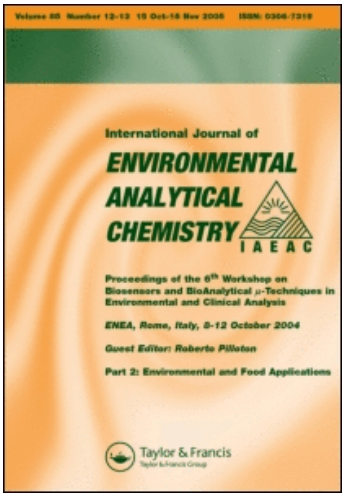

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Determination of $^{13}C/^{12}C$-ratios of anthropogenic organic contaminants in river water samples by GC-irmMS

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This study describes the application of a common analytical procedure adapted for compound-specific stable carbon isotope analyses of riverine contaminants. To evaluate the sensitivity of the analytical method and the precision of the isotopic data obtained, a set of numerous substances at different concentration levels were measured. For most of the anthropogenic contaminants investigated (including chlorinated aliphatics and aromatics, musk fragrances, phthalate-based plasticizers and tetrabutyl tin) acceptable carbon isotope analyses could be obtained down to amounts of approximately 5 ng absolutely applied to the gas chromatograph. These amounts correspond to concentrations in water samples at a natural abundance level of approximately 50–200 ng L^{-1} (low to medium contaminated river systems). However, it has to be considered that the precision and the sensitivity of the analytical method depend partially on the chemical properties of the substances measured. Five recovery experiments were conducted to assess changes in carbon isotope ratios during sample preparation and measurement. The compounds selected for these experiments are known riverine contaminants. Isotopic shifts or higher variations of the isotope ratios as a result of the analytical procedures applied were observed only for a couple of contaminants. Furthermore, compound-specific carbon isotope analyses were performed on eight water extracts of the Rhine river. By comparing the variation of the data of several individual compounds with the deviations obtained from the recovery experiments, it was possible to differentiate contaminants with unaffected isotope ratios and substances with significant alterations of the $\delta^{13}C$-values.

Keywords: Compound-specific isotope analyses; Carbon stable isotopes; GC-irmMS; Organic contaminants; River water

1. Introduction

First studies combining gas chromatography and mass spectrometry to determine variations in the stable carbon isotope composition of organic compounds were reported in 1976 and 1978 [1, 2]. Matthews and Hayes referred to their analytical approach as 'isotope ratio monitoring mass spectrometry' (irmMS), and approximately 10 years later, this technique was commercially implemented. Subsequently, the GC/irmMS technique and the corresponding compound-specific carbon isotope

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analyses were applied to numerous fields of analytical research comprising, for example, food chemistry, organic geochemistry, archaeology, medicine and pharmacology (see also [3, 4], and citations therein).

Previously, compound-specific carbon isotope analyses were also performed in environmental studies. Investigations applied to field samples of the aquatic environment focused mainly on polycyclic aromatic hydrocarbons, monoaromatic compounds and halogenated aliphatics (e.g., [5–8]). In addition, several laboratory studies were reported investigating the isotopic fractionation as a result of environmental processes. Transport and transformation processes (e.g., vaporization, adsorption, abiotic and biotic degradation) of several contaminants comprising also polycyclic aromatic hydrocarbons as well as halogenated aliphatics and aromatics have been investigated (e.g., [9–14]). Carbon isotope ratios are useful data not only for evaluating the fate of organic pollutants in the environment but also for distinguishing between different emission sources discharging the same contaminants. Therefore, isotopic characterization, especially of technical mixtures of chlorinated aromatics, was carried out (e.g., [15, 16]).

All of these isotopic studies were performed on systems with elevated contamination levels or high concentrations measured and mostly on simple compound mixtures. These applications avoid the two major limitations of compound-specific isotope analyses. These limitations lie in the lower sensitivity as compared with traditional GC/MS analyses and in the requirement of complete gas chromatographic separation of the contaminants. However, natural aquatic systems, especially riverine or groundwater systems, are mostly contaminated at a low or medium level, and therefore, the concentrations of individual organic compounds are low. Consequently, information on the isotopic characterization of contaminants existing in these water systems has not been available until now.

