Supplementary Information for

Analysis of Highly Polar Anionic Pesticides in Food of Plant and Animal Origin by Ion Chromatography and Tandem-Mass Spectrometry with emphasis on addressing adverse Effects caused by Matrix Co-extractives

in *Analytical and Bioanalytical Chemistry*

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Table S1: *Brief comparison of the presented method in this manuscript to other IC-MS/MS methods [1-5].*

	Method presented here	**Adams et al. [1]**	**Raijski et al. [2]**	**Bauer et al. I [3]**	**Bauer et al. II [4]**	**Kurz et al. [5]**
Analyte scope	Glyphosate, AMPA, NAGly, glufosinate, MPPA, NAGlu, fosetyl, ethephon, HEPA, cyanuric acid, chlorate, perchlorate, phosphonic acid, bromide, trifluoroacetic acid	Glyphosate, AMPA, *N*-acetyl-AMPA, glufosinate, MPPA, NAGlu, fosetyl, ethephon, cyanuric acid, chlorate, perchlorate, phosphonic acid, clopyralid, bialaphos	Glyphosate, AMPA, *N*-acetyl-AMPA, NAGly, fosetyl, chlorate, perchlorate, phosphonic acid	Ethephon, HEPA, chlorate, perchlorate	Fosetyl, phosphonic acid	Glyphosate, AMPA, glufosinate, fosetyl, clopyralid
Use of IL-IS	all, except bromide	Glyphosate, glufosinate, MPPA, NAGlu, cyanuric acid, chlorate, perchlorate	NAGly	Ethephon, chlorate, perchlorate	Phosphonic acid	-
Matrix scope	Plant and animal origin	Plant origin	Plant origin	Plant origin	Plant origin	Water
IC column and elution	AS19 and AS24, hydroxide elution	AS19, hydroxide elution	AS19 and AS11, hydroxide elution	Metrosep A, bicarbonate in water/ACN elution	Metrosep A, bicarbonate in water/ACN elution	AS24, hydroxide elution
Chromato-graphy system	IC with suppressor	IC with suppressor	IC with suppressor	LC without suppressor	LC without suppressor	IC with suppressor
MS detection	Triplequad	Triplequad	Orbitrap	Triplequad	Triplequad	Triplequad
Make-up solvent	ACN at 1:2 of IC-flow	ACN at 1:2.75 of IC-flow	ACN at 1:1 of IC-flow	-	-	2-propanol at 1:3 of IC-flow

Table S2: *IL-IS used, concentration in spiking solutions, of which 100 µL were added prior to extraction in validation experiments, concentration in 10 g and 5 g sample portion and measured mass transitions in MS/MS, see also [1].*

Compound	Concentration in spiking solutions in µg/mL	Concentration in 10 g sample portion in mg/kg	Concentration in 5 g sample portion in mg/kg	Mass transition
Glyphosate $^{13}C_2$ ^{15}N	20	0.2	0.4	*m/z* 171 → 63
AMPA ^{13}C ^{15}N	40	0.4	0.8	*m/z* 112 → 63
***N*-Acetyl-Glyphosate (NAGly) $^{13}C_2$ ^{15}N**	20	0.2	0.4	*m/z* 213 → 63
Fosetyl D_5	20	0.2	0.4	*m/z* 114 → 82
Ethephon D_4	20	0.2	0.4	*m/z* 147 → 111
HEPA D_4	20	0.2	0.4	*m/z* 129 → 79
Glufosinate D_3	20	0.2	0.4	*m/z* 183 → 63
MPPA D_3	20	0.2	0.4	*m/z* 154 → 63
***N*-Acetyl-Glufosinate (NAGlu) D_3**	20	0.2	0.4	*m/z* 225 → 63
Cyanuric acid $^{13}C_3$	20	0.2	0.4	*m/z* 131 → 43
Chlorate $^{18}O_3$	20	0.2	0.4	*m/z* 89 → 71
Perchlorate $^{18}O_4$	20	0.2	0.4	*m/z* 107 → 89
Phosphonic acid $^{18}O_3$	20	0.2	0.4	*m/z* 87 → 85
Trifluoroacetic acid $^{13}C_2$	10	0.1	0.2	*m/z* 115 → 70

