

Electronic Supplementary Material:

Picogram per Liter Quantification of Pyrethroid and Organophosphate Insecticides in Surface Waters: a Result of large Enrichment with Liquid-Liquid Extraction and Gas Chromatography coupled to Mass Spectrometry using Atmospheric Pressure Chemical Ionization

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ESM.A Chemicals and Solutions

Table A-1: Analytes and internal standards (ISTDs).

Substance	CAS number	Supplier	Quality
Empenthrin	54406-48-3	Dr. Ehrenstorfer	90%
Tefluthrin	79538-32-2	Dr. Ehrenstorfer	98%
Chlorpyrifos-methyl	5598-13-0	Dr. Ehrenstorfer	99.5%
Chlorpyrifos	2921-88-2	Dr. Ehrenstorfer	99%
Allethrin	584-79-2	Dr. Ehrenstofer	95.5%
Imiprothrin	72963-72-5	Dr. Ehrenstofer	97%
Bifenthrin	82657-04-3	Sigma-Aldrich	99%
Tetramethrin	7696-12-0	Dr. Ehrenstofer	99%
Phenothrin	26002-80-2	Dr. Ehrenstofer	96.5%
λ -Cyhalothrin	91465-08-6	Sigma-Aldrich	97.4%
Cyhalothrin	68085-85-8	Sigma-Aldrich	98%
Acrinathrin	101007-06-1	Dr. Ehrenstofer	96.5%
Permethrin	52645-53-1	Sigma-Aldrich	98.3%
α -Cypermethrin	67375-30-8	Sigma-Aldrich	99.7%
Etofenprox	80844-07-1	Sigma-Aldrich	97.9
Esfenvalerate	66230-04-4	Sigma-Aldrich	97%
Fenvalerate	51630-58-1	Dr. Ehrenstofer	98.4%
τ -Fluvalinate	102851-06-9	Dr. Ehrenstofer	94%
Deltamethrin	52918-63-5	Sigma-Aldrich	99.7%
Chlorpyrifos-methyl-d6		Dr. Ehrenstofer	97.5%
Deltamethrin-d5	1217633-23-2	Dr. Ehrenstofer	97%
trans-Cypermethrin-d6	82523-65-7	Dr. Ehrenstofer	98.5%
Fenvalerate-d7		Dr. Ehrenstofer	99%
Etofenprox-d5		Dr. Ehrenstofer	98%
Bifenthrin-d5		Dr. Ehrenstofer	95%
Atrazine-d5	163165-75-1	CIL Cambridge Isotope Laboratories	98%

ESM.B Selection of Internal Standards (ISTDs)

Table B-1: Selection of ISTDs based on relative recoveries of spiked surface water samples (spike level: 500 pg/L) for analytes for which no isotopically labeled ISTDs were available. Besides relative recoveries, the selection of ISTDs was based on the R² of the calibration curve and the standard deviation (relSD) of the spiked replicate samples (relative recovery Spike_1-3 500 pg/L). The ISTD that was chosen for the formation of peak area ratios between analyte and ISTD is marked in green.

Target Analyte	ISTD	R ²	Weight	relative recovery Spike_1 500 pg/L	relative recovery Spike_2 500 pg/L	relative recovery Spike_3 500 pg/L	relative recovery mean	relSD
Empenthrin	Chlorpyrifos-methyl-d6	0.999	1/X	106	115	108	109.7	4.3
Empenthrin	trans-Cypermethrin-d6	0.997	1/X	150	124	139	137.7	9.5
Empenthrin	Deltamethrin-d5	0.994	1/X	133	134	160	142.3	10.8
Empenthrin	Fenvalerate-d7	0.998	1/X	167	134	159	153.3	11.2
Empenthrin	Etofenprox-d5	0.993	1/X	112	125	151	129.3	15.4
Empenthrin	Bifenthrin-d5	0.985	1/X	177	108	160	148.3	24.2
λ-Cyhalothrin	Fenvalerate-d7	0.992	1/X	96	90	88	91.3	4.6
λ-Cyhalothrin	trans-Cypermethrin-d6	0.995	1/X	88	85	78	83.7	6.1
λ-Cyhalothrin	Deltamethrin-d5	0.997	1/X	81	94	93	89.3	8.1
λ-Cyhalothrin	Etofenprox-d5	0.971	1/X	59	76	76	70.3	14
λ-Cyhalothrin	Chlorpyrifos-methyl-d6	0.993	1/X	63	80	62	68.3	14.8
λ-Cyhalothrin	Bifenthrin-d5	0.948	1/X	126	89	109	108	17.1
Tefluthrin	Fenvalerate-d7	0.934	1/X	111	109	110	110	0.9
Tefluthrin	trans-Cypermethrin-d6	0.943	1/X	99	101	97	99	2
Tefluthrin	Deltamethrin-d5	0.935	1/X	88	109	112	103	12.7
Tefluthrin	Bifenthrin-d5	0.870	1/X	114	85	108	102.3	15
Tefluthrin	Chlorpyrifos-methyl-d6	0.923	1/X	69	93	75	79	15.8
Tefluthrin	Etofenprox-d5	0.899	1/X	73	99	103	91.7	17.8
Chlorpyrifos-methyl	Chlorpyrifos-methyl-d6	0.998	1/X	99	106	96	100.3	5.1
Chlorpyrifos-methyl	Deltamethrin-d5	0.996	1/X	127	125	145	132.3	8.3
Chlorpyrifos-methyl	trans-Cypermethrin-d6	0.998	1/X	138	113	122	124.3	10.2
Chlorpyrifos-methyl	Fenvalerate-d7	0.999	1/X	152	120	137	136.3	11.7
Chlorpyrifos-methyl	Etofenprox-d5	0.991	1/X	93	102	119	104.7	12.6
Chlorpyrifos-methyl	Bifenthrin-d5	0.946	1/X	197	118	168	161	24.8