In this context, the present investigation focused on the evaluation of a common analytical procedure adapted for the preparation of material utilizable for compound-specific stable carbon isotope analyses of riverine contaminants. In particular, the sensitivity of the analytical method and the precision of the isotopic data obtained were studied. So far, this analytical procedure has been applied to river water samples for qualitative and quantitative GC/MS analyses [17, 18].

2. Experimental

2.1. *Samples*

River water samples were taken on 9 March 2001 at eight sampling locations from the Rhine river (Germany) between Koblenz and Venlo (see figure 1). Two-litre water samples were scooped up from below the water surface at a distance of approximately 2 m from the river side and bottled in pre-cleaned glass flasks. Filled sample flasks were sealed free of air bubbles with glass stoppers. They were then stored in the darkness at a temperature of approximately 4°C.

2.2. *Extraction*

A sequential liquid/liquid extraction procedure was applied to approximately 1000-mL aliquots of the water samples using the solvents *n*-pentane and dichloromethane

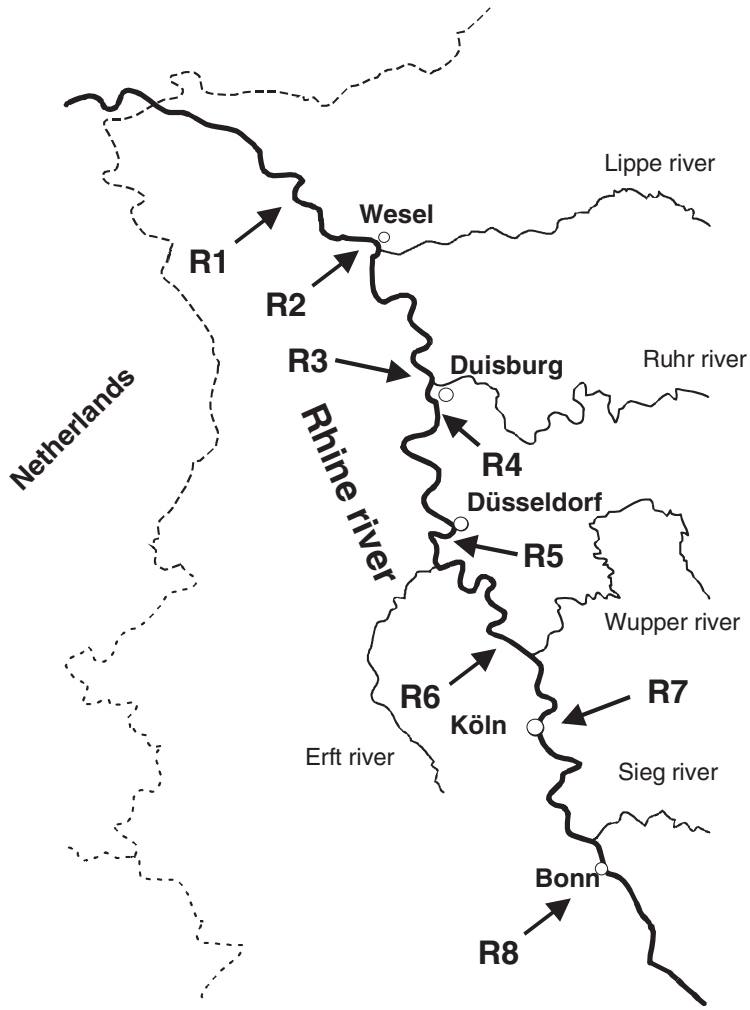

Figure 1. Sampling locations at the Rhine river.

