Supplementary information

Solid-phase extraction method for stable isotope analysis of pesticides from large volume environmental water samples

Clara Torrentó[a*†], Rani Bakkour[b], Gaétan Glauser[c], Aileen Melsbach[d], Violaine Ponsin[a], Thomas B. Hofstetter[b], Martin Elsner[d,e], Daniel Hunkeler[a]

[a]Centre for Hydrogeology and Geothermics (CHYN), University of Neuchâtel, 2000 Neuchâtel, Switzerland

[b]Eawag, Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Switzerland

[c]Neuchâtel Platform of Analytical Chemistry (NPAC), University of Neuchâtel, 2000 Neuchâtel, Switzerland

[d]Helmholtz Zentrum München, Institute of Groundwater Ecology, 85764 Neuherberg, Germany

[e]Technical University of Munich, Chair of Analytical Chemistry and Water Chemistry, 81377 Munich, Germany

*Corresponding author: Clara Torrentó, Phone: +41 327182649, Fax: +41 327182603, e-mail: clara.torrento@gmail.com

[†]Present Address: Grup MAiMA, Departament Mineralogia, Petrologia i Geologia Aplicada, Facultat de Ciències de la Terra, Universitat de Barcelona (UB), C/ Martí i Franquès s/n, 08028, Barcelona, Spain.

Content (20 Pages)

1. Chemical and reagents

For concentration analysis and SPE efficiency tests, the following Pestanal-quality standards were purchased from Sigma-Aldrich (Seelze, Germany): atrazine (ATR), desethylatrazine (DEA), desisopropylatrazine (DIA), acetochlor (ACETO), metolachlor (METO), chloridazon (CLZ), 2,6-Dichlorobenzamide (BAM), the single isotopically labeled surrogates (atrazine-d_5, alachlor-d_{13} and chloridazon-d_5) and the internal standard (terbuthylazine). Certified standards of desphenylchloridazon (DPC), methyldesphenylchloridazon (M-DPC) and chloridazon-d_5 were purchased from Dr. Ehrenstorfen GmbH (Wesel, Germany). For each analyte, 1 mg mL^{-1} standard stock solutions were prepared in ethyl acetate (EtAc) or ethanol (in acetonitrile:ultrapure water 50:50 v/v for DPC) and stored in dark at -18 °C for six months. Working solutions were prepared by dilution of the stock solution in ethanol. These solutions were renewed every two months. Calibration solutions were prepared in methanol:ultrapure water (70:30 v/v) mixture with concentrations ranging from 10 to 1000 µg L^{-1}.

The following standards were used as in-house standards for isotope measurements and to prepare spike solutions for the SPE-CSIA method validation: ATR and CLZ (purity not available, Cfm Oskar Tropitzsch GmbH, Marktredwitz, Germany), DEA (purity not available, Synchem, Felsberg, Germany), ACETO and METO (96.3% and 96.2%, respectively, Chemos GmbH, Regenstauf, Germany), BAM (purity not available, Fluorochem Ltd., Derbyshire, UK) and DPC (99.8%, BASF SE, Limgurgerhof, Germany). Stock solutions were prepared in EtAc, methanol (MeOH) or ethanol for all the standards, except DPC, for which ultrapure water was used. Working solutions were prepared by dilution of the stock solutions in EtAc or MeOH (ultrapure water for DPC).

Empty polyethylene cartridges (6 mL and 60 mL) and matching polyethylene frits (20-µm pore size) were obtained from Grace (Columbia, SC, USA). A 12-positions SPE vacuum manifold station from Phenomenex was used. MeOH, EtAc and ethanol of analytical grade were used. Ultrapure water was prepared by ultrafiltration with a Millipore DirectQ apparatus (Millipore, Bedford, MA, USA).

The following SPE sorbents were tested (Table S1): Supelclean ENVI-Carb (0.5 g, 120 m^2 g^{-1}, 6-mL cartridges, Supelco, Bellefonte, PA), Strata SDB-L (0.5 g, 500 m^2 g^{-1}, 6-mL cartridges Phenomenex, Torrance, CA, USA), Bakerbond SDB-1 (0.2 g, 915 m^2 g^{-1}, 6-mL cartridges, J.T. Baker, Phillipsburg,

NJ, US), Lichrolut EN (0.2 g, 1200 m^2 g^{-1}, 6-mL cartridges, Merck, Darmstadt, Germany), Sepra ZT (760-820 m^2 g^{-1}, Phenomenex) bulk phase and Oasis HLB (0.2 g, 830 m^2 g^{-1}, 6-mL cartridges, Waters, Milford, MA).

Table S1. Physical characteristics of the sorbents used in this study.

Sorbent	Supplier	Base material	Polar character[a]	Particle size (µm)	Surface area (m^2 g^{-1})	Pore diameter (Å)
Supelclean ENVI-Carb	Supelco	GCB	hydrophobic	na	120	na
Strata-SDB-L	Phenomenex	PS-DVB	hydrophobic	83	500	260
Sepra ZT (bulk phase of StrataX)	Phenomenex	ST-DVB c.m.	hydrophilic	30	760-820	82
Oasis HLB	Waters	PS-DVB-VP	hydrophilic	30	830	80
Bakerbond SDB-1	J.T. Baker	HC-PS-DVB-EVB	hydrophobic	40-150	915	90
LiChrolut EN	Merck	HC-PS-DVB-EVB	hydrophobic	40-120	1200	na

[a]Fontanals et al.[1]; na: value not available in the data supplied by manufacturers. GCB: graphitized carbon; PS-DVB: polystyrene-divinylbenzene; ST-DVB c.m.: styrene-divinylbenzene, chemically modified (polymeric skeleton, modified with the incorporation of pyrrolidone groups); PS-DVB-VP: divinylbenzene-vinylpyrrolidone; HC-PS-DVB-EVB: hypercrosslinked polystyrene-divinylbenzene-ethylvinylbenzene

2. Analytical methods

Concentration analyses. Concentration of the target compounds in the SPE eluates were determined by ultra-high pressure liquid chromatography quadrupole time of flight mass spectrometry (UHPLC-QTOF-MS). A Synapt G2 Q-TOF mass spectrometer (Waters, Milford, MA, USA) equipped with an electrospray (ESI) probe was used. The mass spectrometer was operated in positive ionization mode using the MS full scan mode over a mass range of 50-600 Da with a scan time of 0.4 sec. The following QTOF-MS conditions were used: capillary voltage of +2800 V, cone voltage of +30 V, extraction cone voltage of +3.0 V, source temperature of 120 °C, desolvation temperature of 550 °C, desolvation gas flow of 13.3 L min^{-1} and cone gas flow of 20 L h^{-1}. The Q-TOF was coupled to an Acquity UPLC™ system (Waters). An Acquity UPLC BEH C18 column (50 mm × 2.1 mm, 1.7 µm, Waters) was used, at a flow rate of 0.4 mL min^{-1} in gradient mode. A guard column (5 mm × 2.1 mm, 1.7 µm) of identical phase chemistry was placed before the column. The mobile phase consisted of two solvents: solvent A (water and formic acid 0.05%) and solvent B (acetonitrile and formic acid 0.05%). The following gradient was used: 2-65% B in 4.5 min, 65-100% B in 1 min, holding at 100% B for 1.5 min and re-equilibration at 2% B for 1.5 min. The column temperature was maintained at 25 °C and the injection volume was 2 µL. The system was controlled by Masslynx 4.1 (Waters). Quantification was performed by the internal standard method, based on peak areas, using terbuthylazine as internal standard. For quantification, extracted ion chromatograms (EIC) were generated using mass windows of 0.02 Da around the mass-to-charge ratios (m/z) of the analytes. The quantifier and qualifier ions and the instrument method detection limit for each analyte are shown in Table S2.

