

Automated determination of EU Water Framework Directive priority contaminants in surface water at pg/L detection limits using Stir Bar Sorptive Extraction (SBSE) GC/MS/MS



Abstract

Highly sensitive determination of around 100 contaminants at trace concentrations from low double-digit to low triple-digit pg/L was performed in surface water. Priority compounds from the EU water framework directive (EU-WFD) as well as substances from other legislations were included. A sequential stir bar sorptive extraction (SBSE) approach employing the GERSTEL Twister was used for analyte enrichment from 100 mL water samples. The Twister was thermally desorbed, compounds were separated via GC and detected by tandem mass spectrometry (MS/MS) utilizing the highly sensitive Agilent 7010 Mass Spectrometer with High Efficiency Source (HES).

The analysis method was comprehensively validated to meet the requirements of the EU-WFD for inland surface water. Repeat analysis of water samples spiked at concentrations close to the LOQs of the respective analytes demonstrated relative standard deviations between 1 and 15 % with an average of 6.9 %. (n=6) Trueness was mainly between 90 and 110 % with an average of X. The correct quantitation of particle-adsorbed compounds like PAHs was examined and confirmed using certified reference sediment.

Cypermethrin, heptachlor, heptachlorepoide and also dicofol in other surface waters were the only compounds for which the required LOQs could not be reached. To date there is no technique known to the authors that reaches the required LOQs of 0.06 pg/L for heptachlor and heptachlorepoide. The developed analysis method was successfully applied to real world water samples.

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Keywords

Priority Pollutants, Environmental,
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Introduction

Regulatory challenge

In 2000 the European Community issued a directive "to establish a framework for the protection of inland surface waters, transitional waters, coastal waters and groundwater". This was amended and modified by further directives in 2008 and 2013 [1-3]. Goals are the reduction of surface and ground water pollution as well as to "protect and enhance the status of aquatic ecosystems" and "the protection of territorial and marine waters". A continuous improvement of water quality shall be pursued to maintain "sufficient supply of good quality surface water and groundwater as needed for sustainable, balanced and equitable water use".

To document the present state and to verify the achievements every EU member country is obliged amongst others to test for the "chemical status" of the surface water in each river basin district. The meaning of "chemical status" is substantiated with a list of so called "priority substances" which need to be monitored regularly. Maximum contaminant concentrations derived from toxicological considerations and aimed at implementation for every EU surface water in the long-run are defined as so called "environmental quality standards" (EQS). Annual average (AA-EQS) and maximum allowable concentrations (MAC-EQS) were established distinguishing between "inland surface waters" like rivers and lakes and "other surface waters" like coastal waters.

Analysis methods employed in the context of EU-WFD need to fulfill certain performance criteria [4]: "Member States shall ensure that the minimum performance criteria for all methods of analysis applied are based on an uncertainty of measurement of 50 % or below ($k = 2$) estimated at the level of relevant environmental quality standards and a limit of quantification equal or below a value of 30 % of the relevant environmental quality standards". Where those criteria are not met for any matrix, Member States shall ensure that monitoring is carried out using best available techniques not entailing excessive costs".

naphthalene	600 ng/L
chlorpyrifos-ethyl	9 ng/L

Required LOQs for inland surface water resulting from these specifications, calculated from AA-EQS are challenging for the majority of priority compounds: naphthalene 600 ng/L, chlorpyrifos-ethyl 9 ng/L, p,p'-DDT 3 ng/L, endosulfan 1.5 ng/L, dichlorvos 0.18 ng/L, benzo[a]pyrene 0.051 ng/L, cypermethrin 0.024 ng/L and heptachlor and heptachlorepoxyde 0,00006 ng/L. "The water EQS [...] are expressed as total concentrations in the whole water sample", meaning that particle-adsorbed analytes must be quantified correctly as well.

Analytical methodologies for implementing regulatory requirements

Relevant analytes for the method described here were derived from the aforementioned EU-WFD, the Commission Implementing Decision (EU) 2015/495 ("watch list") [5], the German Oberflächengewässerverordnung (OGewV, version July 2011) [6] and further sources. Only those compounds were included, which could be extracted by polydimethylsiloxane (PDMS) Twisters and separated via gas chromatography. Polar substances of the EU-WFD need to be analyzed by LC-MS/MS, e.g. isoproturon and diuron, volatile compounds like dichloromethane by headspace-GC/MS and metal ions like lead and mercury by inductively coupled plasma mass spectrometry (ICP-MS) or atomic absorption spectroscopy (AAS). The final list contained mainly persistent organic pollutants (POPs) like organochlorine pesticides, PAHs, PCBs and PBDEs as well as other pesticides and industrial pollutants.

Enrichment and detection of GC/MS amenable analytes

For analyte enrichment we employed stir bar sorptive extraction (SBSE) using the GERSTEL Twister®. It is a glass-encased magnetic stir bar coated with an extraction phase of polydimethylsiloxane (PDMS) or ethyleneglycol-polydimethylsiloxane (EG-silicone). While stirring the sample analytes partition between the extractant phase, in this case PDMS, and the liquid sample phase just as in liquid-liquid extraction. Subsequently Twisters are thermally desorbed in the GERSTEL Thermal Desorption Unit (TDU) and analytes are refocused in the Cooled Injection System (CIS) followed by transfer to the GC column and triple quadrupole MS detection in multi reaction monitoring (MRM) mode. Using this setup, all extracted analytes are completely transferred onto the GC-MS/MS making this automated technique highly sensitive.



Figure 1. Twister PDMS stir bar, 1 cm length, 1 mm phase thickness.