Target Analyte	ISTD	R ²	Weight	relative recovery Spike_1 500 pg/L	relative recovery Spike_2 500 pg/L	relative recovery Spike_3 500 pg/L	relative recovery mean	relSD
Chlorpyrifos	Deltamethrin-d5	0.991	1/X	114	134	99	115.7	15.2
Chlorpyrifos	Etofenprox-d5	0.961	1/X	78	105	82	88.3	16.5
Chlorpyrifos	Fenvalerate-d7	0.985	1/X	145	129	96	123.3	20.3
Chlorpyrifos	trans-Cypermethrin-d6	0.988	1/X	130	124	84	112.7	22.2
Chlorpyrifos	Bifenthrin-d5	0.963	1/X	184	117	117	139.3	27.8
Chlorpyrifos	Chlorpyrifos-methyl-d6	0.991	1/X	86	120	66	90.7	30.1
Allethrin¹⁾	Chlorpyrifos-methyl-d6	0.933	1/X	151	128	172	150.3	14.6
Bifenthrin	Bifenthrin-d5	0.978	1/X	133	139	132	134.7	2.8
Bifenthrin	Fenvalerate-d7	0.960	1/X	99	136	103	112.7	18
Bifenthrin	trans-Cypermethrin-d6	0.964	1/X	90	128	92	103.3	20.7
Bifenthrin	Deltamethrin-d5	0.970	1/X	83	142	110	111.7	26.4
Bifenthrin	Etofenprox-d5	0.935	1/X	60	114	89	87.7	30.8
Bifenthrin	Chlorpyrifos-methyl-d6	0.967	1/X	64	121	73	86	35.6
Tetramethrin¹⁾	Chlorpyrifos-methyl-d6	0.825	1/X	65	79	144	96	43.9
Tetramethrin	trans-Cypermethrin-d6	0.789	1/X	102	93	204	133	46.4
Tetramethrin	Fenvalerate-d7	0.793	1/X	113	101	232	148.7	48.7
Phenothrin	Fenvalerate-d7	0.982	1/X	117	123	105	115	8
Phenothrin	trans-Cypermethrin-d6	0.985	1/X	106	116	93	105	11
Phenothrin	Bifenthrin-d5	0.976	1/X	155	124	132	137	11.7
Phenothrin	Deltamethrin-d5	0.989	1/X	98	129	111	112.7	13.8
Phenothrin	Etofenprox-d5	0.961	1/X	71	104	90	88.3	18.8
Phenothrin	Chlorpyrifos-methyl-d6	0.987	1/X	76	109	74	86.3	22.8
Acrinathrin	Deltamethrin-d5	0.984	1/X	82	86	78	82	4.9
Acrinathrin	Etofenprox-d5	0.993	1/X	61	70	65	65.3	6.9
Acrinathrin	Fenvalerate-d7	0.990	1/X	99	82	74	85	15
Acrinathrin	trans-Cypermethrin-d6	0.987	1/X	90	77	66	77.7	15.5
Acrinathrin	Chlorpyrifos-methyl-d6	0.980	1/X	64	73	52	63	16.7
Acrinathrin	Bifenthrin-d5	0.894	1/X	126	80	90	98.7	24.5

Target Analyte	ISTD	R ²	Weight	relative recovery Spike_1 500 pg/L	relative recovery Spike_2 500 pg/L	relative recovery Spike_3 500 pg/L	relative recovery mean	relSD
Permethrin	Deltamethrin-d5	0.982	1/X	104	105	108	105.7	2
Permethrin	Etofenprox-d5	0.958	1/X	76	85	88	83	7.5
Permethrin	Chlorpyrifos-methyl-d6	0.980	1/X	81	90	72	81	11.1
Permethrin	trans-Cypermethrin-d6	0.977	1/X	113	95	90	99.3	12.2
Permethrin	Fenvalerate-d7	0.975	1/X	124	101	101	108.7	12.2
Permethrin	Bifenthrin-d5	0.985	1/X	166	102	129	132.3	24.3
α-Cypermethrin	Bifenthrin-d5	0.954	1/X	111	102	111	108	4.8
α -Cypermethrin	Fenvalerate-d7	0.989	1/X	85	103	90	92.7	10
α -Cypermethrin	trans-Cypermethrin-d6	0.992	1/X	77	97	80	84.7	12.7
α -Cypermethrin	Deltamethrin-d5	0.995	1/X	71	107	95	91	20.1
α -Cypermethrin	Etofenprox-d5	0.964	1/X	52	86	77	71.7	24.6
α -Cypermethrin	Chlorpyrifos-methyl-d6	0.993	1/X	55	91	63	69.7	27.1
Etofenprox	Deltamethrin-d5	0.991	1/X	134	140	138	137.3	2.2
Etofenprox	Etofenprox-d5	0.997	1/X	99	115	115	109.7	8.4
Etofenprox	Fenvalerate-d7	0.996	1/X	161	134	131	142	11.6
Etofenprox	trans-Cypermethrin-d6	0.993	1/X	146	126	116	129.3	11.8
Etofenprox	Chlorpyrifos-methyl-d6	0.994	1/X	105	119	92	105.3	12.8
Etofenprox	Bifenthrin-d5	0.941	1/X	208	132	161	167	23
Esfenvalerate	Fenvalerate-d7	0.999	1/X	98	104	104	102	3.4
Esfenvalerate	trans-Cypermethrin-d6	0.999	1/X	89	98	93	93.3	4.8
Esfenvalerate	Bifenthrin-d5	0.945	1/X	126	102	128	118.7	12.2
Esfenvalerate	Deltamethrin-d5	0.999	1/X	81	108	110	99.7	16.2
Esfenvalerate	Chlorpyrifos-methyl-d6	0.997	1/X	63	92	73	76	19.4
Esfenvalerate	Etofenprox-d5	0.987	1/X	59	88	90	79	22