Table S2. Parameters for UHPLC-QTOF-MS analysis: quantifier and qualifier ions, retention time and the instrument method detection (LOD) and quantification (LOQ) limits. Molecular mass and log K_{OW} of the target herbicides and metabolites are also shown.

compound	molecular mass (g mol^{-1})	log Kow	quantifier ion (m/z)	qualifier ion (m/z)	retention time	LOD (µg L^{-1})	LOQ (µg L^{-1})
Desphenylchoridazon (DPC)	145.55	-0.40	146.012	148.008	0.77	9.4	28.1
Methyldesphenylchoridazon (M-DPC)	159.57	-0.30	160.028	162.024	1.07	3.1	9.0
Desisopropylatrazine (DIA)	173.60	1.15	174.050	176.052	1.66	4.1	11.4
Desethylatrazine (DEA)	187.63	1.51	188.070	222.042	2.13	2.1	6.0
2,6-Dichlorobenzamide (BAM)	190.03	0.77	189.983	191.980	1.76	5.9	18.2
Chloridazon (CLZ)	221.66	1.14	222.039	188.070	2.12	1.5	4.3
Atrazine (ATR)	215.68	2.61	216.103	218.100	3.33	1.0	3.0
Acetochlor (ACETO)	269.77	3.03	224.080	284.140	4.63	4.8	14.0
Metolachlor (METO)	283.79	3.13	284.138	306.120	4.57	1.5	4.4
Atrazine-d_5	220.71	-	221.135	-	3.29	-	-
Alachlor-d_{13}	282.85	-	251.180	-	4.59	-	-
Chloridazon-d_5	226.67	-	227.075	-	2.12	-	-

Isotope analyses. Carbon and nitrogen isotope measurements of ATR, ACETO, METO, DEA and BAM in EtAc were performed by GC/IRMS according to a modified method from Meyer et al.[2], Reinnicke et al.[3] and Schreglmann et al.[4]. A Thermo Finnigan TRACE GC Ultra coupled to a Delta V Plus IRMS via a Finningan GC Combustion III interface (Thermo Fisher Scientific, Bremen, Germany) was used. For all $\delta^{13}C$ and $\delta^{15}N$ measurements, a self-made Ni/Ni/Pt reactor[5] was operated at 1180 °C and reoxidized for 20 min with a continuous O_2 stream after every measurement. For N isotope analysis, a standard reduction reactor (Thermo) was operated at 650 °C and liquid N_2 was used for cryogenic trapping of CO_2. Liquid samples in EtAc (1 µL for C and 5 µL for N) were injected with a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland) in a split/splitless injector operated for 1 min in splitless and then in split mode with a split flow of 50 mL min^{-1} at a temperature of 230 °C. Helium was used as carrier gas at constant pressure (200 kPa). The GC was equipped with an OV-1701OH deactivated fused-silica guard column (660 µm OD, 530 µm ID, BGB Analytik), a 60 m × 0.32 mm Rxi-5ms column (Crossbond diphenyl dimethyl polysiloxane, 1 µm df, Restek), and an OV-1701OH deactivated fused-silica postcolumn (450 µm OD, 320 µm ID, BGB Analytik). The oven temperature program was 50 °C

min^{-1} from 80 to 230 °C, 2 °C min^{-1} to 270 °C (10 min) and 20 °C min^{-1} to 280 °C (10 min). Peak identification was based on retention times in comparison with external standards. The GC/IRMS system and data collection were controlled using Isodat 3.0 software (Thermo).

Carbon isotope ratios of DPC in water were measured by LC/IRMS.[6] Briefly, high-performance liquid chromatography (HPLC) was carried out on a Dionex system consisting of an Ultimate 3000 HPLC pump and an Ultimate 3000 autosampler (all from Thermo), fitted with a Sentry guard column (3 µm, 20 mm) and an Atlantis T3 column (3 µm, 100 mm, Waters) and eluted at 500 µL min^{-1} isocratically with pH 2 phosphoric acid solution at room temperature. Isotopic ratio measurements were carried out on a Delta V Advantage IRMS (Thermo) coupled to the LC system by an Isolink interface (Thermo). The separated peaks were quantitatively oxidized using oxidant (90 g L^{-1} $Na_2S_2O_8$) and acid (1.5 M H_3PO_4), each introduced at a flow rate of 30 µL min^{-1} in the oxidation reactor held at 99.9 °C. The injection volume ranged between 10 and 100 µL.

For measuring nitrogen isotope ratios of DPC, derivatization with trimethylsilyldiazomethane was performed prior to GC/IRMS analysis.[6] Briefly, the sample was reconstituted in 1mL MeOH. At a temperature of 70 °C and with an excess of trimethylsilyldiazomethane (TMSD) (160-fold $n_{TMSD}/n_{analyte}$), a nearly complete reaction with accurate $\delta^{15}N$ values was obtained. Afterwards, the sample was evaporated using a gentle stream of nitrogen gas. Prior to GC/IRMS measurements, the samples were reconstituted in 30 – 150 µL acetone. Depending on the sample concentration, the sample was either injected in splitless mode or on-column. For both cases, the sample was injected onto a DB-1701 column (30 m × 25 mm × 1 µm) (J&W Scientific, Santa Clara, CA) placed in a GC/IRMS system. The system consisted of a TRACE GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) coupled with a Finnigan MAT 253 isotope ratio mass spectrometer (IRMS) (Thermo Fisher Scentific, Bremen, Germany). The instrument was operated with a He carrier gas (grade 5.0) at a flow rate of 1.4 mL min^{-1}. The GC temperature program started at 100°C and held for 1 min, followed by a temperature ramp of 25 °C min^{-1} to 240 °C held for 0 min followed by a ramp of 10 °C to 280 °C (held for 5 min). In case of on-column injection, the temperature program starts at 40 °C, held for 1 min, ramped by 25 °C min^{-1} to 240 °C, held for 0 min, ramped with 10 °C and held for 5 min.

All reported isotope ratios are expressed as arithmetic means of replicate measurements with 1 standard deviation (±σ) in $\delta^{13}C$ and $\delta^{15}N$ values relative to the international standards Vienna Pee Dee Belemnite (VPDB) and air, respectively. For both GC/IRMS and LC/IRMS, calibration was performed by using in-house standards and reference gas peaks spread throughout the chromatograms. The trueness of the isotope measurements is expressed as the deviation of isotope signatures measured by GC/IRMS and LC/IRMS from reference isotope signatures of the calibrated in-house standards of known carbon and nitrogen isotope ratios, which were previously determined by EA/IRMS based on two-point normalization using the international organic reference materials USG 40 (L-glutamic acid), USG 41 (L-glutamic acid) and IAEA 600 (caffeine), provided by the International Atomic Agency (Vienna, Austria).[6] Table S3 lists the $\delta^{13}C$ and $\delta^{15}N$ values of these standards measured by EA/IRMS.