Complete transfer of extracted analytes onto GC column

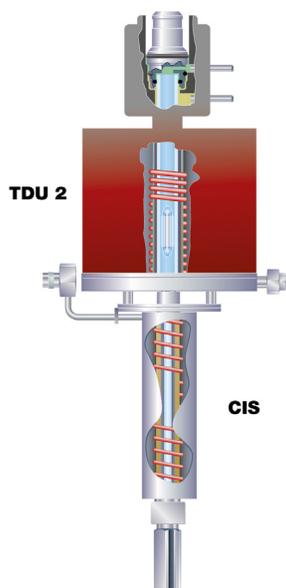


Figure 2. Schematic drawing of the Thermal Desorption Unit (TDU and the Cooled Injection System (CIS) used for Twister desorption and analyte transfer to the GC.

In order to access a wide variety of compounds and to ensure the extraction of particle-adsorbed compounds, the technique of sequential SBSE developed by Ochiai et. al. [7] was utilized. A single sample aliquot of 100ml is extracted sequentially by two Twisters under different conditions, for example, with or without salt or solvent addition. Subsequently, both Twisters are desorbed simultaneously resulting in a single chromatogram covering an extended range of analytes.

The aim of our method development was to achieve the determination of around 100 analytes by SBSE-TDU-GC-MS/MS in a single analytical run. The required LOQs for compounds listed in the EU-WFD should be reached while the sample preparation method should be as simple and straightforward as possible. Finally, the extraction and correct quantification of particle-adsorbed compounds should be ensured as required by the EU-WFD.

Experimental

Materials and Solvents

For sample extraction 1 cm / 1 mm (length / phase thickness) PDMS Twisters were used (GERSTEL p/n 011333-001-00). The Twisters were conditioned at 280 °C under a nitrogen flow overnight using a GERSTEL® Tube Conditioner (TC 2) and stored in the original storage vial until use. Samples were filled into individual 100 mL vials (GERSTEL 093640-062-00), a Twister was added to each vial before closing it with a crimp cap (GERSTEL 011912-002-00). A heated stirring plate was utilized for Twister extraction (GERSTEL 049000-000-NS), along with a dedicated twister removal tool (GERSTEL 013820-001-00) and drying device (GERSTEL 049000-200-00). Organic modifier for the second extraction step was added by a multi dispenser (GERSTEL 049000-100-00) equipped with 50 mL pipette tips (GERSTEL 049000-101-00).

To reduce background contamination all glassware was heated at 300 °C in a laboratory oven overnight prior to use. In the same manner pipette tips were heated at 120 °C.

All solvents used were of analytical grade, purchased from different suppliers, including Merck, LGC Standards, Alfa Aesar and Carl Roth.

Preparation of samples and calibration standards

For calibration a multi-analyte standard in acetone was prepared from certified reference standards and solutions made up from individual neat compounds. Concentrations were adapted to the respective LOQ of each analyte. Three working solutions were diluted from this stock solution to be used for spiking calibration samples. A multi-component internal standard solution was prepared from deuterated or ¹³C labeled analyte analogues in acetone.

Calibration was run over the complete analysis method. To avoid external contamination a 100 g aliquot of pure water was weighed in directly from the primary bottle without using a pipette or similar device. Subsequently, different amounts of working solution and 30 µL of the internal standard solution were added. Background analyte peaks resulting from the water matrix and the laboratory surrounding should be small compared to the lowest calibration level. Additionally, the water used for calibration should be as similar as possible to the water of the real samples. Therefore, very clean groundwater, tap water or mineral water were found to be suitable for calibration whereas groundwater gave the best results regarding trueness of analysis values. Samples to be analyzed should have a pH between 5.5 and 7. If necessary the pH value can be adjusted by addition of 0.1 M hydrochloric acid or 0.1 M potassium hydroxide solution. Again 100 g aliquots were weighed in and 30 µL of internal standard solution added before extraction.

A 1 cm / 1 mm PDMS Twister was added to the vial, which was closed with a crimp cap and placed on a Twister stirring plate for extraction at 1000 rpm for 5 h. After that the Twister was briefly rinsed in HPLC water, transferred to a clean desorption tube and dried in a stream of nitrogen for around 10 s since it was found that, in this case, the standard drying procedure employing a lint free tissue was prone to contaminate the Twister. Desorption tubes were stored contamination free on the TDU rack of the MultiPurpose Sampler (MPS). To perform the second step in the sequential SBSE method, a 15 mL volume of an organic modifier was added to each sample vial for subsequent extraction with a second PDMS Twister at 1000 rpm and elevated temperature over a 17 h period. After rinsing in HPLC water the second Twister was combined with the Twister from the first extraction in the same desorption tube and dried as previously mentioned. Twisters placed in TDU tubes were stored in sealed TDU racks on the MPS until they underwent automated thermal desorption.

Instrumentation

Analyses were performed using a 7890 GC coupled to a 7010 Triple Quadrupole MS (both Agilent Technologies), a Thermal Desorption Unit (TDU 2), Cooled Injection System (CIS 4) and MultiPurpose Sampler (MPS Robotic) (all GERSTEL).

The MPS automatically delivered Twisters loaded in desorption tubes from the storage rack to the TDU. During thermal desorption, analytes were refocused in the CIS on a standard deactivated liner filled with glass wool. An HP-5ms Ultra Inert 30 m, di=0.25 mm, df=0.25 µm from Agilent was utilized for compound separation. Retention times were locked with chlorpyrifos-methyl (at 18.111 min). Helium was used as carrier gas and quenching gas in the MS collision cell and nitrogen was used as collision gas.



Figure 3. Analysis system used for this application consisting of a GERSTEL® MultiPurpose Sampler (MPS Robotic), a Thermal Desorption Unit (TDU 2), a Cooled Injection System (CIS 4) and an Agilent® Technologies 7890 GC configured with a 7010 triple quadrupole MS.