(according to [18]). Each extraction step was carried out in a separating funnel with 50 mL of the solvent. Subsequently, the organic layers were separately dried by filtration over 1 g of anhydrous granulated sodium sulphate (Merck, Darmstadt, Germany), and 50 μL of an internal standard solution containing 12.0 ng μL^{-1} d_{34}-*n*-hexadecane in *n*-hexane was added. Prior to GC/MS and GC/irmMS analyses, the extracts were reduced to a final volume of approximately 10–20 μL by rotary evaporation at room temperature. Inaccuracies of injection or sample volumes during gas-chromatographic analysis were considered by the internal standard.

2.3. *Materials*

All reference substances were purchased from Promochem (Germany), Merck (Germany) and Aldrich (Germany). To minimize sample contamination only

pre-cleaned glass, metal and Teflon equipment was used in the laboratory. All solvents were purchased from Merck (Germany), and distilled over a 0.5 m packed column (reflux ratio approximately 1 : 25). The purity was tested by gas chromatographic analyses. Anhydrous granulated sodium sulphate (Merck, Germany) was cleaned with pure *n*-hexane.

Blank analyses were run to determine background concentrations of the investigated compounds. Only phthalates were detected in the blank with concentrations up to 5%, as compared with the investigated river water samples.

2.4. *Recovery experiments*

Recovery experiments were performed by spiking high-purity water (Lichrosolv, Merck, Germany) with concentrations of 800–1500 ng L^{-1} of the respective reference compounds dissolved in methanol (see table 1). After an equilibration time of 12 h, the extraction procedure was performed as described above, and the extracts were analysed by GC/MS and GC/irmMS. Former investigations revealed recovery rates of 25–95% for the individual substances [18, 19].

2.5. *GC-MS analyses*

GC/MS analyses were performed on a Trace MS mass spectrometer (Thermoquest, Egelsbach, Germany) linked to a Mega Series gas chromatograph (Carlo Erba, Milano, Italy) which was equipped with a 30 m × 0.22 mm i.d. × 0.25 μm film BPX5 fused silica capillary column (SGE, Weiterstadt, Germany). The chromatographic conditions were: 0.5 μL split/splitless injection at 60°C, splitless time 60 s, 3 min hold, then programmed at 3°min^{-1} to 300°C, and helium carrier gas velocity 40 cm s^{-1}.

The mass spectrometer was operated in electron impact ionization mode (EI^+, 70 eV) with a source temperature of 200°C scanning 35–700 amu at a rate of 0.5 s $decade^{-1}$.

2.6. *GC-irmMS analyses*

Compound-specific carbon isotope analyses were carried out on a Delta Plus XL mass spectrometer (ThermoFinnigan, Bremen, Germany) equipped with a GCC III combustion interface and linked to a gas chromatograph 6980A (Agilent, Waldbronn, Germany). Gas-chromatographic separation was performed either on a 30 m × 0.25 mm i.d. × 0.25 μm film HP1 fused silica capillary column (precision and recovery experiments) or on a 30 m × 0.22 mm i.d. × 0.25 μm film BPX5 fused silica capillary column (Rhine river water samples). Chromatographic conditions were: 2 μL split/splitless injection at 60°C, splitless time 60 s, 3 min hold, then programmed at 3°min^{-1} to 300°C, helium carrier gas velocity for both capillary columns was set to 35 cm s^{-1}. The oxidation of the eluting substances was carried out at 940°C facilitated by a CuO/NiO/Pt-catalyst. Unless specifically noted, all stable carbon ratio measurements of field samples and recovery experiments were performed in triplicate. Isotopic data of pure reference compounds were obtained by 10 repetitions of the measurements.