Target Analyte	ISTD	R ²	Weight	relative recovery Spike_1 500 pg/L	relative recovery Spike_2 500 pg/L	relative recovery Spike_3 500 pg/L	relative recovery mean	relSD
τ-Fluvalinate	Fenvalerate-d7	0.995	1/X	90	101	88	93	7.5
τ-Fluvalinate	Bifenthrin-d5	0.949	1/X	94	80	87	87	8
τ-Fluvalinate	trans-Cypermethrin-d6	0.997	1/X	80	93	77	83.3	10.2
τ-Fluvalinate	Deltamethrin-d5	0.997	1/X	72	101	89	87.3	16.7
τ-Fluvalinate	Etofenprox-d5	0.980	1/X	60	93	83	78.7	21.5
τ-Fluvalinate	Chlorpyrifos-methyl-d6	0.986	1/X	56	86	60	67.3	24.2
Deltamethrin	Deltamethrin-d5	0.998	1/X	99	102	97	99.3	2.5
Deltamethrin	Etofenprox-d5	0.972	1/X	72	83	79	78	7.1
Deltamethrin	Fenvalerate-d7	0.992	1/X	118	98	92	102.7	13.3
Deltamethrin	trans-Cypermethrin-d6	0.995	1/X	108	92	82	94	14
Deltamethrin	Chlorpyrifos-methyl-d6	0.995	1/X	77	87	65	76.3	14.4
Deltamethrin	Bifenthrin-d5	0.954	1/X	155	97	114	122	24.4

¹⁾ For the more polar pyrethroids allethrin, tetramethrin and imiprothrin no linear calibration curves were achieved no matter which ISTD was used. To determine absolute recoveries_{LLE} for allethrin, tetramethrin and imiprothrin, peak area ratios were formed with atrazine-d5 ($\log K_{ow} = 2.6$; Chemistry Dashboard, U.S. Environmental Protection Agency (<https://comptox.epa.gov/dashboard>)).

ESM.C Analyte Protection Mix

Table C-1: Compounds that are contained in the Analyte Protection Mix.

Compound	CAS number	Supplier	Final concentration in methanol / solvent mixture
D-sorbitol	50-70-4	Sigma Aldrich	10 mg/mL
3-ethoxy-1,2-propanediol	1874-62-0	Sigma Aldrich	100 mg/mL
D-(+)-gluconic acid lactone	90-80-2	Sigma Aldrich	10 mg/mL
D-(+)-ribonic acid γ -lactone	5336-08-3	Sigma Aldrich	10 mg/mL

The final solution was diluted 1:5 (v/v) and 30 μ L of Analyte Protection Mix were employed per 1 mL of solvent.

Optimization of the Source Parameters

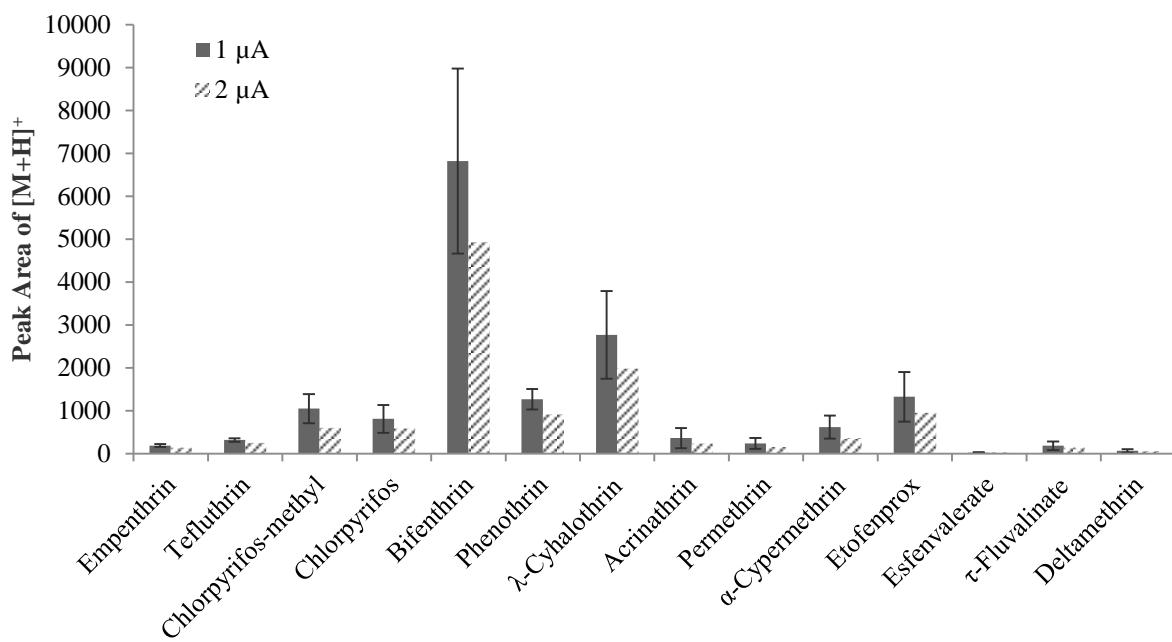


Figure D-1: Effect of different APCI corona discharge currents (1 and 2 μ A) on the analyte peak areas of $[M+H]^+$ in an externally prepared calibration standard (0.1 ng/mL) during MRM acquisition. Sample replicates n = 2 (1 μ A) and n=1 (2 μ A). For bifenthrin, permethrin and etofenprox fragments were used as precursor ions.