Table S3: *IC-MS/MS and LC-MS/MS conditions employed in this study.*

IC-MS/MS conditions [1,2]		
Column/Pre-column	Thermo Scientific Dionex IonPac AS19 2x 250 mm with AG19 2x 50 mm and AS24 2x 250 mm with AG24 2x 50mm	
Potassium hydroxide (KOH) gradient for separation	**Time**	**Molarity of KOH in mmol/L**
	0	15
	8	15
	13	36
	21	36
	21.5	70
	25	70
	25.5	15
	30	15
Flow rate (IC)	0.3 mL/min	
Injection volume	5 µL of 5-fold diluted extracts in ultrapure water	
Eluent(source)	Thermo Scientific Dionex EGC 500 KOH eluent generator cartridge	
Suppressor	Dionex ASRS 300; 2mm	
Temperature	Column oven: 32 °C, Suppressor: 15 °C	
Flow rate (of make-up solvent)	0.15 mL/min ACN (MS-grade)	
Ion source	ESI Turbo V ion source, negative mode	
Curtain gas (nitrogen) pressure	30 psi	
Ion Spray Voltage	-4500 V	
Gas supply	Gas 1 (nebulizer): 60 psi; Gas 2 (heater): 60 psi	
Temperature of Gas 2	600 °C	
LC-MS/MS Conditions HILIC/Torus: Method M1.6 of QuPPe-PO-Method [1]		
LC-MS/MS Conditions PGC/Hypercarb: Method M1.3 of QuPPe-PO-Method [1]		

Table S4: *Mass transitions of the analytes recorded with MS/MS, see also [1].*

Compound	Mass Transition 1 (target)	Mass Transition 2	Mass Transition 3
Glyphosate	*m/z* 168 → 63	*m/z* 168 → 150	*m/z* 168 → 124
AMPA	*m/z* 110 → 63	*m/z* 110 → 79	*m/z* 110 → 81
NAGly	*m/z* 210 → 63	*m/z* 210 → 150	*m/z* 210 → 124
Fosetyl	*m/z* 109 → 81	*m/z* 109 → 63	*m/z* 109 → 79
Ethephon	*m/z* 143 → 107	*m/z* 145 → 107	*m/z* 143 → 79
HEPA	*m/z* 125 → 63	*m/z* 125 → 95	*m/z* 125 → 107
Glufosinate	*m/z* 180 → 63	*m/z* 180 → 95	*m/z* 180 → 85
MPPA	*m/z* 151 → 63	*m/z* 151 → 133	*m/z* 151 → 107
NAGlu	*m/z* 222 → 63	*m/z* 222 → 136	*m/z* 222 → 59
Cyanuric acid	*m/z* 128 → 42	*m/z* 128 → 85	
Chlorate	*m/z* 83 → 67	*m/z* 85 → 69	*m/z* 85 → 67
Perchlorate	*m/z* 99 → 83	*m/z* 101 → 85	*m/z* 99 → 67
Bromide**	*m/z* 81 → 81	*m/z* 79 → 79	
Phosphonic acid	*m/z* 81 → 79	*m/z* 81 → 63	
Trifluoroacetic acid	*m/z* 113 → 69	*m/z* 113 → 113*	

*no other transition available

** high collision energy was used [1]

Table S5: *Overview of retention times and contents of exemplary anionic or potentially anionic matrix components in lemon, soybean, rhubarb, Swiss chard and cucumber according to literature [3] in mg/100g and concentrations in undiluted QuPPe extracts in mg/mL (assuming quantitative extraction). Values were calculated for 10 g sample, except for soybean, where 5 g were used.*