Analysis conditions

TDU:	
Temperature	90 °C; 80 °C/min; 300°C (6 min)
Pneumatics	Solvent Venting/Dry Purge for 2 min
CIS:	
Temperature	-40°C; 12°C/s; 300°C (5 min); 12°C/s; 280°C (37 min)
Pneumatics	Solvent Vent with 80 mL/min at column head pressure Purge flow to split vent 200 mL/min @ 3.01 min Gas Saver 100 mL/min @ 13 min
Liner	Standard deactivated liner filled with glass wool
GC:	
Temperature	60 °C (1 min); 40 °C/min; 120 °C; 5 °C/min; 310 °C, postrun 325 °C
Pneumatics	He, 1 mL/min, constant flow, postrun 1.5 mL/min for 3min Retention Time Locked (RTL) with chlorpyrifos-methyl at 18.111 min
Column	HP-5ms Ultra Inert 30 m, di=0.25 mm, df=0.25 µm (PN)
MS/MS:	
Ionization	Electron impact (EI) mode at 70 eV, except for cypermethrin and benzo[a]pyrene 110 eV
Mode	Multi reaction monitoring (MRM), see table 1
Source	300 °C
Quadrupole	150 °C
Collision Gas	N ₂ , 1.5 mL/min
Quenching Gas	He, 4 mL/min
Transferline	300 °C

Results and Discussion

A thorough method development was essential to meet all challenges posed by this application. Many parameters were evaluated and optimized to achieve best possible sensitivity over a broad range of compounds, e.g. polar and nonpolar, volatile and nonvolatile. We explored TDU/CIS parameters by an experimental design approach, chose optimal MRMs for every compound and tested different modifiers to access particle-adsorbed analytes. With the final method we determined around 100 relevant compounds in surface waters in the sub ng/L range. Comprehensive validation data was collected and the method was applied successfully to real world samples. EU-WFD requirements for inland surface water were fulfilled for all included analytes except cypermethrin, heptachlor and heptachlorepoide for which the required LOQs at 0.06pg/L and 2.4pg L could not be reached.

Limits of Quantification

Limits of quantification and limits of detection were calculated from calibration lines near the expected LOQ as per the requirements of DIN 32645 [8]. If the blank analysis revealed a significant analyte peak, LOQs were determined based on repeat analyses (n=8) of blank samples. At the LOQ, the quantifier/qualifier peak area ratio must be in a range between 80 and 120 % of the expected ratio for at least one qualifier. Furthermore, the relative standard deviation (%RSD) of repeat analyses at the LOQ level must be below 20% and the trueness between 80 and 120%. If these additional specifications were not met at the calculated LOQ the LOQ value was increased to a concentration at which all specifications were met.

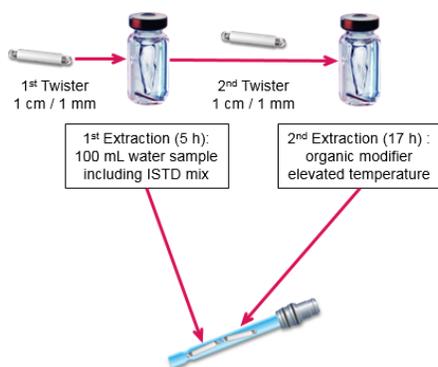


Figure 4. Sequential SBSE extraction workflow employed for analysis of surface water samples.

If feasible, i.e. if the background concentration of an analyte was low, river water was used as matrix when establishing LOQ. For certain compounds like PAHs the background value of the available river water was too high. In these cases, either tap or mineral water were used as matrix for LOQ determination. Generally, all validation data strongly depend on the level of background contamination and its uniformity. Moreover, the background contamination of the laboratory and the analysts' skill and experience will influence the quality of the validation data

The following table presents the determined LOQs for all analytes and their required LOQs from EU-WFD and other legislation. In cases where the EQS refers to a sum of multiple analytes the required LOQ was calculated separately for each of these analytes. This is a very strict interpretation assuming that only one of the respective analytes is present and all others are not.

<< See Table 2 Results in the Appendix >>

The LOQ for cypermethrin of 0.12 ng/L missed the required LOQ of 0.024 ng/L for inland surface waters by a modest factor 5. By employing negative chemical ionization (NCI) instead of EI the required LOQ is likely to be achievable. To date there is presumably no technique available at all to reach the required LOQ of 0.06 pg/L for heptachlor and heptachlorepoxide for inland surface waters.

The following chromatograms (figures 5a-q) show representative analyte peaks (quantifier and qualifier MRMs) near their respective LOQs.

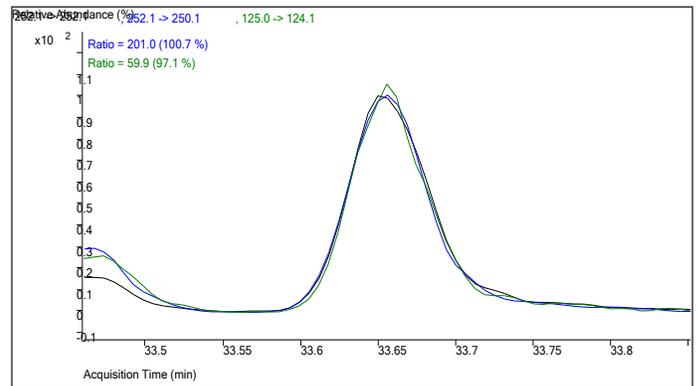


Figure 5a. Benzo[a]pyrene peak at 0.040 ng/L in mineral water. LOQ: 0.033 ng/L.

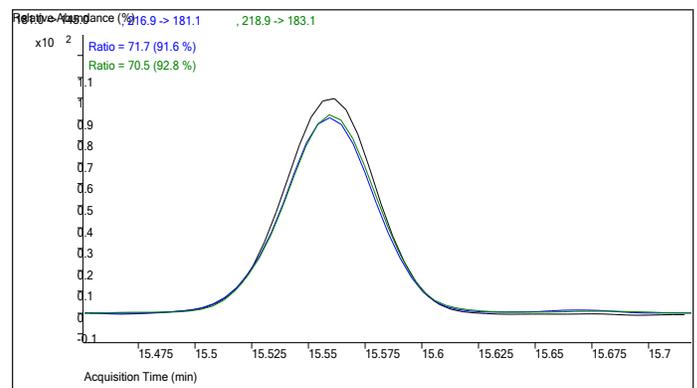


Figure 5b. gamma-HCH peak at 0.052 ng/L in mineral water. LOQ: 0.052 ng/L.