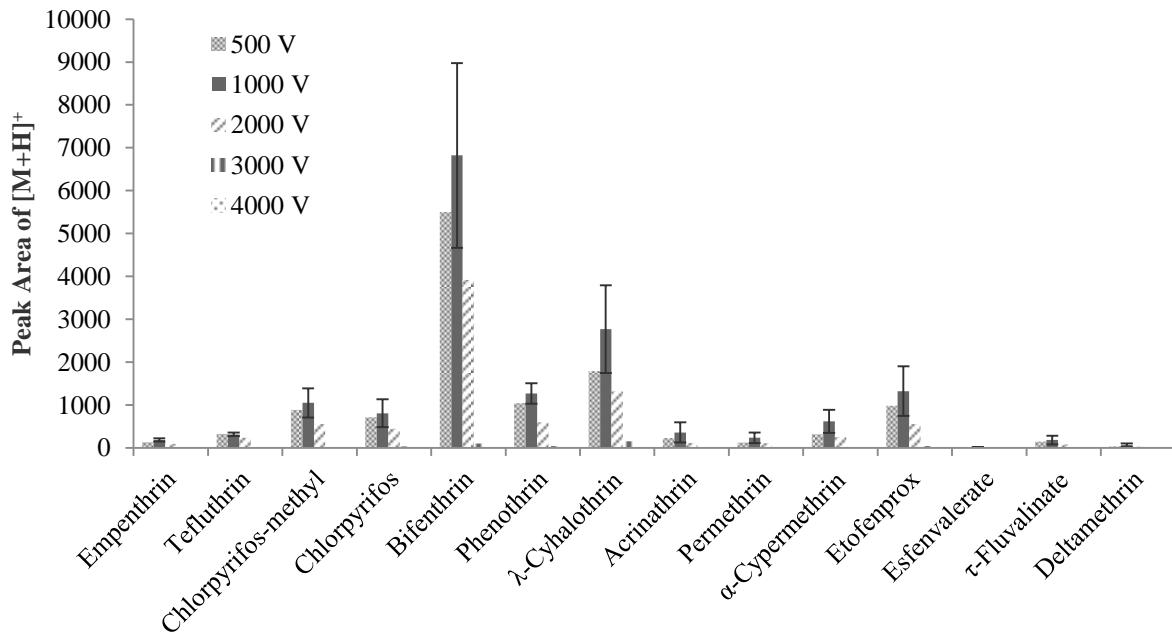


Figure D-2: Effects of different capillary voltages (500, 1000, 2000, 3000 and 4000 V) on the analyte peak areas of $[M+H]^+$ in an externally prepared calibration standard (0.1 ng/mL) during MRM acquisition. Sample replicates n = 2 (1000 V) and n=1 (500, 2000, 3000 and 4000 V). For bifenthrin, permethrin and etofenprox fragments were used as precursor ions.

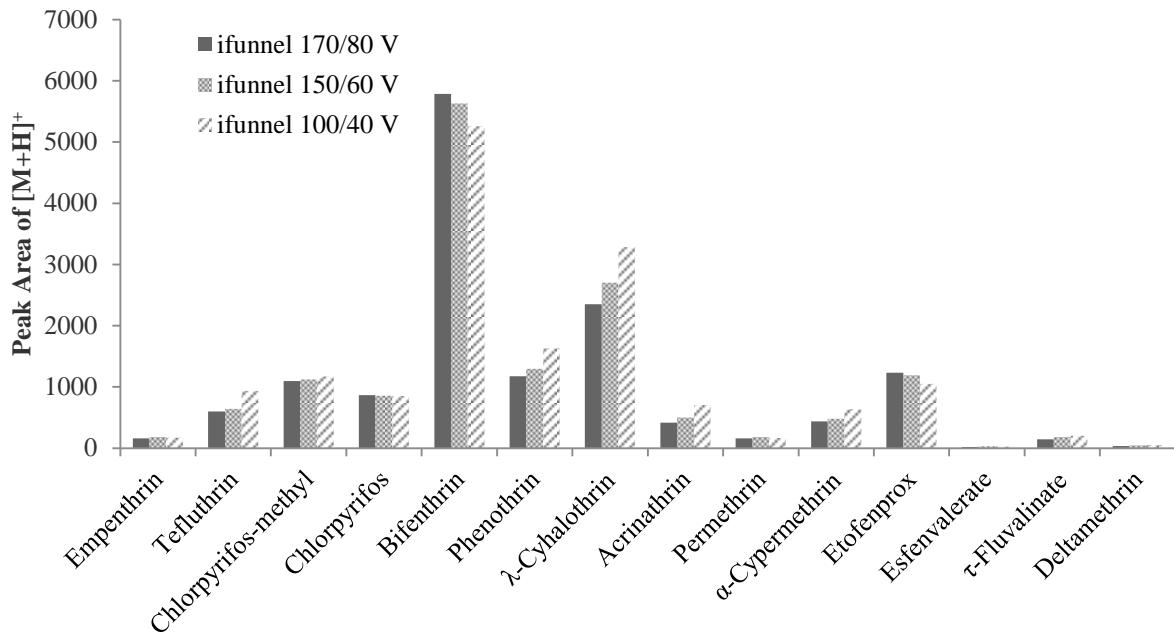


Figure D-3: Effects of different ifunnel parameters (170/80, 150/60 and 100/40 V positive high pressure RF/positive low pressure RF) on the analyte peak areas of $[M+H]^+$ in an externally prepared calibration standard (0.1 ng/mL) during MRM acquisition. Sample replicates n = 1. For bifenthrin, permethrin and etofenprox fragments were used as precursor ions.