The carbon isotope ratio of the reference gas (carbon dioxide) was calibrated with a certified reference standard purchased from Chiron (Trondheim, Norway) containing

Table 1. δ^{13}C-values of selected organic compounds measured at different concentration levels.[a]

Concentration levels (n = repetitions)		*n*-Pentadecane	*n*-Docosane	*n*-Hexacosane	*n*- Tetradecanoic acid methylester	*n*- Pentadecanoic acid methylester	*n*-Hexadecanoic acid methylester	2,2,4,4,6,8,8- Heptamethylnonane	Dimethylphthalate	Diethylphthalate	Di-*iso*-butylphthalate	Di-*sec*-butylphthalate	Di-*n*-butylphthalate	Bis-(2-ethylhexyl)phthalate	Cashmerane	Celestolid	Pantolide	Tetrabutyl tin	Hexachlorobutadiene	Pentachlorobenzene	Hexachlorobenzene
	Relative carbon fraction of compounds (%)	85	85	85	74	75	76	85	62	65	69	69	69	74	82	84	84	55	16	29	26
1 ng level	Amount (ng)	0.8	0.9	0.9	1.3	1.2	1.0	0.9	0.8	0.9	1.1	0.9	0.9	1.0	1.0	1.1	0.9	0.8	0.8	1.3	0.8
	δ^{13}C-values (‰ VPDB)	nd	nd	nd	−27.6	−29.9	−28.6	nd	nd	nd	−24.8	−26.4	−25.6	−27.0	−27.8	−24.8	−28.0	nd	nd	nd	nd
(n = 10)	SD (‰ VPDB)				1.7	1.7	1.5				2.4	2.0	2.6	1.7	1.2	0.8	0.7				
5 ng level	Amount (ng)	4.1	4.4	4.5	6.6	5.9	5.2	4.4	3.9	4.7	5.3	4.6	4.4	5.0	5.1	5.6	4.3	3.9	3.8	6.6	3.9
	δ^{13}C-values (‰ VPDB)	−30.0	−24.5	−30.4	−28.9	−30.1	−29.9	−28.5	−24.6	−25.8	−26.8	−27.3	−27.7	−26.9	−27.8	−24.8	−27.8	−24.0	−23.9	−20.8	−24.3
(n = 10)	SD (‰ VPDB)	1.0	0.8	0.8	1.2	1.1	0.8	0.5	1.1	0.7	0.6	0.5	0.6	0.4	0.3	0.5	0.5	0.4	2.2	1.0	2.3
10 ng level	Amount (ng)	8.2	8.7	8.9	13.1	11.7	10.5	8.7	7.8	9.5	10.6	9.1	8.7	10.0	10.2	11.1	8.8	7.9	7.6	13.2	7.7
	δ^{13}C-values (‰ VPDB)	−30.00	−24.7	−29.4	−30.6	−29.8	−30.2	−29.7	−25.9	−26.1	−27.2	−28.0	−27.8	−27.5	−27.9	−25.6	−29.0	−25.2	−24.6	−21.5	−25.0
(n = 10)	SD (‰ VPDB)	0.3	0.3	0.5	0.6	0.5	0.6	0.6	0.4	0.3	0.4	0.6	0.2	0.4	0.3	0.4	0.6	1.2	3.2	1.0	3.2
25 ng level	Amount (ng)	20.4	21.8	22.3	32.8	29.3	26.2	21.8	19.4	23.7	26.6	22.8	21.8	25.1	25.5	27.8	21.4	19.7	19.1	33.1	19.3
	δ^{13}C-values (‰ VPDB)	−30.8	−25.1	−29.5	−30.6	−29.5	−30.1	−30.1	−26.1	−25.8	−27.0	−28.1	−27.9	−27.2	−27.4	−24.1	−27.5	−25.1	−25.8	−22.2	−25.9
(n = 10)	SD (‰ VPDB)	0.5	0.3	0.3	0.5	0.6	0.3	0.7	0.4	0.2	0.2	0.3	0.3	0.3	1.04	0.67	0.9	0.8	2.9	0.8	0.8

[a] All substances are well-known riverine contaminants (nd: not detected).

n-undecane (−26.11% *vs* VPDB), *n*-pentadecane (−30.22% *vs* VPDB) and *n*-eicosane (−33.06% *vs* VPDB). All data presented are expressed relative to the VPDB standard.