Table S3. C and N EA/IRMS results of the standards used for isotope measurements and for validation of the SPE-CSIA method.

standard	EA/IRMS $\delta^{13}C$ (‰) ± sd	EA/IRMS $\delta^{15}N$ (‰) ± sd
DPC	-17.8±0.1	-3.8±0.1
M-DPC	-21.2±0.1	1.0±0.1
DEA	-32.1±0.2	-9.4±0.1
BAM	-27.5±0.3	-6.4±0.2
CLZ	-27.3±0.2	-5.6±0.1
ATR	-28.4±0.3	-1.8±0.1
ACETO	-25.0±0.1	0.5±0.1
METO	-28.0±0.1	0.7±0.2

3. Optimization of the extraction methods

SPE procedure for large-volume samples. The optimal procedure consisted of the following steps. Empty 60-mL cartridges were packed with 8 g of each sorbent (first Sepra ZT and second SDB-1 in the flow direction). Both sorbents were separated by a polyethylene frit and frits were also added on the bottom and on top of the cartridges. The cartridges were first rinsed two times with 15 mL EtAc, conditioned with four times 15 mL MeOH and finally equilibrated with four times 15 mL ultrapure water. The cartridges were attached to Teflon sampling tubing using Teflon cap adapters and large-volume water samples (5-10 L) were pumped through the cartridges at 5 mL min^{-1}. Two 20 L Nalgene™ heavy duty vacuum carboys served as a water trap between the vacuum pump and the vacuum manifold (see picture below). Thereafter, the sorbent was washed with four times 15 mL ultrapure water and dried under vacuum overnight. The cartridges were eluted with eight times 15 mL EtAc (3 mL min^{-1}). Eluates were then reduced to 0.1 mL using a Syncore Analyst R-12 (Büchi, Flawil, Switzerland) evaporator, transferred by several EtAc cleaning steps to 7.5-mL Pyrex glass tubes (VWR Scientific, Rochester, NY, USA) and finally evaporated until dryness using a CentriVap Benchtop vacuum concentrator (Labconco, Kansas City, MO, USA). Dry extracts were stored frozen until isotope analyses. The reconstitution procedure for (derivatization)-GC/IRMS was done in several steps. First, eleven reconstitutions steps in EtAc (except two times 250 µL of MeOH) were performed to a final reconstitution volume of 5 mL. For each step, the solution was shaken for 1 min by vortex, soaked for 10 min in an ultrasonic bath and finally shaken again for 1 min by vortex. The final solution was filtered with a 0.45 µm regenerated cellulose (RC) filter, blown down to approximately 1 mL, filtered through a 0.2 µm RC filter and lastly blown down to the final volume (ranging between 40 and 150 µL). For LC/IRMS measurements, dry extracts were reconstituted in the required volumes of ultrapure water (ranging between 150 and 500 µL) and filtered through a 0.2 µm RC filter before injection. Recovery was estimated from GC/IRMS and LC/IRMS responses.

SPE procedure for large-volume samples

Figure S1. pH effect. Extraction efficiencies (mean recoveries, %) of LiChrolut and Bakerbond SDB-1 0.2g-cartridges for 20-mL distilled water samples spiked to 12.5 µg L^{-1} of each analyte (25 µg L^{-1} for DCP). Cartridges were eluted with 3 mL EtAc. Error bars show RSDs (n=3).

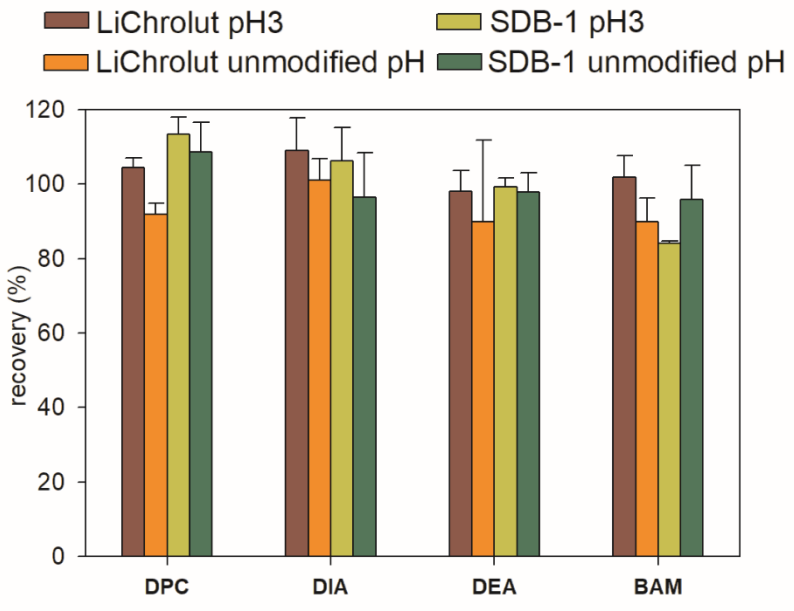
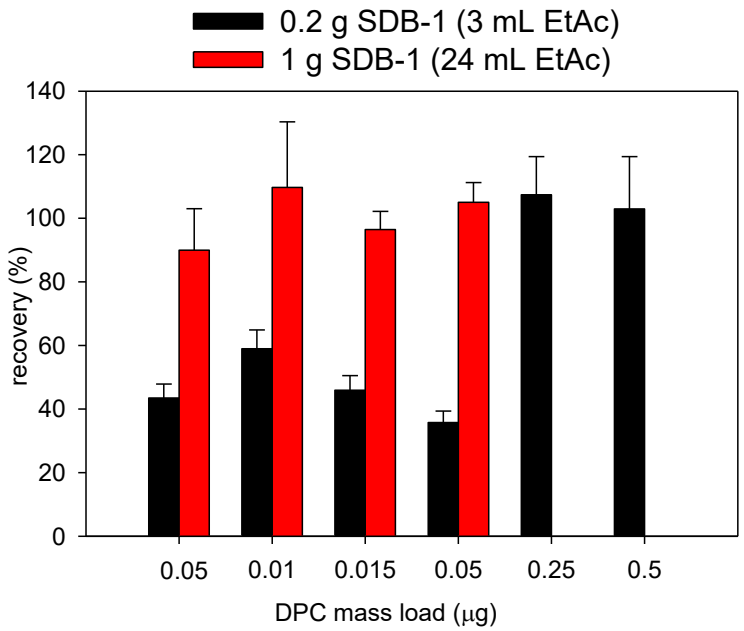


Figure S2. Sorbent mass effect. Extraction efficiencies (mean recoveries, %) for 20-25-mL distilled water samples spiked with 0.005 to 0.5 µg DPC using two different Bakerbond SDB-1 masses: 0.2 g (black bars) and 1 g (red bars). Cartridges were eluted with 3 mL EtAc. Error bars show RSDs (n=2). Note that for DPC load masses above 0.05 µg, cartridges with 1 g SDB-1 were not tested because high recoveries were already obtained using cartridges with 0.2 g SDB-1.