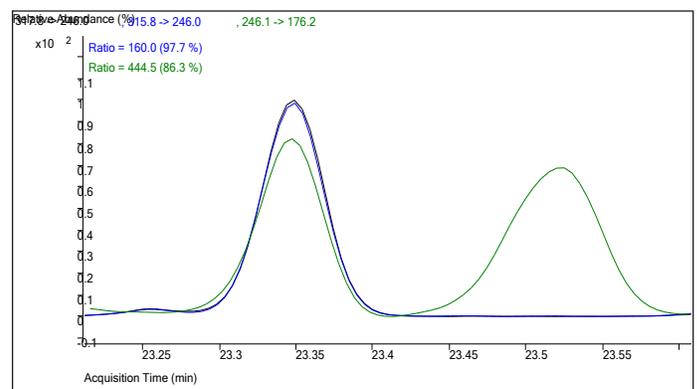


Figure 5c. p,p'-DDE peak at 0.026 ng/L in mineral water. LOQ: 0.017 ng/L.

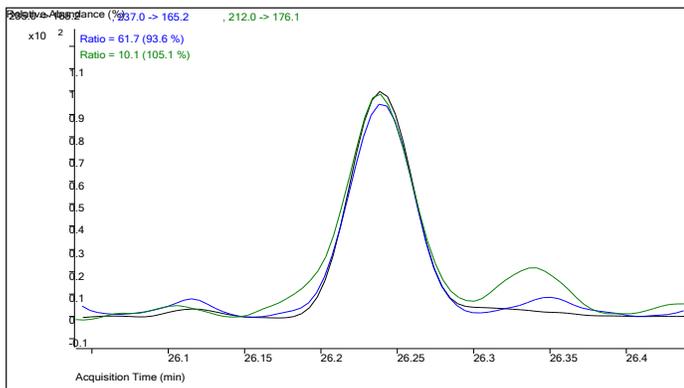


Figure 5d. p,p'-DDT Peak at 0.068 ng/L in river water. LOQ: 0.067 ng/L.

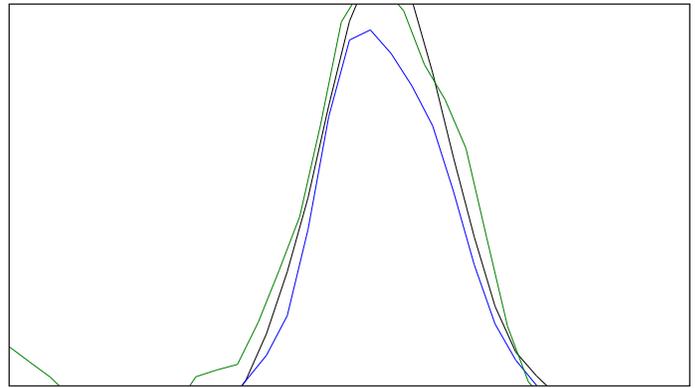


Figure 5g. Cybutryne peak at 0.030 ng/L in mineral water. LOQ: 0.030 ng/L.

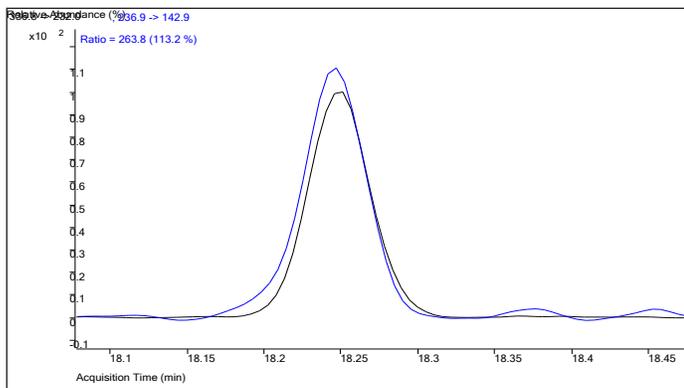


Figure 5e. Heptachlor peak at 0.052 ng/L in river water. LOQ: 0.052 ng/L.

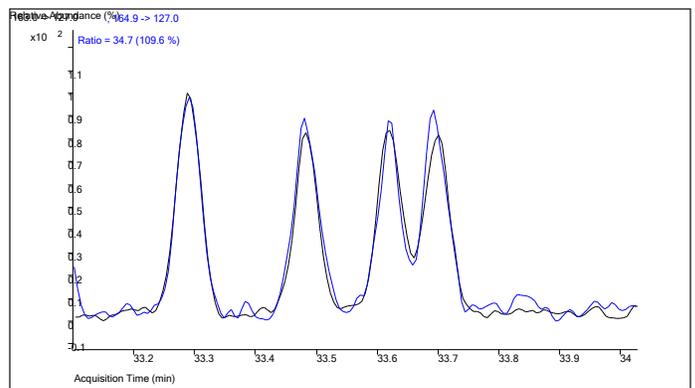


Figure 5h. Cypermethrin peaks at 0.12 ng/L in river water. LOQ: 0.12 ng/L.