3. Results and discussion

3.1. *Stable carbon isotope analyses of selected anthropogenic contaminants*

To evaluate the sensitivity and precision of compound-specific carbon isotope analyses of river water contaminants, a set of numerous anthropogenic and biogenic substances at different concentration levels were measured. Besides alkanes and fatty acid methyl esters, xenobiotical compounds were considered, including chlorinated aliphatics and aromatics, musk fragrances, phthalate-based plasticizers and tetrabutyl tin. To prevent any gas chromatographic coelutions, the compounds were divided into appropriate subsets prior to the measurements. The resolution of the individual chromatograms is illustrated in figure 2. All analyses were performed at four different concentration levels representing either a sufficient amount with respect to the technical conditions of the GC-irmMS system (approximately 25 ng; see table 1) or amounts prevalently detected during analyses of low contaminated river water extracts (1–5 ng; see table 1). Considering the analytical procedures applied to the river water samples (see section 3.2), the amounts selected represent concentration ranges of approximately 10–1000 ng L^{-1} in field water samples.

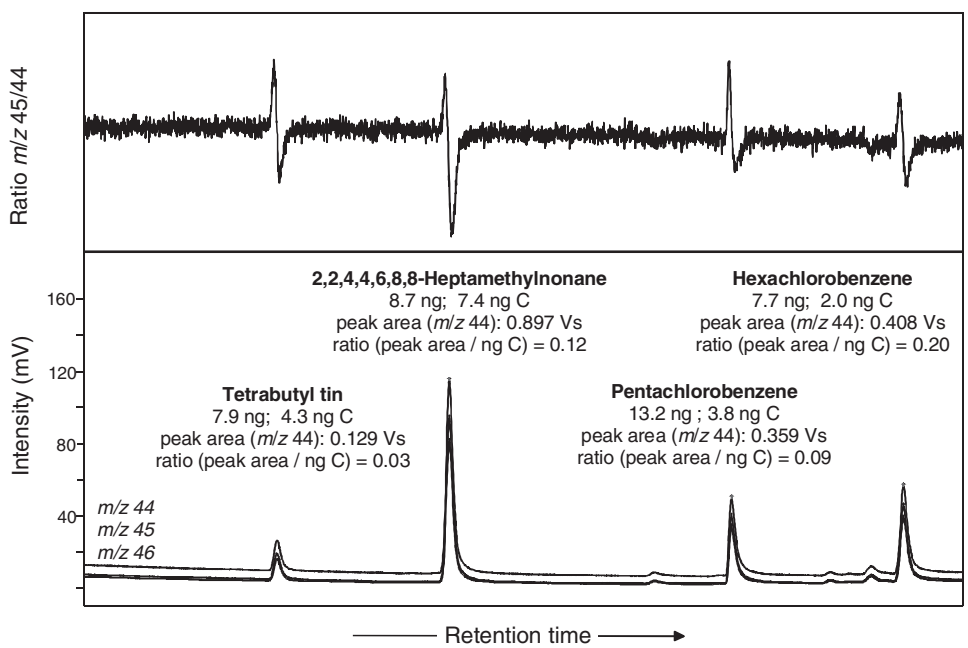

Figure 2. GC-irmMS chromatogram and corresponding m/z 44/45 ratio chromatogram obtained from a standard solution at the 10 ng level. Additionally, data of amounts (ng), corresponding amounts of carbon (ng C), the peak area derived from the m/z 44 trace (Vs) as well as the ratios of peak areas and carbon amounts (Vs/ng C) are given for all individual compounds.