4. CSIA methods

Table S4. Trueness, precision and reproducibility of carbon and nitrogen isotope measurements of standards of the target herbicides and metabolites determined by GC/IRMS ($\delta^{13}C$ and $\delta^{15}N$ of ATR, DEA, ACETO, MET, BAM and M-DPC) derivatization-GC/IRMS ($\delta^{15}N$ of DPC) and LC/IRMS ($\delta^{13}C$ of DPC). Precision is reported as arithmetic mean (±σ) of n measurements that were conducted with constant and optimal analytes concentration under exactly the same instrumental conditions. Trueness (Δδ) is expressed as mean deviation of the measured isotope signatures from the reference isotope signatures determined by EA/IRMS. Data points are shown in Figure S3. Reproducibility is reported as the arithmetic mean (±σ) of n measurements that were conducted at concentrations within the linearity range (Fig. S4 and S5) during a period of t months. For the purposes of this study, M-DPC was only evaluated for N CSIA, as the correction standard for the determination of the $\delta^{15}N$-DPC values after DPC derivatization to M-DPC.[6] The derivatization of DPC to M-DPC did not cause any isotope fractionation as the pure non-derivatized M-DPC standard shows a similar offset of the nitrogen isotope value from the reference value as the derivatized DPC standard. Consequently, the offset is caused by incomplete oxidation of M-DPC. The offset of the $\delta^{15}N$ M-DPC standard from its reference value determined with EA-IRMS was used to correct the $\delta^{15}N$-DPC.[6]

	mM	n	$\delta^{13}C$ (‰)	sd	$\Delta\delta^{13}C$ (‰)	$\delta^{13}C$±sd (‰) for reproducibility	mM	n	$\delta^{15}N$ (‰)	sd	$\Delta\delta^{15}N$ (‰)	$\delta^{15}N$±sd (‰) for reproducibility
ATR	0.2	94	-28.3	0.5	+0.1	-27.9±0.6, n=215, t=1	1.4	84	-1.0	0.1	+0.9	-0.9±0.3, n=151, t=2
ACETO	0.2	94	-25.5	0.4	-0.5	-25.3±0.7, n=200, t=1	1.2	83	0.7	0.2	+0.2	0.7±0.3, n=150, t=2
METO	0.2	97	-28.5	0.4	-0.5	-28.3±0.5, n=202, t=1	1.2	84	1.1	0.3	+0.4	1.2±0.3, n=139, t=2
DEA	0.5	52	-30.1	0.3	+2.0	-30.0±0.4, n=139, t=1	1.1	71	-8.3	0.1	+1.1	-8.3±0.1, n=91, t=1.5
BAM	1.0	52	-29.1	0.5	-1.6	-28.9±0.8, n=125, t=1	1.0	11	-6.1	0.8	+0.3	-5.9±0.7, n=20, t=0.2
DPC	0.7[a]	75	-14.6	0.4	+3.2	-14.5±0.4, n=233, t=1.2	1.7	12	-5.4	0.4	-1.6	-5.6±0.4, n=27, t=3
M-DPC				-			4.4	46	-0.6	0.4	-1.6	-0.6±0.4, n=46, t=0.1

[a]Corresponding to an injection volume of 10 µL and 27.5 nmol C on column

Figure S3. Trueness and precision of carbon and nitrogen isotope measurements of standards of the target herbicides and metabolites determined by GC/IRMS ($\delta^{13}C$ and $\delta^{15}N$ of ATR, DEA, ACETO, MET, BAM and M-DPC) derivatization-GC/IRMS ($\delta^{15}N$ of DPC) and LC/IRMS ($\delta^{13}C$ of DPC). Measurements were conducted with constant and optimal analytes concentration under exactly the same instrumental conditions (see Table S4). Black and blue lines indicate EA/IRMS and mean measured $\Delta\delta$ values, with intervals of ±0.5‰ and ±1.0‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively (dashed lines).

