramadol	16	28	0.265
			Trimethoprim	2	39	0.229
			Venlafaxine	4	26	0.295
			Warfarin	4	41	0.446
			Xylometazoline	3	33	0.325
			Zuclopenthixol	36	63	0.364