All δ^{13}C-values determined are presented in table 1 and range between approximately −20 and −30‰. The precision of the stable carbon isotope ratios detected is characterized by the standard deviation obtained by 10 repetitions of the measurements. These values covered the range of 0.2–3.2‰. Generally, increasing standard deviation values were observed with decreasing amounts as a result of the technical limitations imposed by the irmMS system. Data obtained for the 25- and 10 ng levels are generally characterized by satisfactory deviations between 0.2 and 0.7‰. On the contrary, at the 5 ng level, an increasing number of analyses revealed deviations of more than 0.7‰. Furthermore, at the 1 ng level, nearly all compounds were not detected at all, or their δ^{13}C-values exhibited standard deviations higher than 1.0‰.

Additionally, significant variations of the standard deviation values were observed with respect to the individual compounds. The precision of δ^{13}C-values of *n*-alkanes, 2,2,4,4,6,8,8-heptamethylnonane, fatty acid methyl esters, phthalates and musk fragrances was generally acceptable down to amounts of approximately 5 ng, with standard deviation values below 1.0‰. On the contrary, already at the 25 and 10 ng levels, the stable carbon isotope ratios of the chlorinated compounds (Cl_5- and Cl_6-benzene, Cl_6-butadiene) as well as of tetrabutyl tin were measured with high standard deviations of 0.8–3.2% Some influence on the precision of carbon isotope analyses by halogen and tin atoms became evident, possibly as a result of the low fraction of carbon in the polychlorinated substances (16–29%; see table 1) minimizing the effective amount of CO_2 measured in the irmMS. However, this assumption is contradicted by the varying standard deviations of analysis of individual compounds with comparable carbon amounts. For example, the precision of pentachlorobenzene analysis at the 25 ng level (representing approximately 10 ng carbon) of 0.7‰ SD was significantly higher as compared with analysis of cashmerane at the 10 ng level (also representing approximately 10 ng carbon) with a standard deviation of 0.28‰. Therefore, a major influence on the oxidation processes due to interactions of halogens or tin with the catalysts (primarily with the copper) can be assumed. This assumption is partially supported by a quantitative comparison of combustion yields, as illustrated in figure 2. Comparing the peak areas (*m*/*z* 44 trace) of pentachlorobenzene, hexachlorobenzene, tetrabutyl tin and 2,2,4,4,6,8,8-heptamethylnonane with the corresponding amounts of carbon (3.8, 2.0, 4.3, and 7.4 ng, respectively), significantly lower ratios are obvious for the tin-containing compound. This observation suggested incomplete combustion of tin organic compounds as the result of poisoning effects on the catalyst. The same effect cannot be stated for halogenated compounds. Penta- and hexachlorinated benzene generated a similar area to C-amount ratio as compared with the branched alkane representing very similar combustion yields for these compounds.

However, because many priority pollutants belong to the group of polyhalogenated substances, the low degree of precision in the concentration ranges investigated affects the compound-specific carbon isotope analysis of an important class of anthropogenic contaminants.

Considering both the compound and amount related effects compromising the precision of carbon isotope analyses, the sensitivity of the analytical method used can be appointed to an amount down to approximately 5 ng for numerous anthropogenic contaminants. However, it should be noted that in comparison, the precision of the analyses of halogenated and tin-containing compounds is generally lower.

3.2. *Recovery experiments*

An important precondition for the successful determination of carbon isotope ratios is the prevention of isotopic shifts as a result of the analytical procedures applied. Therefore, five recovery experiments were performed to detect changes in carbon isotope ratios during sample preparation and measurement. The compounds selected for these experiments are known riverine contaminants and comprise hexachlorobutadiene, several musk fragrances, phthalates and other plasticizers, a flame retardant and a pesticide. All recovery samples were spiked with concentrations of approximately 800–1500 ng L^{-1} for each compound representing a common abundance level in river systems.