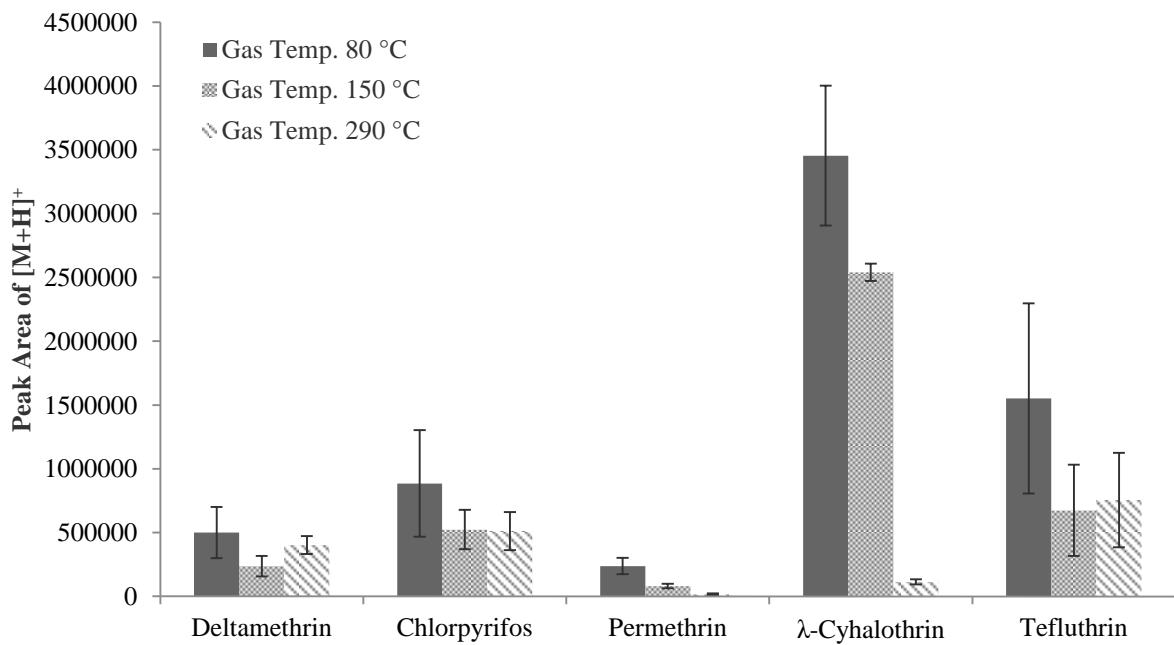


Figure D-4: Effects of different gas temperatures (80, 150 and 290 °C) on the analyte peak areas of $[M+H]^+$ in an externally prepared calibration standard (10 ng/mL) exemplary shown for deltamethrin, chlorpyrifos, permethrin, λ -cyhalothrin and tefluthrin during full scan acquisition. 80 °C was discarded due to condensation of water used as modifier in the source and due to risk of corrosion. Sample replicates n = 3. For permethrin no $[M+H]^+$ was formed and a fragment was scanned.

ESM.E Positioning of the Ion Transfer Capillary

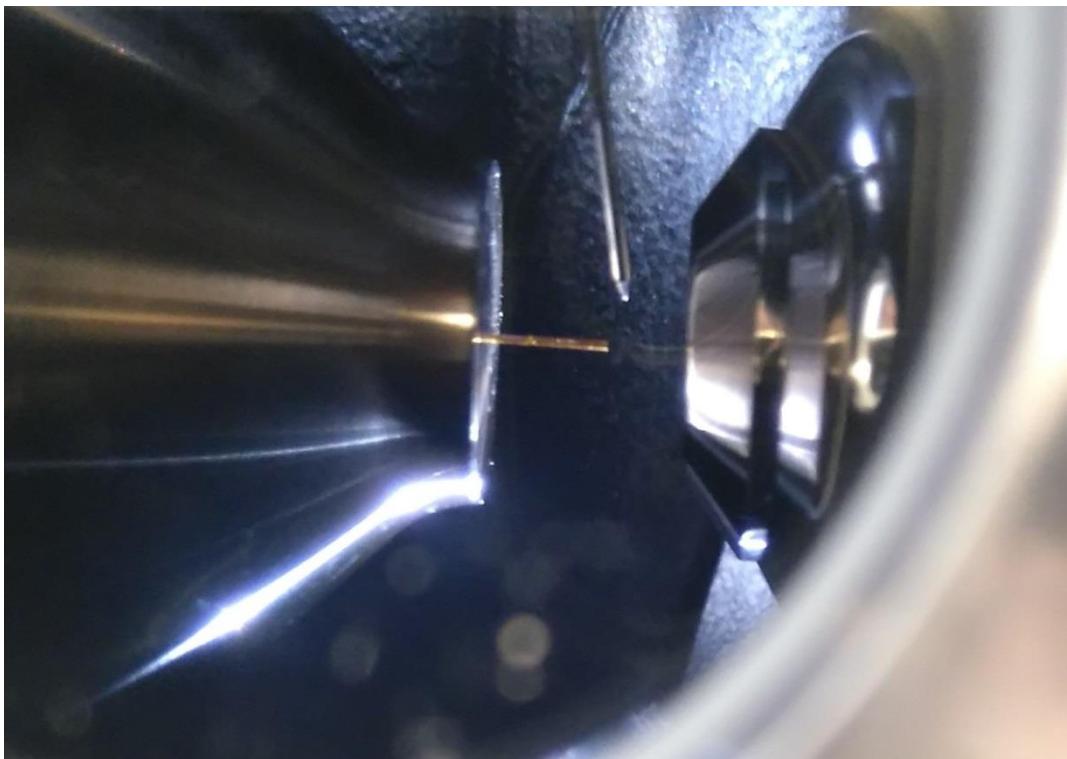


Figure E-1: Ideal positioning of (i) the ion transfer capillary to the mass spectrometer inlet and (ii) the corona discharge needle.