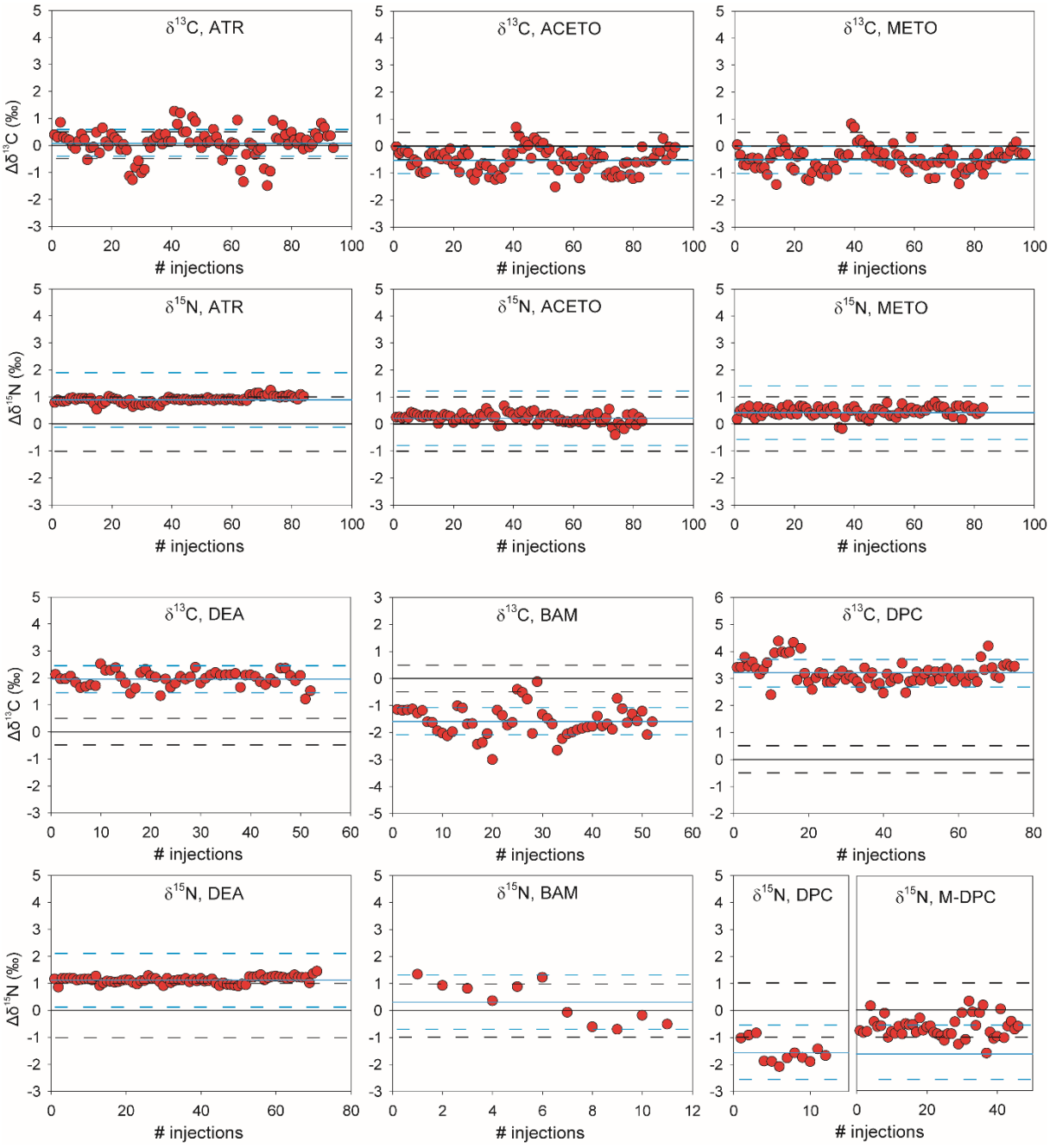


Figure S4. Amount-dependency of the precision (i.e. linearity range) and determination of the limit of precise $\delta^{13}C$ analysis ($Limit_{instrument}$) of the in-house standards of ATR (A), ACETO (B), METO (C), DEA (D), BAM (E), analyzed by GC/IRMS, and of DPC (F), analyzed by LC/IRMS. The linear regression between amplitudes and injected concentrations is also shown (dashed black lines). Gray bars indicate $Limits_{intrument}$ determined according to the moving mean procedure[7] with intervals of ±0.5‰ (blue lines). Moving means are indicated by dashed blue lines. Gray areas indicate the linearity ranges. Error bars show standard deviation (±σ) of quintuplicate measurements (quadruplicate for DPC).

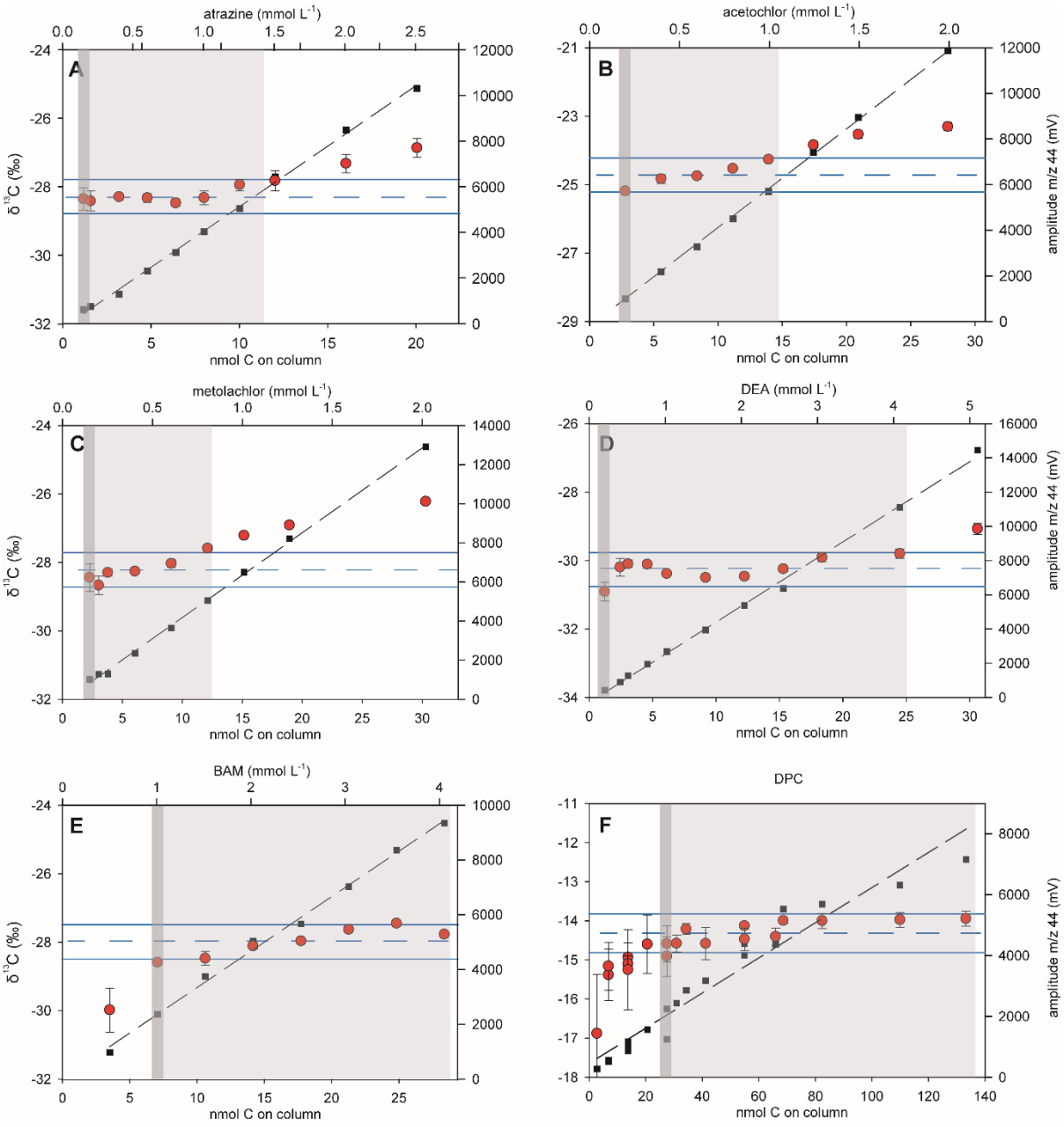


Figure S5. Amount-dependency of the precision (i.e. linearity range) and determination of the limit of precise $\delta^{15}N$ analysis ($Limit_{instrument}$) of the in-house standards of ATR (A), ACETO (B), METO (C), DEA (D), BAM (E), analyzed by GC/IRMS, and of DPC (F), analyzed by derivatization-GC/IRMS. The linear regression between amplitudes and injected concentrations is also shown (dashed black lines). Gray bars indicate $Limits_{intrument}$ determined according to the moving mean procedure[7] with intervals of ±1‰ (blue lines). Moving means are indicated by dashed blue lines. Gray areas indicate the linearity ranges. Error bars show standard deviation (±σ) of quintuplicate measurements (triplicate for DPC).