Matrix component	Retention time*	Lemon		Soybean		Rhubarb	
		Literature [mg/100g]	QuPPe extract [mg/mL]	Literature [mg/100g]	QuPPe extract** [mg/mL]	Literature [mg/100g]	QuPPe extract [mg/mL]
Citric acid	25.3	4683	23.4			130	0.7
Malic acid	15.5	200	1.0			1250	6.3
Phosphate	19.8			550	1.4	22	0.1
Nitrate	10.9					215	1.1
Chloride	6.1					60	0.3
Sulfate	14.5						
Oxalic acid	16.1					270	1.4

Matrix component	Retention time*	Swiss chard		Cucumber	
		Literature [mg/100g]	QuPPe extract [mg/mL]	Literature [mg/100g]	QuPPe extract [mg/mL]
Citric acid	25.3			20	0.1
Malic acid	15.5			240	1.2
Phosphate	19.8	39	0.2	15	0.1
Nitrate	10.9	487	2.4	19	0.1
Chloride	6.1			37	0.2
Sulfate	14.5				
Oxalic acid	16.1				

*Using ion chromatography on a AS19 column, and detection via conductivity

** using 5 g analytical portion

Table S6: *Full table of effect of the make-up solvents ACN, methanol, 2-propanol at ratios of ~1:4 to ~5:4 compared to a constant IC flow rate of 0.3 mL/min. The % value of the peak areas were compared with injections in pure water (no make-up solvent), which was set at 100%. Each measurement was performed in triplicate and mean values (and relative standard deviation (RSD) in brackets) were given for the most prominent MS/MS transitions. The best values are highlighted in bold letters.*

Compound	**Solvent**	**Flow-rate of make-up solvent (external pump)***				
		0.08 mL	**0.15 mL**	**0.23 mL**	**0.3 mL**	**0.38 mL**
		Make-up solvent flow-rate compared to IC effluent flow-rate (1:1 = equal)				
		~1:4	**2:4 (1:2)**	**~3:4**	**4:4 (1:1)**	**~5:4**
		Share of make-up solvent on total flow after admixture				
		20%	**33%**	**43%**	**50%**	**56%**
		Normalized peak areas (no make-up solvent set at 100%); RSD in % in brackets				
Glyphosate	Acetonitrile	**189%** (7%)	**180%** (6%)	**197%** (5%)	175% (1%)	162% (3%)
	Methanol	**153%** (5%)	123% (5%)	103% (2%)	86% (7%)	71% (7%)
	2-Propanol	100% (19%)	**150%** (10%)	86% (1%)	87% (3%)	79% (4%)
AMPA	Acetonitrile	**196%** (4%)	168% (3%)	**201%** (4%)	**186%** (5%)	175% (6%)
	Methanol	**144%** (4%)	117% (4%)	107% (5%)	86% (1%)	65% (15%)
	2-Propanol	105% (13%)	**131%** (5%)	98% (14%)	88% (9%)	100% (7%)
NAGly	Acetonitrile	**223%** (5%)	169% (3%)	181% (12%)	138% (2%)	146% (7%)
	Methanol	**236%** (7%)	**232%** (3%)	**248%** (9%)	201% (12%)	152% (11%)
	2-Propanol	112% (20%)	**182%** (11%)	124% (10%)	136% (6%)	125% (8%)
Fosetyl	Acetonitrile	206% (5%)	**224%** (3%)	**236%** (3%)	**225%** (8%)	**216%** (5%)
	Methanol	**150%** (7%)	**156%** (1%)	**163%** (1%)	**152%** (1%)	132% (4%)
	2-Propanol	55% (29%)	76% (21%)	95% (19%)	**114%** (12%)	**109%** (18%)
Ethephon	Acetonitrile	**181%** (3%)	**179%** (2%)	**177%** (1%)	**167%** (3%)	146% (0.1%)
	Methanol	136% (17%)	**163%** (2%)	**161%** (2%)	**153%** (2%)	114% (8%)
	2-Propanol	97% (20%)	**189%** (8%)	118% (7%)	125% (14%)	101% (11%)
HEPA	Acetonitrile	**170%** (2%)	**169%** (4%)	**180%** (4%)	**175%** (2%)	160% (3%)
	Methanol	139% (5%)	**151%** (4%)	133% (4%)	117% (4%)	99% (6%)
	2-Propanol	105% (17%)	**182%** (10%)	136% (4%)	144% (9%)	132% (10%)
Glufosinate	Acetonitrile	**146%** (4%)	113% (2%)	116% (6%)	96% (4%)	84% (12%)
	Methanol	**126%** (5%)	105% (4%)	103% (7%)	87% (7%)	68% (13%)
	2-Propanol	101% (19%)	**158%** (10%)	127% (8%)	133% (2%)	134% (6%)
MPPA	Acetonitrile	**164%** (6%)	**153%** (3%)	**161%** (4%)	**147%** (2%)	141% (7%)
	Methanol	121% (9%)	**134%** (4%)	**136%** (3%)	123% (2%)	103% (7%)
	2-Propanol	93% (18%)	**155%** (9%)	91% (3%)	94% (11%)	82% (9%)