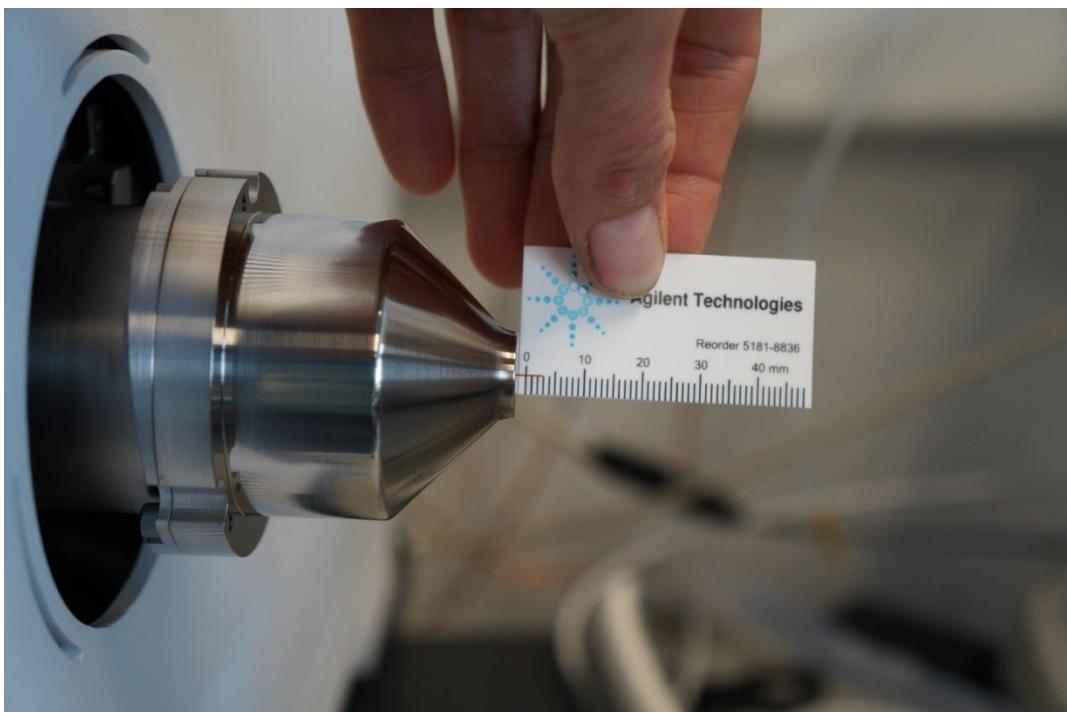


Figure E-2: Ideal positioning of the ion transfer capillary.

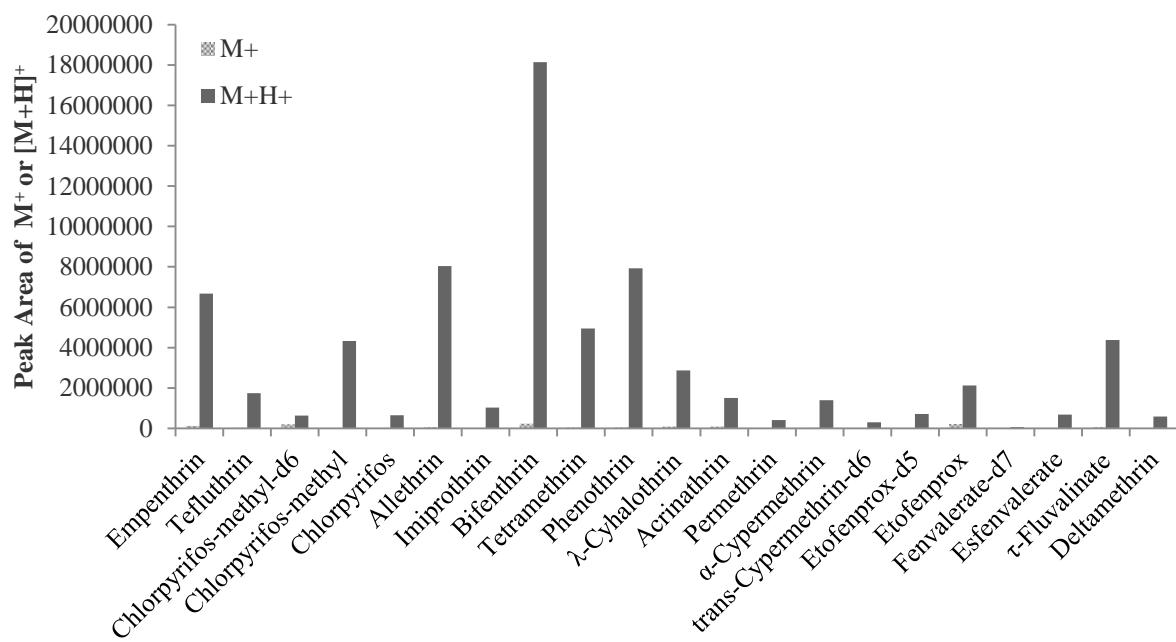


Figure F-1: Comparison of the peak areas of M^+ and $[M+H]^+$ of analytes and ISTDs in an externally prepared calibration standard (1000 ng/mL) during full scan acquisition. Water (1% formic acid) was used as modifier in the ionization source. Sample replicates n=1. For bifenthrin, permethrin and etofenprox no $[M+H]^+$ were formed and fragments were scanned.