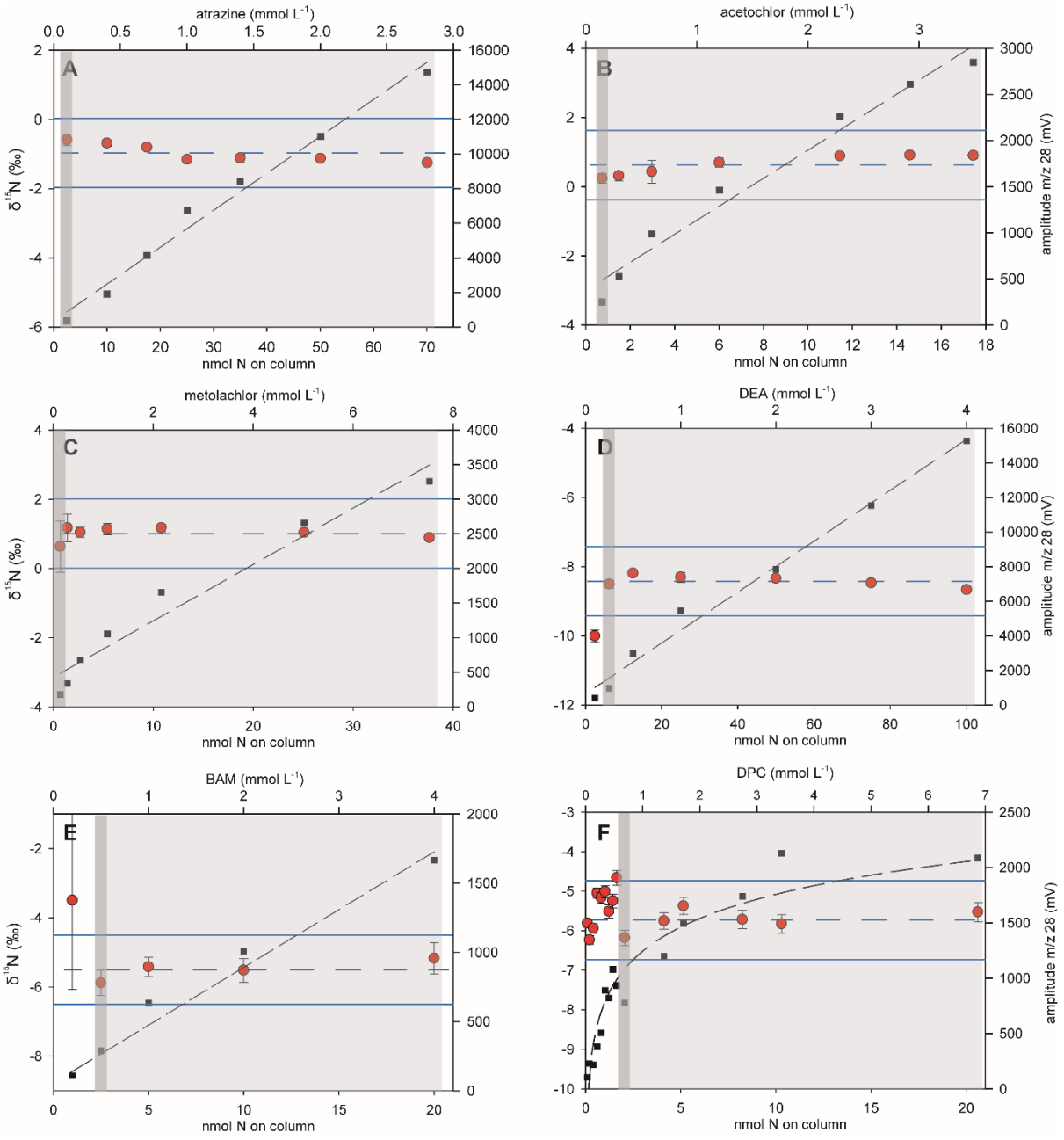


Table S5. Instrumental ($Limits_{instrument}$) and SPE-CSIA method limits ($Limits_{method}$) of precise carbon and nitrogen isotope analysis of the target compounds in 10 L environmental water samples. $Limits_{instrument}$ were determined according to the moving mean procedure[7] with intervals of ±0.5‰ and ±1‰, respectively. $Limits_{instrument}$ are expressed as injected concentration (mM) and as corresponding mass of C and N on-column (nmol). Corresponding peak amplitudes (mV) are also shown. $Limits_{method}$ are expressed as concentration of the target herbicides and metabolites in water.

	$\delta^{13}C$					**$\delta^{15}N$**				
	$Limit_{instrument}$			**$Limit_{method}$[b]**		**$Limit_{instrument}$**			**$Limit_{method}$[b]**	
	mM	**nmol C on-column**	**Amp m/z 44 ±σ (mV)**	**nM**	**µg L^{-1}**	**mM**	**nmol N on-column**	**Amp m/z 28 ±σ (mV)**	**nM**	**µg L^{-1}**
ATR	0.15	1.2	626±30	1.3	0.3	0.10	2.5	349±2	0.8	0.2
ACETO	0.2	2.8	987±21	1.7	0.5	0.15	0.7	250±2	1.3	0.3
METO	0.15	2.3	1015±54	1.3	0.4	0.14	0.7	178±1	1.2	0.3
DEA	0.2	1.2	421±4	1.7	0.3	0.25	6.3	975±6	2.1	0.4
BAM	1	7.1	2393±40	8.4	1.6	0.50	2.5	288±3	4.0	0.8
DPC	0.14[a]	27.5	1249±177	8.2	1.2	0.69	2.1	778±31	21	3.2

[a]Corresponding to an injection volume of 50 µL
[b]Assuming a reconstitution volume of 80 µL and SPE recoveries of 95% (Table 3), except for DPC (150 µL and 25%, respectively)

5. Validation of the SPE-CSIA procedure

Table S6. Carbon and nitrogen isotope ratios of the target compounds after SPE-CSIA of drainage water samples spiked with sub-microgram per liter concentrations of the in-house standards. Isotope ratios of the bracketing standards intercalated in the same sequence than the spiked samples are also shown. $\delta^{13}C$ and $\delta^{15}N$ values of ATR, ACETO, METO, DEA, BAM and M-DPC were measured by GC/IRMS, whereas $\delta^{13}C$ and $\delta^{15}N$-DPC values were measured by LC/IRMS and derivatization-GC/IRMS, respectively. All the samples were run in quadruplicate (triplicate for $\delta^{15}N$ of DEA, BAM and DPC). Recovery was estimated from GC/IRMS and LC/IRMS responses. Trueness ($\Delta\delta$) is expressed as mean deviation of isotope signatures measured with GC/IRMS or LC/IRMS from the reference values determined by EA/IRMS. Asterisk symbols indicate samples for which amplitudes were out of the linearity range. na= not analyzed (given that some analyses were performed at different times or at different laboratories, not all the samples were analyzed for both C and N CSIA).

	Recovery (%)	$\delta^{13}C$ (‰)	sd	$\Delta\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	sd	$\Delta\delta^{15}N$ (‰)
ATR							
5 µg L^{-1}, 10 L	109	-27.7	0.4	+0.7	-0.9	0.1	+1.0
1 µg L^{-1}, 10 L	80	-28.9	0.8	-0.5	-0.2	1.3	+1.7
0.5 µg L^{-1}, 2 × 10 L	88	-28.6	1.0	-0.2	0.0	0.8	+1.9
0.5 µg L^{-1}, 10 L	31	-27.3*	0.4*	+1.1*	na	na	na
Bracketing standards		-27.5	0.2	+1.0	-0.6	0.3	+1.3
ACETO							
5 µg L^{-1}, 10 L	75	-24.1	0.0	+0.9	1.0	0.1	+0.5
1 µg L^{-1}, 10 L	105	-24.9	0.3	+0.1	1.2	0.6	+0.7
0.5 µg L^{-1}, 2 × 10 L	63	-24.9	0.3	+0.1	1.2	0.4	+0.7
0.5 µg L^{-1}, 10 L	131	-25.2	0.2	-0.2	na	na	na
Bracketing standards		-26.1	0.5	-1.1	1.0	0.2	+0.5
METO							
5 µg L^{-1}, 10 L	123	-27.0	0.2	+1.0	1.1	0.2	+0.4
1 µg L^{-1}, 10 L	114	-28.7	0.7	-0.6	1.3	0.4	+0.6
0.5 µg L^{-1}, 2 × 10 L	89	-27.4	1.5	+0.6	1.6	0.3	+0.9
0.5 µg L^{-1}, 10 L	109	-27.1	0.1	+0.9	na	na	na
Bracketing standards		-28.2	0.4	-0.2	1.3	0.3	+0.7
DEA							
10 µg L^{-1}, 10 L	101	-29.8	0.3	+2.2	-9.7	0.2	+0.1
5 µg L^{-1}, 10 L	76	-30.9	0.6	+1.2	-8.7	0.5	+1.1
2.5 µg L^{-1}, 10 L	102	-28.7	1.5	+3.4	-8.4	0.3	+1.4
1 µg L^{-1}, 10 L	86	na	na	na	-7.2	0.1	+2.6
1 µg L^{-1}, 10 L	27	-29.2	0.6	+2.8	na	na	na
1 µg L^{-1}, 10 L	22	-30.0	0.2	+2.1	na	na	na
1 µg L^{-1}, 10 L	92	-29.6	0.3	+2.5	na	na	na
0.5 µg L^{-1}, 10 L	66	-29.7	0.1	+2.4	na	na	na
Bracketing standards		-29.9	0.4	+2.2	-9.3	0.4	+0.6