NAGlu	Acetonitrile	**216%** (5%)	141% (4%)	136% (9%)	112% (10%)	99% (16%)
	Methanol	208% (6%)	267% 0.2%)	**380%** (6%)	**346%** (9%)	263% (8%)
	2-Propanol	118% (13%)	**183%** (11%)	147% (2%)	154% (5%)	147% (7%)
Chlorate	Acetonitrile	**200%** (8%)	**194%** (4%)	**209%** (2%)	**209%** (4%)	**208%** (5%)
	Methanol	179% (7%)	216% (3%)	**276%** (3%)	**262%** (2%)	**249%** (3%)
	2-Propanol	124% (22%)	**239%** (5%)	197% (2%)	205% (8%)	184% (1%)
Perchlorate	Acetonitrile	**434%** (6%)	299% (14%)	251% (8%)	217% (5%)	219% (2%)
	Methanol	**339%** (6%)	**329%** (13%)	**326%** (13%)	218% (14%)	158% (9%)
	2-Propanol	169% (11%)	**241%** (2%)	178% (3%)	188% (6%)	179% (5%)
Phosphonic	Acetonitrile	**188%** 0.3%)	**186%** (1%)	**190%** (3%)	170% (5%)	158% (3%)
Acid	Methanol	**182%** (9%)	**190%** (4%)	169% (1%)	141% (3%)	114% (9%)
	2-Propanol	68% (18%)	89% (22%)	105% (16%)	**122%** (6%)	**114%** (15%)

* the flow-rate of AXP-MS pump was only adjustable to two decimals. Therefore, increments derived from the standard value of 0.3 mL (i.e. identical with the flow rate of the mobile phase) were rounded to two decimals)

Table S7: *Validation data of target transitions in strawberry and milk using IC-MS/MS in comparison to LC-MS/MS (PGC and HILIC) (validated levels do not represent lowest successfully validated levels). Recoveries were determined using external matrix-matched calibrations using matching isotopically labelled standards for each analyte.*