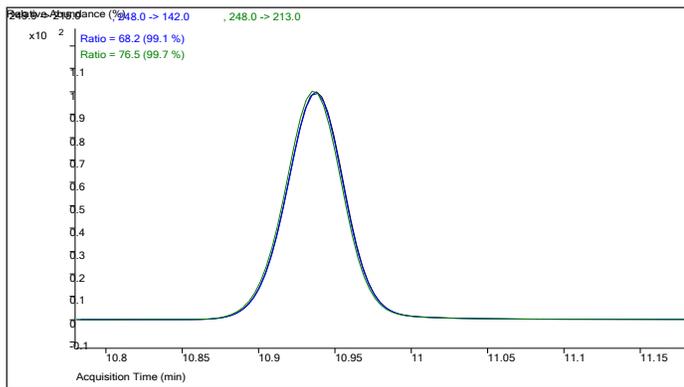


Figure 5f. Pentachlorobenzene peak at 0.16 ng/L in river water. LOQ: 0.075 ng/L.

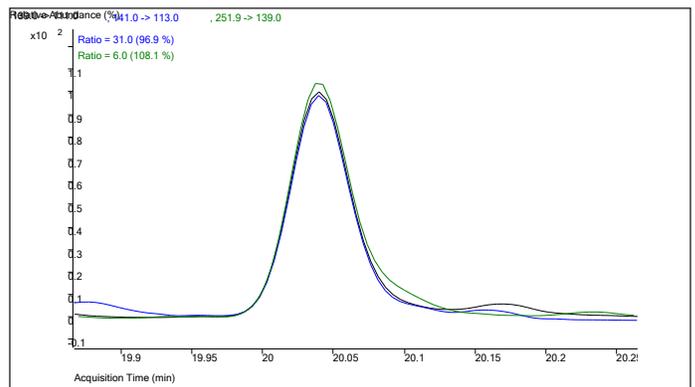


Figure 5i. Dicolfol peak at 0.15 ng/L in river water. LOQ: 0.15 ng/L.

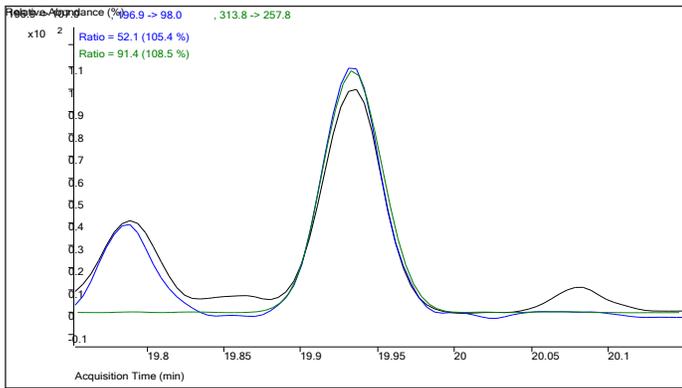


Figure 5j. Chlorpyrifos-ethyl peak at 0.024 ng/L in river water. LOQ: 0.024 ng/L.

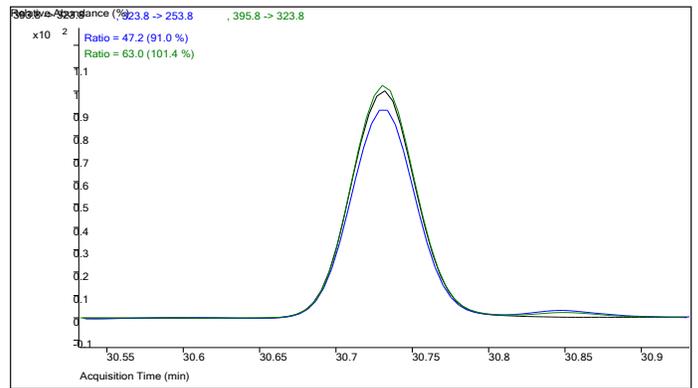


Figure 5m. PCB 189 peak at 0.060 ng/L in river water. LOQ: 0.054 ng/L.

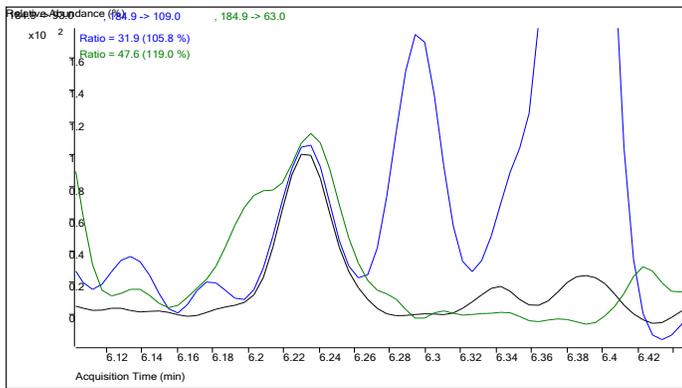


Figure 5k. Dichlorvos peak at 0.073 ng/L in river water. LOQ: 0.073 ng/L.

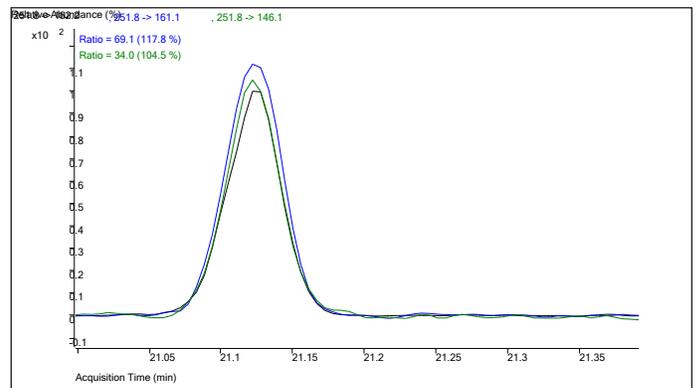


Figure 5n. Pendimethalin peak at 0.10 ng/L in river water. LOQ: 0.094 ng/L.