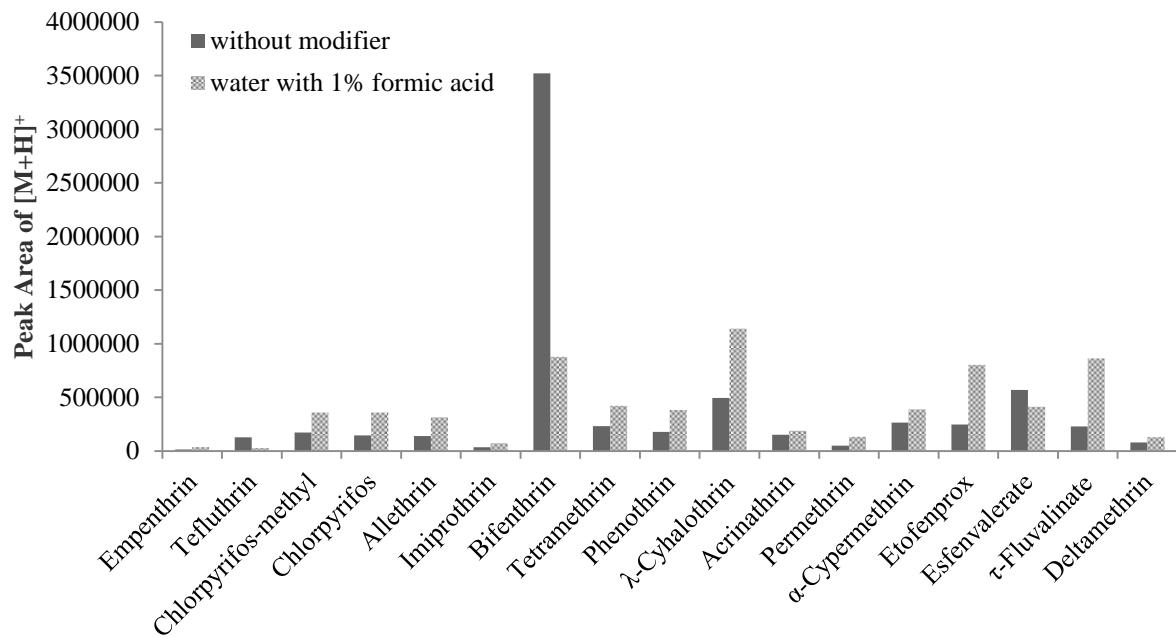


Figure F-2: Effect of water (1% formic acid), which was used as a modifier in the ionization source, on the analyte peak areas of $[M+H]^+$ in an externally prepared calibration standard (500 ng/mL) in the presence and absence of the modifier during full scan acquisition. Sample replicates n=1. For bifenthrin, permethrin and etofenprox no $[M+H]^+$ were formed and fragments were scanned.