					IC (AS19)		**LC HILIC (Torus DEA)**		**LC PGC (Hypercarb)**	
Analyte	Mass transition	Matrix	Validated Level in mg/kg	No. of repli-cates	Average Recovery (%)	RSD (%)	Average Recovery (%)	RSD (%)	Average Recovery (%)	RSD (%)
AMPA	*m/z* 110 → 63	**Strawberry**	0.05	5	107	2.5	109	4.3	107	5.4
			0.1	5	102	2.1	101	1.8	98	5.1
Cyanuric acid	*m/z* 128 → 42		0.05	5	97	6.5	*	*	104	3.7
			0.1	5	101	8.3	*	*	98	4.0
Ethephon	*m/z* 143 → 107		0.01	5	95	12	99	9.5	99	7.6
			0.02	5	95	5.4	94	6.3	100	5.9
Fosetyl	*m/z* 109 → 81		0.01	5	98	3.4	107	1.8	102	4.2
			0.02	5	99	2.1	101	1.9	100	1.5
Glufosinate	*m/z* 180 → 63		0.03	5	101	3.7	99	9.7	102	0.9
			0.06	5	100	1.5	97	5.0	98	3.0
Glyphosate	*m/z* 168 → 63		0.05	5	98	8.8	99	3.7	103	2.1
			0.1	5	97	4.7	93	7.0	99	1.4
HEPA	*m/z* 125 → 63		0.02	5	104	15	110	5.0	67	20
			0.04	5	102	3.9	97	4.2	92	5.4
MPPA	*m/z* 151 → 63		0.02	5	97	5.6	102	8.6	102	3.1
			0.04	5	103	3.8	94	4.2	98	2.6
NAGlu	*m/z* 222 → 63		0.02	5	108	4.3	122	2.9	105	1.4
			0.04	5	100	1.5	99	6.7	100	1.3
NAGly	*m/z* 210 → 63		0.05	5	99	4.2	108	3.2	104	1.7
			0.1	5	103	5.1	100	2.0	101	0.9
AMPA	*m/z* 110 → 63	**Milk**	0.05	5	97	3.8	99	6.6	102	4.2
Cyanuric acid	*m/z* 128 → 42		0.05	5	98	12	*	*	111	8.5
Ethephon	*m/z* 143 → 107		0.01	5	94	5.9	107	7.3	***	***
Fosetyl	*m/z* 109 → 81		0.01	5	97	1.4	108	3.3	96	2.6
Glufosinate	*m/z* 180 → 63		0.03	5	96	2.8	92	5.9	100	5.7
Glyphosate	*m/z* 168 → 63		0.05	5	96	3.8	100	1.0	102	3.9
HEPA	*m/z* 125 → 63		0.02	5	100	7.1	97	7.4	106	8.7
MPPA	*m/z* 151 → 63		0.02	5	97	1.5	102	5.4	97	2.7
NAGlu	*m/z* 222 → 63		0.02	5	95	4.1	99	7.5	97	4.9
NAGly	*m/z* 210 → 63		0.05	5	97	3.6	101	6.9	95	5.9
Phosphonic acid	*m/z* 81 → 79		0.05	5	94	2.3	90	10	**	**
			0.1	5	101	1.8	93	11	**	**
Chlorate	*m/z* 85 → 51		0.03	5	86	6.0	72	13	**	**
			0.06	5	100	1.3	94	4.2	**	**
Perchlorate	*m/z* 101 → 85		0.01	5	102	5.2	**	**	**	**
			0.02	5	104	1.7	**	**	**	**
Bromide	*m/z* 79 → 79		5	5	98	3.8	85	3.7	**	**
			10	5	101	6.4	92	4.9	**	**
Trifluoracetic acid	*m/z* 113 → 69		0.025	5	103	21.8	***	***	*	*
			0.05	5	102	1.3	***	***	*	*

* not included in this method
** not measured
*** validation at that level not successful

Table S8: *Number of positive findings in routine analysis of incurred residues in market samples, using IC-MS/MS (AS19) and LC-MS/MS (Hypercarb) by analyte.*

Analyte	**IC-MS/MS**			**LC-MS/MS PGC (Hypercarb)**		
	LOQ [mg/kg]*	>LOQ	<LOQ***	LOQ [mg/kg]*	>LOQ	<LOQ***
AMPA	0.01	0	0	0.01	0	2
Cyanuric acid	0.05	8	0	0.005	31	11
Ethephon	0.01	2	0	0.01	1	3
Fosetyl	0.01	1	2	0.01	1	4
Glufosinate	0.01	0	3	0.01	0	0
Glyphosate	0.01	0	5	0.02	0	1
HEPA	0.01	0	6	0.01	2	0
MPPA	0.01	1	3	0.01	2	2
NAGlu	0.02	0	2	0.02	1	0
NAGly	0.02	0	0	0.02	0	0
Bromide**	5	1	21	5	0	19
Chlorate	0.005	27	1	0.01	15	20
Perchlorate	0.005	36	8	0.01	17	19
Phosphonic acid	0.01	76	13	0.05	32	17
Trifluoracetic acid	0.02	31	30	-	-	-