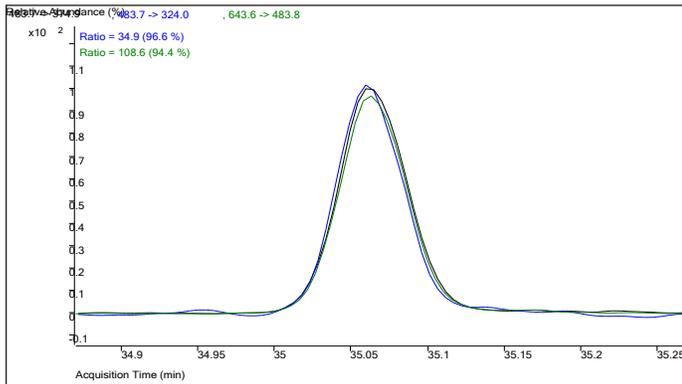


Figure 5l. PBDE 154 peak at 0.021 ng/L in river water. LOQ: 0.020 ng/L.

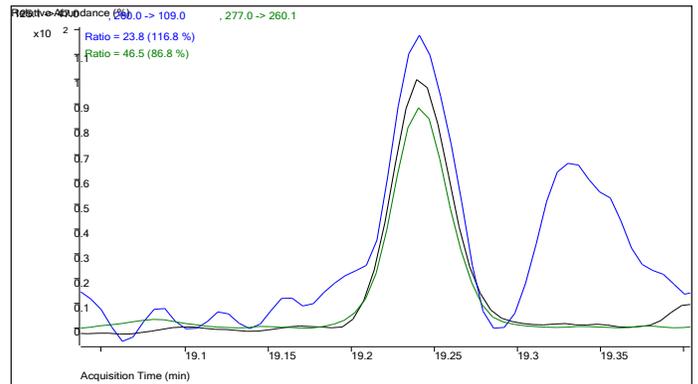


Figure 5o. Fenitrothion peak at 0.027 ng/L in river water. LOQ: 0.024 ng/L.

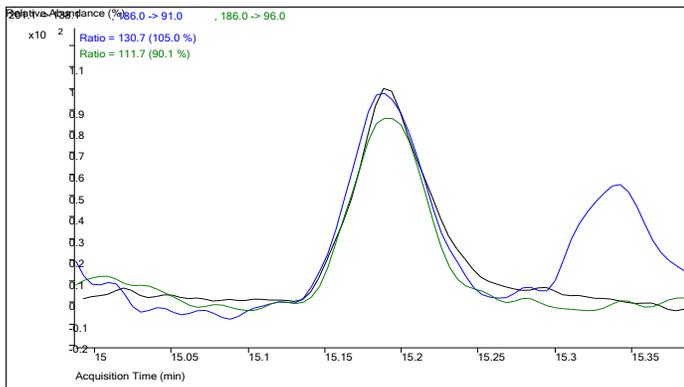


Figure 5p. Simazine peak at 2.4 ng/L in river water. LOQ: 1.9 ng/L.

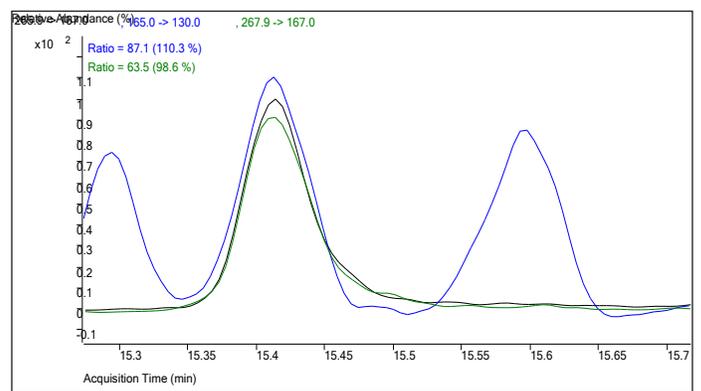


Figure 5q. Pentachlorophenol peak at 6.4 ng/L in mineral water. LOQ: 3.0 ng/L.

Repeatability and Trueness

Repeat analyses (n=6) of mineral water samples spiked at levels close to the respective LOQs revealed relative standard deviations between 1 and 15 % for all compounds with an average of 6.9%. Trueness was between 90 and 110% for most analytes.

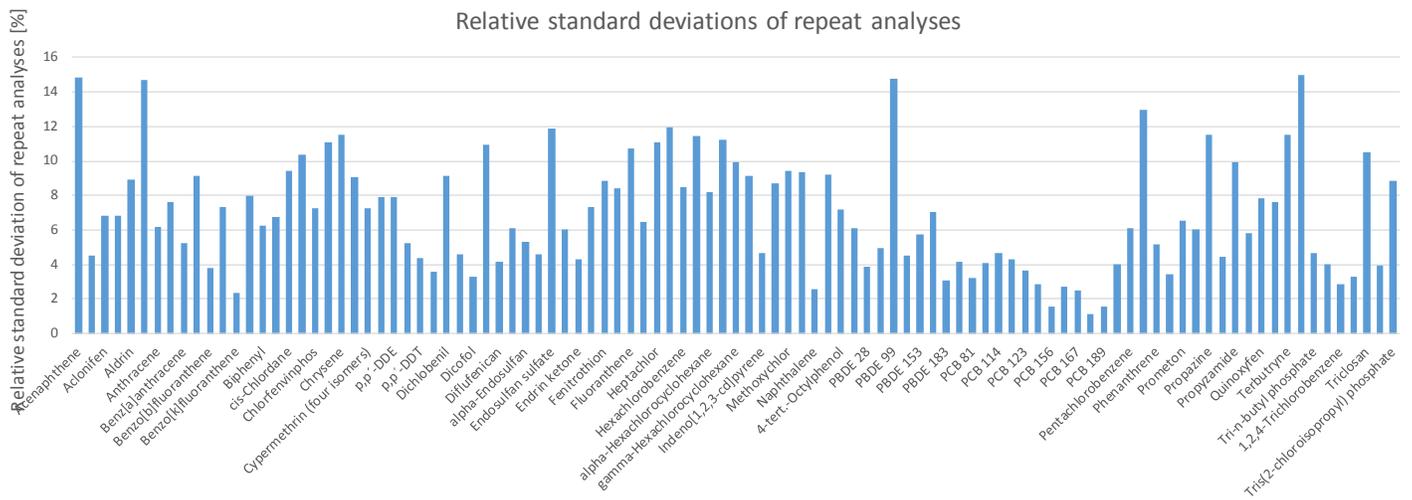


Figure 6. Relative standard deviations of analysis values of spiked mineral water near the respective LOQs (n=6).