Table S6 (cont.)

	Recovery (%)	$\delta^{13}C$ (‰)	sd	$\Delta\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	sd	$\Delta\delta^{15}N$ (‰)
BAM							
10 µg L^{-1}, 10 L	134	-27.4	0.3	+0.1	-5.6	0.2	+0.9
7.5 µg L^{-1}, 10 L	97	-26.4	0.7	+1.1	-5.9	0.5	+0.5
5 µg L^{-1}, 10 L	136	-26.2	0.4	+1.3	-5.9	0.2	+0.5
4 µg L^{-1}, 10 L	83	-20.3*	0.5*	+7.2*	-5.6	0.6	+0.9
1 µg L^{-1}, 10 L	56	-27.6*	0.7*	-0.2*	na	na	na
0.5 µg L^{-1}, 2 × 10 L	74	-28.5*	0.9*	-1.0*	na	na	na
Bracketing standards		-28.4	0.8	-0.9	-6.4	0.7	0.0
DPC							
50 µg L-1, 10 L	26	-14.4	0.3	+3.4	na	na	na
50 µg L-1, 10 L	55	-15.0	0.3	+2.8	na	na	na
25 µg L-1, 10 L	54	-15.2	0.2	+2.6	na	na	na
10 µg L-1, 10 L	52	-14.9	0.1	+2.9	na	na	na
5 µg L-1, 10 L	28	-17.3*	0.6*	+0.5*	na	na	na
5 µg L-1, 10 L	66	-15.1	0.5	+2.8	na	na	na
5 µg L-1, 10 L	71	-15.0	0.2	+2.8	na	na	na
0.5 µg L-1, 2 × 10 L	29	-14.8*	3.2*	+3.1*	na	na	na
0.5 µg L-1, 2 × 10 L	31	-19.6*	0.3*	-1.8*	na	na	na
Bracketing standards ($\delta^{13}C$-DPC)		-14.5	0.2	+3.3	-	-	-
20 µg L^{-1}, 7.7 L	12	na	na	na	-6.5	0.1	-2.7
10 µg L^{-1}, 10 L	17	na	na	na	-6.1	0.5	-2.3
5 µg L^{-1}, 10 L	37	na	na	na	-5.5	0.2	-1.7
2.5 µg L^{-1}, 10 L	14	na	na	na	-4.7	0.2	-0.8
1 µg L^{-1}, 10 L	19	na	na	na	-4.3	0.4	-0.5
Bracketing standards ($\delta^{15}N$-MDPC)		-	-	-	-1.7	0.8	-2.6

Figure S6. Examples of resulting GC/IRMS and LC/IRMS chromatograms for both standards and spiked drainage water samples. (A) GC/IRMS chromatogram (m/z 44) of an in-house standard containing 115 mg L^{-1} METO, 160 mg L^{-1}ACETO, 160 mg L^{-1}DEA, 170 mg L^{-1} ATR and 290 mg L^{-1} BAM, (B) GC/IRMS chromatogram (m/z 44) of an spiked drainage water sample (10 L spiked at 0.5 µg L^{-1} DEA, ATR, ACETO and METO; complete recovery of ACETO and METO, whereas 30-65% recovery for DEA and ATR) , (C) LC/IRMS chromatogram (m/z 44) of an in-house 100 mg L^{-1} DPC standard (10 µL injected), (D) LC/IRMS chromatogram (m/z 44) of an spiked drainage water sample (10 L spiked at 10 µg L^{-1} DCP, 50 µL injected; 52 % recovery), (E) GC/IRMS chromatogram (m/z 28) of an in-house 200 mg L^{-1} M-DPC standard, (F) GC/IRMS chromatogram (m/z 28) of an spiked sample after derivatization (10 L spiked at 10 µg L^{-1} DCP, 17 % recovery, derivatization with 140 µL).