ESM.G Blank Samples

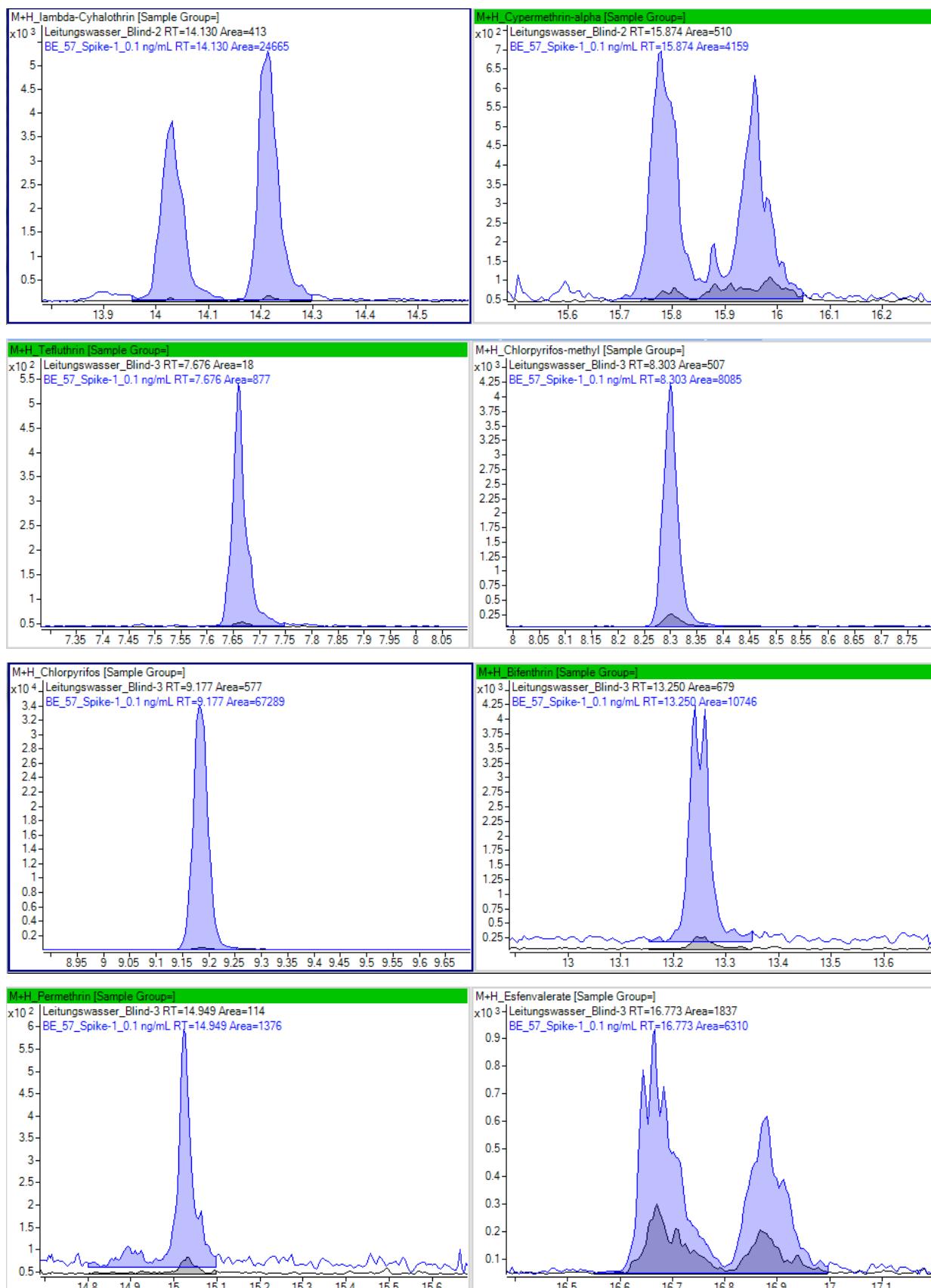


Figure G-1: MRM chromatograms comparing blank samples (grey, filled) and surface water samples (blue, filled) spiked to a concentration of 25 pg/L. Displayed are the quantifier transitions of all analytes which were detected in the blank samples that went through the whole sample preparation.

ESM.H Inter-day Relative Recoveries and Precisions

Table H-1: Inter-day relative recoveries and precisions determined within several months using surface water samples spiked to a concentration of 500 pg/L. Sample replicates n=3.

	inter-day recovery [%]	inter-day precision [%]
Empenthrin	91	18
Tefluthrin	90	8
Chlorpyrifos-methyl	93	7
Chlorpyrifos	95	5
Bifenthrin	101	32
Phenothrin	105	14
λ -Cyhalothrin	86	9
Acrinathrin	85	21
Permethrin	108	8
α -Cypermethrin	95	9
Etofenprox	104	13
Esfenvalerate	101	20
τ -Fluvalinate	95	2
Deltamethrin	108	7

ESM.I Intra-day Relative Recoveries and Precisions around the Method Limit of Quantification

Table I-1: Intra-day relative recoveries and precisions around the method limit of quantification. Surface water samples were spiked in duplicate to a concentration of 25 pg/L.

	intra-day recovery [%]	intra-day precision [%]
Empenthrin	- ^{*)}	- ^{*)}
Tefluthrin	89	4
Chlorpyrifos-methyl	118	21
Chlorpyrifos	- ^{**)}	- ^{**)}
Bifenthrin	107	14
Phenothrin	106	5
λ -Cyhalothrin	117	17
Acrinathrin	91	11
Permethrin	- ^{***)}	- ^{***)}
α -Cypermethrin	101	14
Etofenprox	95	8
Esfenvalerate	90	3
τ -Fluvalinate	95	5
Deltamethrin	85	8

^{*)} not determined

^{**) spike level too low compared to the chlorpyrifos concentration already present in the surface water samples}

^{***)} spike level < method limit of quantification

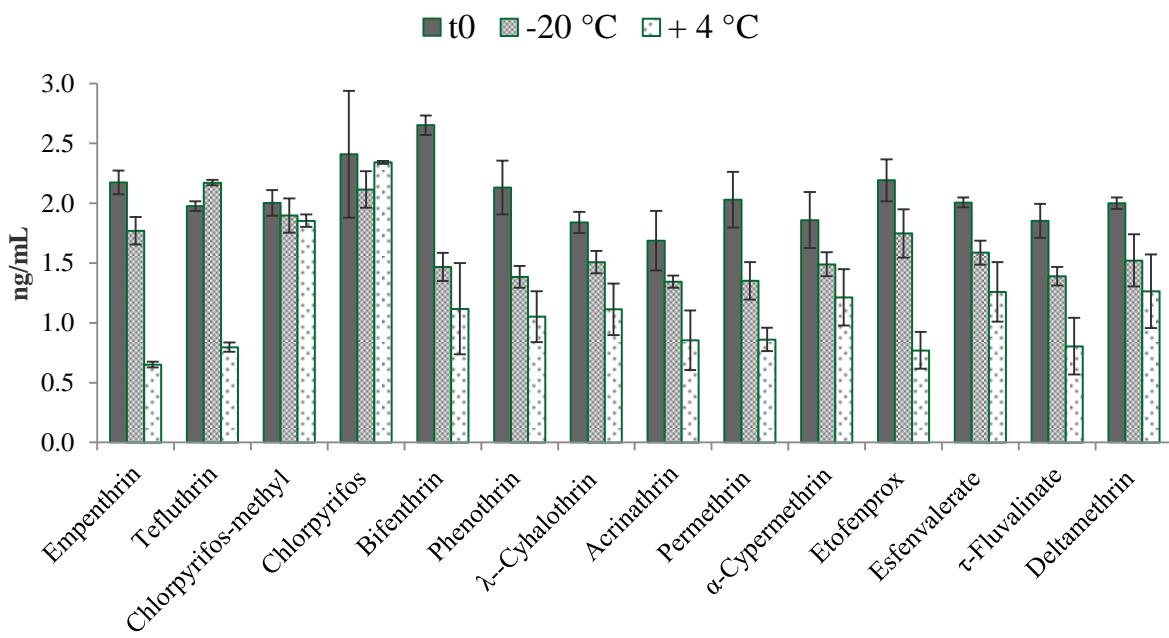


Figure J-1: Surface water spiked to a concentration of 500 pg/L (corresponding to 2 ng/mL in the vial with an enrichment factor of 4000) which was directly analyzed (dark grey), stored at -20 °C in the dark for one week (pattern fill grey) or stored at +4 °C in the dark for one week (white with grey dots). Sample replicates n=3.