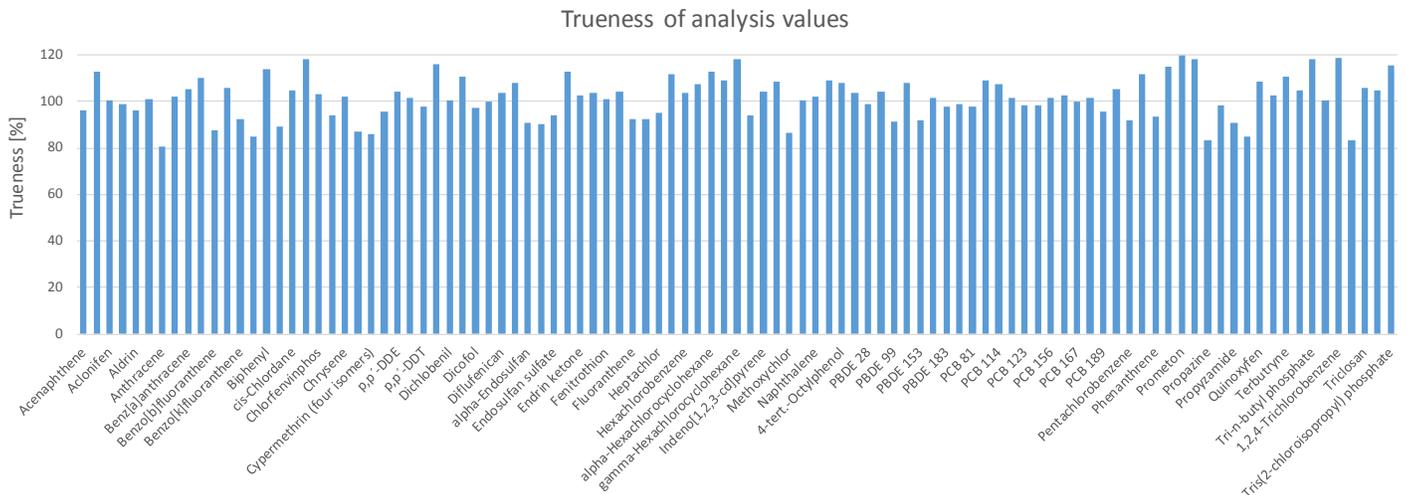


Figure 7. Average trueness of analysis values of spiked mineral water near the respective LOQs (n=6).

Uncertainty of measurement

Relative uncertainty of measurement was calculated from six repeat analyses with the aid of an Excel sheet of the University Stuttgart, Germany (Freeware, © 2015 Dr. M. Koch, Institut für Siedlungswasserbau, Universität Stuttgart, www.aqsbw.de [9]). According to 2009/90/EC [4] the relative uncertainty of measurement at the respective EQS may not exceed 50% which is fulfilled for analytes from the EU-WFD.

<< See Table 3 Results in the Appendix >>

Calibration

The calibration range was dependent on the compound LOQ and for most compounds, the calibration range span 50 fold to 250-fold their LOQ.

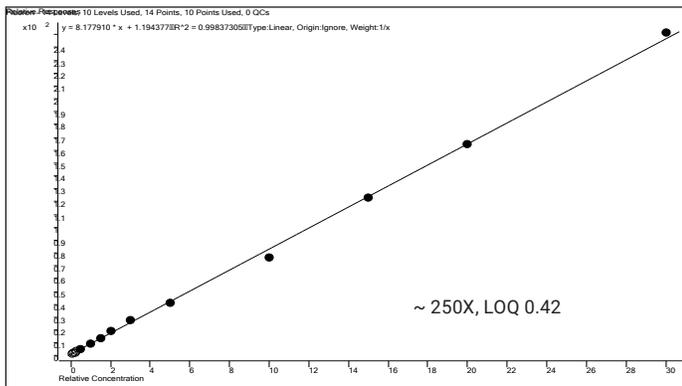


Figure 8a. Linear calibration function for fluorene in the range between 0.5 and 30 ng/L.

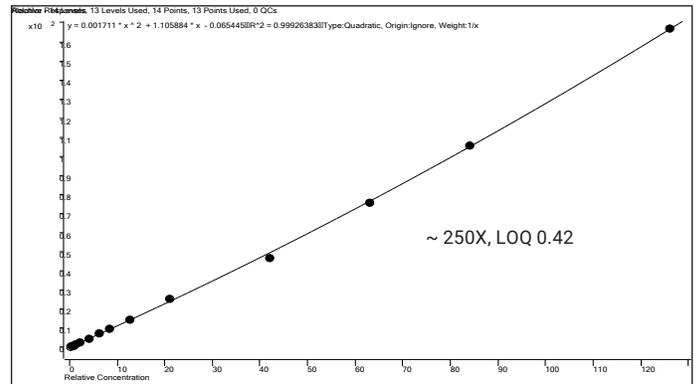


Figure 8b. Steepened quadratic calibration function for alachlor in the range between 0.53 and 126 ng/L.

Quantitation of particle-adsorbed analytes

The correct quantitation of particle-adsorbed compounds like PAHs was examined and confirmed with the aid of certified reference sediment (WEPAL SETOC 745, channel sludge). Analysis results were given for PAHs and some OCPs by the certificate.