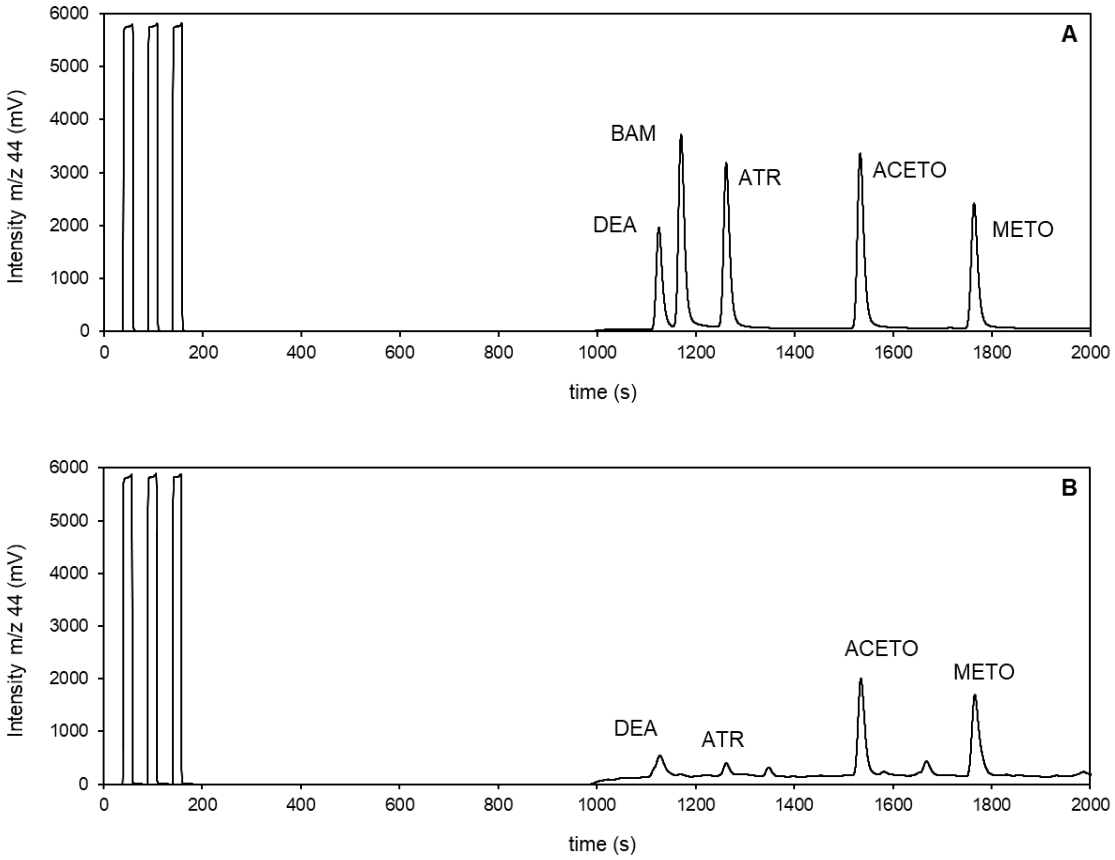
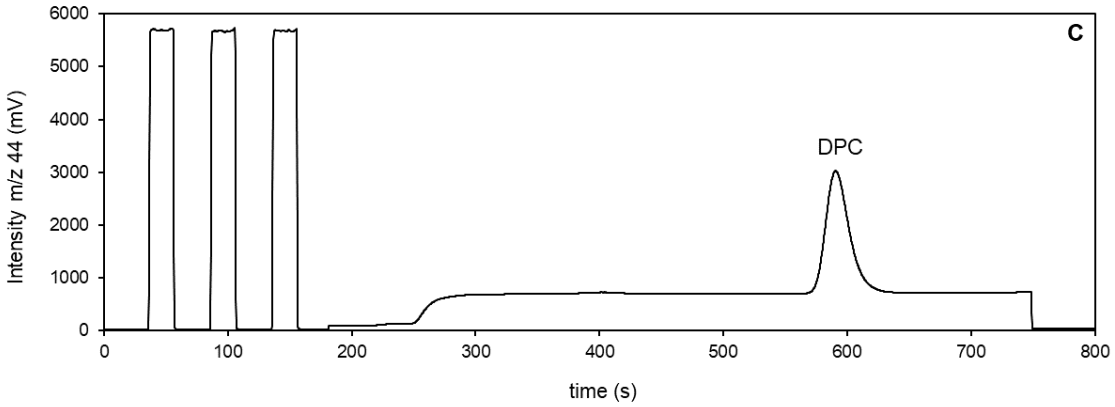
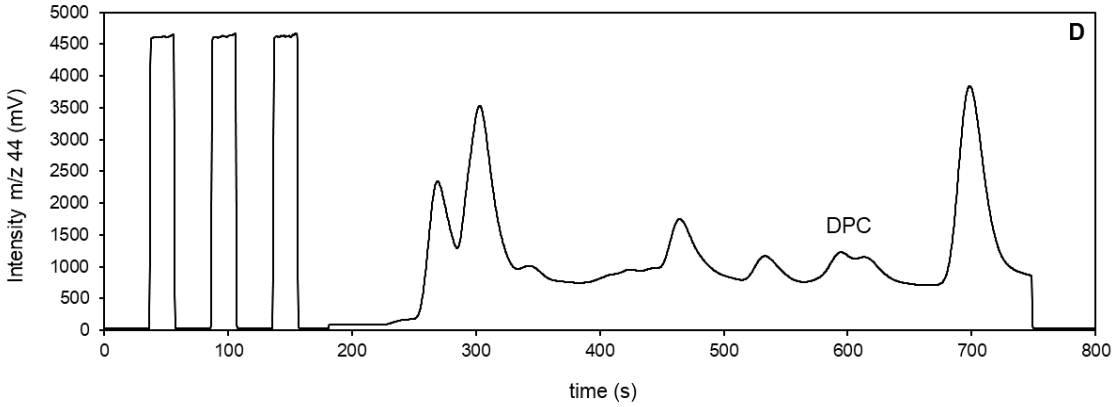
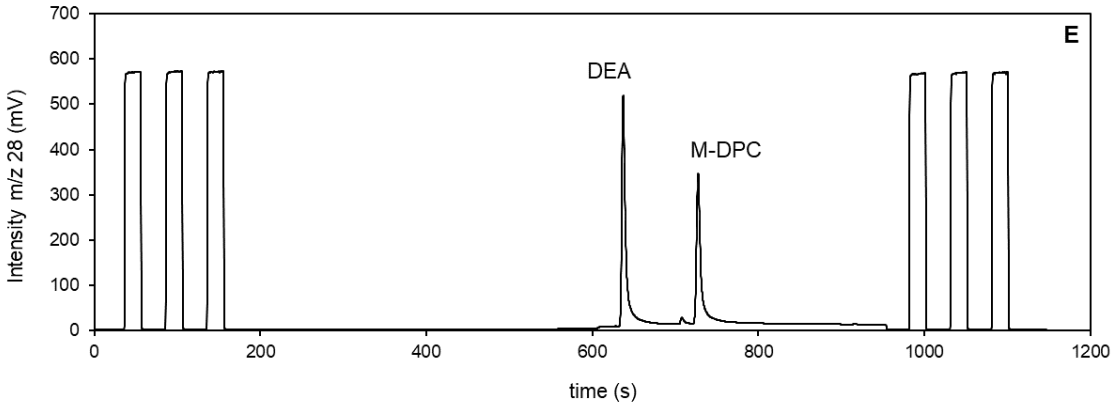
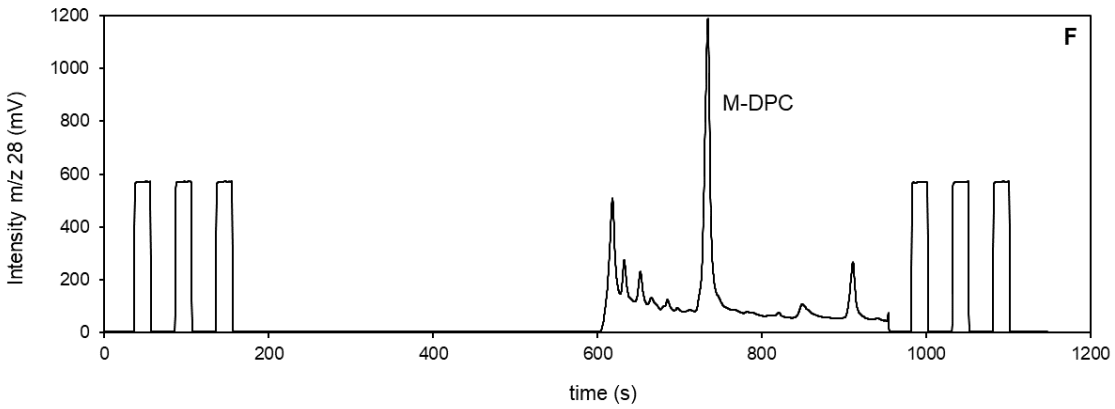


Figure S6 (cont.)


C
DPC
Intensity m/z 44 (mV)
time (s)


D
DPC
Intensity m/z 44 (mV)
time (s)


E
DEA
M-DPC
Intensity m/z 28 (mV)
time (s)


F
M-DPC
Intensity m/z 28 (mV)
time (s)

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