*the LOQ indicated here refers to fruits and vegetables and refers to the lowest successfully validated level
**the LOQ was arbitrarily set at 5 mg/kg due to the natural background levels of bromide; values "<LOQ" were in the range between >0.5 and <5 mg/kg
*** Identified analytes at semiquantitative levels (<LOQ, but typically not lower than 10%LOQ)

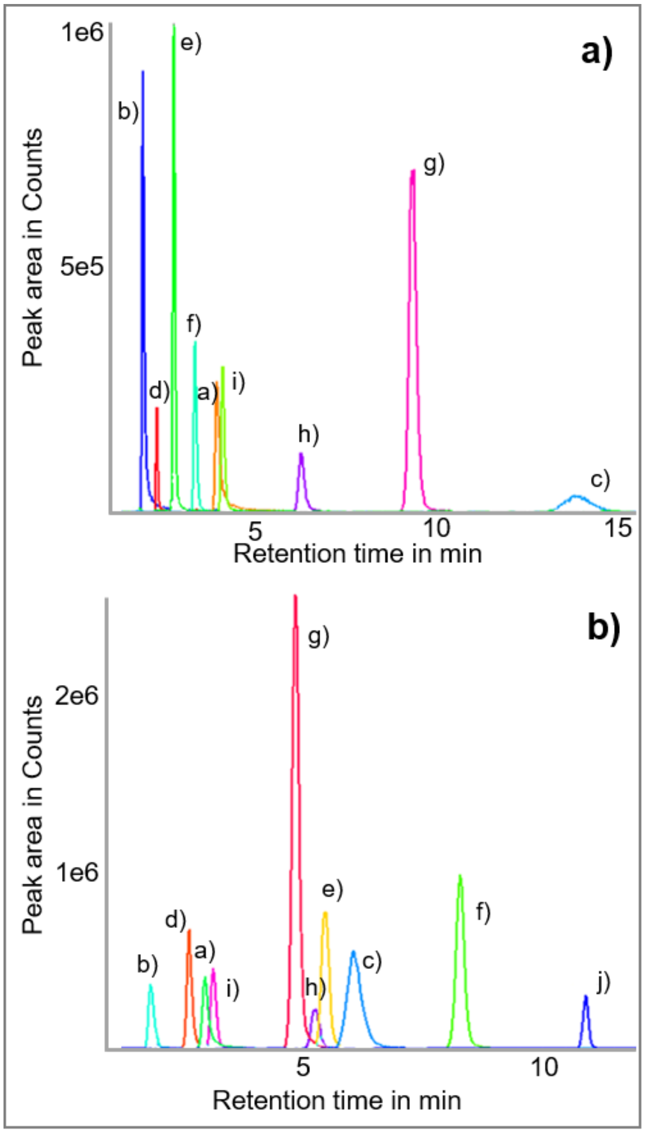

Fig. S1: *LC/MSMS chromatograms of ten pesticide standards* ***(a)*** *on a Torus DEA (HILIC) column and* ***(b****) a Hypercarb (PGC) column. Compounds: a) glyphosate, b) AMPA, c) NAGly, d) glufosinate, e) MPPA, f) NAGlu, g) fosetyl, h) ethephon, i) HEPA, j) cyanuric acid, each at 0.1 µg/mL in solvent*