Clean tap water samples of 100 mL were spiked with 5, 10 or 15 mg reference sediment and 30 μ L of the internal standard solution. This corresponds to particle content levels of 50, 100 or 150 mg/L, which is a realistic range for real surface water samples. The resulting water concentration of the contaminants - if all pollutants would have been 100% released from the particulates - would have been in the range of 0.05 -10.7 ng/L.

The average analysis results obtained from four repeat analyses of spiked samples were compared to the theoretical analysis values calculated from the certificate of the sediment. For virtually all compounds, good agreement was achieved despite the very low concentrations.

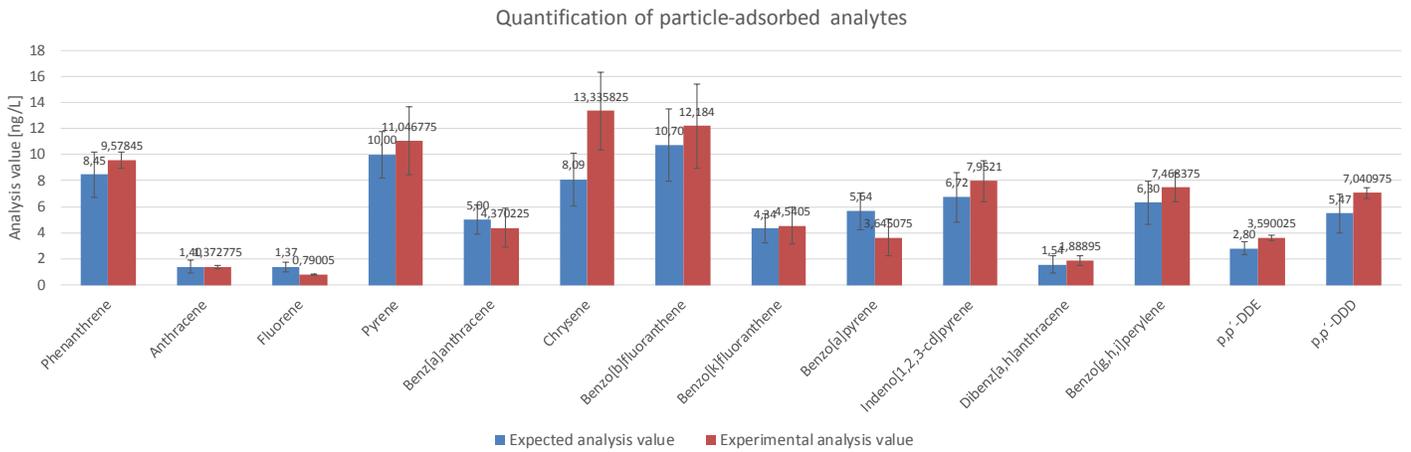


Figure 9a. Quantification of particle-adsorbed analytes shown for a certified reference sediment (WEPAL SETOC 745, channel sludge) at a concentration of 100 mg particles per liter water sample. Comparison between the theoretical analysis values calculated from the certificate and the experimental analysis values. Error bars represent the standard deviation of the measurements.

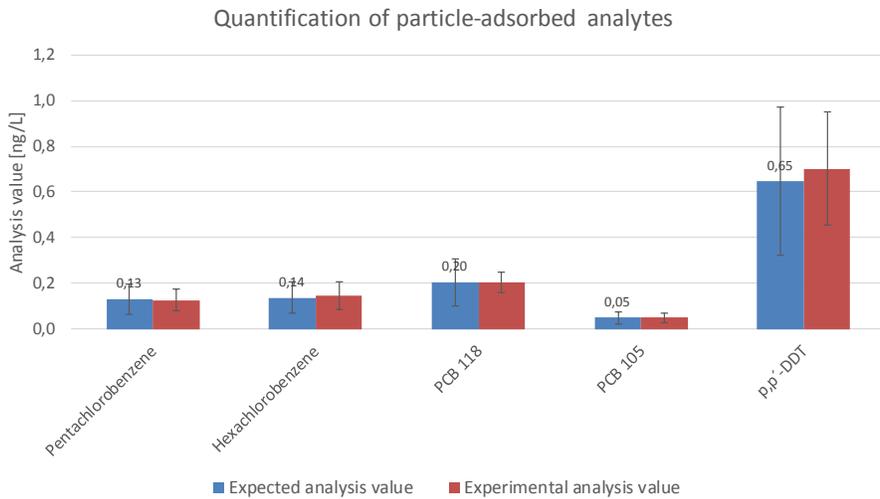


Figure 9b. Quantification of particle-adsorbed analytes shown for a certified reference sediment (WEPAL SETOC 745, channel sludge) at a concentration of 100 mg particles per liter water sample. Comparison between the theoretical analysis values calculated from the certificate and the experimental analysis values. Error bars represent the standard deviation of the measurements.

<< See Table 4 Results in the Appendix >>

Conclusion

The developed Twister-TDU-GC-MS/MS method enables the determination of around 100 relevant contaminants from as little as 100 mL surface water sample, including particle-adsorbed compounds, in a single analytical run. The Agilent Technologies 7010 triple quadrupole MS and the GERSTEL Twister represent the ideal combination for reaching LOQs in the low double-digit to low triple-digit pg/L range for the vast majority of analytes. Comprehensive validation data were collected and the method was successfully applied to real world samples. Relative standard deviations laid between 1 and 15 % for all compounds with an average of 6.9 % for N=6 at concentrations close to the LOQ. Trueness was mainly between 90 and 110 % (give average). Requirements of the EU-WFD for inland surface waters were fulfilled for all analytes with the exception of cypermethrin, heptachlor and heptachlorepoxide, for which the required LOQs are extremely low. "Other surface waters" as mentioned by the EU-WFD, such as coastal waters, were not tested with this analysis workflow. The developed method is straightforward, requiring only limited manual work. The method is flexible and can readily be used to determine additional contaminants of relevance for the field of water analysis.

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