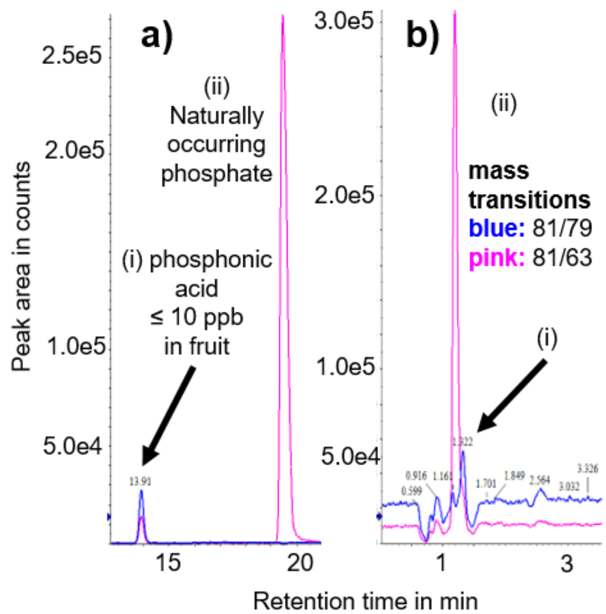

Fig. S2: (a) *IC-MS/MS (AS19; 5-fold diluted QuPPe extract) and (**b**) LC-MS/MS (PGC; 10-fold diluted QuPPe extract) chromatograms of phosphonic acid and phosphate in an extract of a fruit preparation for infants with phosphonic acid being contained at a level ~ 0.01 mg/kg and natural contents of phosphate. While the target transition of phosphonate (m/z 81 → 79) is largely unaffected by phosphate, the qualifier transition (m/z 81 → 63) is strongly interfered by phosphate and therefore useless in case of a partial co-elution of phosphate and phosphonate. The resolution on the PGC column can vary depending on the column condition. The particular example represents an extreme case, combining not only a very poor resolution, but also a very low phosphonate level and the presence of phosphate at very high levels*

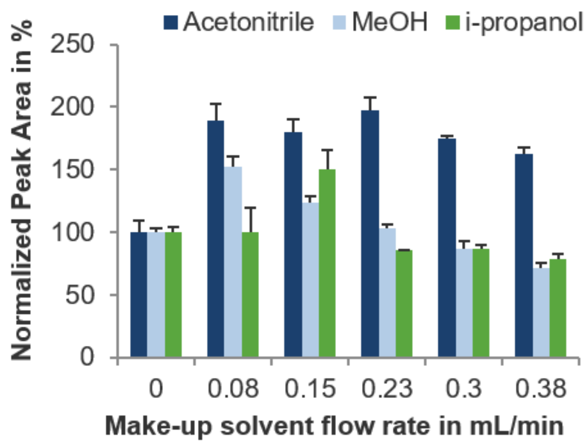

Fig. S3*: Average normalized peak areas (n=3) of glyphosate according to the applied flow rate of the make-up pump. Values measured without the make-up pump (flow rate 0 mL/min) function as reference and are set at 100%*

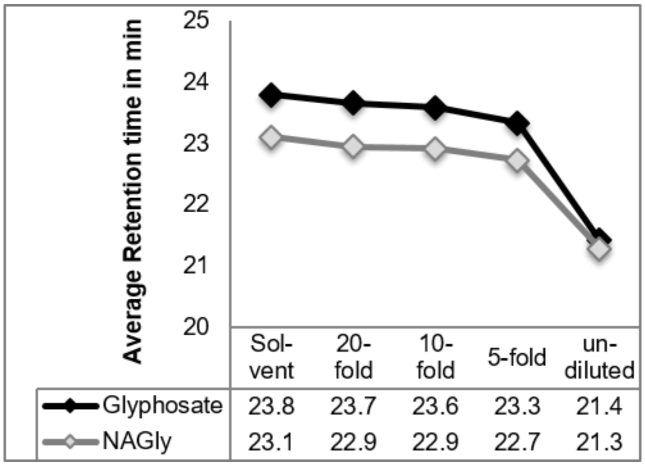

Fig. S4: *Average retention times (t_R in min) of glyphosate and NAGly in undiluted and differently diluted lemon extracts (n=3)*

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