The sequential extraction with *n*-pentane and dichloromethane resulted in an exclusive occurrence in one extract for most of the compounds selected. All musk fragrances investigated, most of the phthalates, *N*,*N*-diethyltoluamide (DEET), 2,2,4-trimethyl-1,3-pentandioldi-*iso*-butyrate, 2,6-di-*tert*-butyl-4-methylphenol and hexachlorobutadiene were detected in the pentane fractions. The more polar compounds 2,4,7,9-tetramethyl-5-decyne-4,7-diol, tri-*n*-butylphosphate and tris(2-chloroethyl)phosphate appeared exclusively in the dichloromethane fraction. 2-(2-Ethoxybutoxy)ethylacetate and dimethylphthalate were detected in both extracts with varying concentrations. All δ^{13}C-values obtained after the recovery procedure are presented in tables 2–4 in the order of their occurrence in the separate extracts. Because the gas chromatographic separations of 2,6-di-*tert*-butyl-4-methylphenol and cashmerane as well as galaxolide and tonalide were insufficient regarding the strict chromatographic requirements of compound-specific isotope analyses, the carbon isotope ratios were summarized for the unresolved peaks, respectively.

To illustrate the variations of the carbon isotope ratios measured after execution of the five recovery experiments, figure 3 presents the differences of the δ^{13}C-values of the recovered substances as compared with the untreated compounds noted as $\Delta\delta^{13}$C-values.

A first group of contaminants including nearly all phthalates and musk fragrances, as well as 2-(2-ethoxybutoxy)ethyl acetate and 2,2,4-trimethyl-1,3-pentandioldi-*iso*-butyrate were recovered with only minor changes in δ^{13}C-values as compared with the untreated substances. Additionally, acceptable standard deviations were obtained for the analyses of these compounds (see tables 2–4), and a slight tendency to lower δ^{13}C-values was observed for almost all compounds of this first group. Pantolide, tri-*n*-butylphosphate, and bis(2-ethylhexyl)phthalate represent a second group of substances. This group is characterized by a distribution of the δ^{13}C-values with differences up to 2‰ as compared with the original data of the untreated substances. General trends towards lower as well as higher values were observed.

A third group is characterized by significant excursions of the carbon isotope ratios of up to 3‰ after application of the analytical procedures. These comprise hexachlorobutadiene, 2,4,7,9-tetramethyl-5-decyne-4,7-diol, cashmerane/2,6-di-*tert*-butyl-4-methylphenol, tris(2-chloroethyl)phosphate and diethyltoluamide (DEET). Interestingly, both halogenated compounds are included in this group reflecting also difficulties in precise carbon isotope analyses of chlorinated contaminants. The variations of the δ^{13}C-values of the coeluting compounds cashmerane and ionol might be the effect of changing composition of the gas-chromatographic peak as the result of different or fluctuating recovery rates. On the contrary, the physico-chemical properties and,

Table 2. δ^{13}C-values of compounds exclusively detected in the *n*-pentane extracts of the recovery experiments.

Recovery experiments		Hexachlorobutadiene	Cashmeran/2,6-di-*tert*-butyl-4-methylphenol	Celestolide	Pantolide	Galaxolide/Tonalide	Diethylphthalate	Di-*iso*-butylphthalate	Di-*sec*-butylphthalate	Di-*n*-butylphthalate	Bis(2ethylhexyl)phthalate	*N*,*N* -Diethyltoluamide (DEET)	2,2,4-trimethyl-1,3-pentandioldi-*iso*-butyrate
Original	δ^{13}C-values (‰ VPDB)	−32.1	−27.5	−26.0	−28.9	−27.8	−29.8	−27.5	−27.5	−27.6	−26.9	−25.8	−27.4
	SD (‰ VPDB)	1.3	0.4	0.5	0.3	0.2	0.2	0.1	0.5	0.3	0.4	1.6	0.1
1	δ^{13}C-values (‰ VPDB)	nd	−28.7	−25.5	−28.7	−28.5	−29.7	−28.2	−27.6	−28.3	−27.1	nd	nd
	SD (‰ VPDB)		0.5	0.1	0.2	0.1	0.3	0.2	0.1	0.3	0.4		
2	δ^{13}C-values (‰ VPDB)	−31.4	−28.6	−25.4	−27.1	−27.7	−29.8	−27.5	−26.5	−27.4	−27.0	−25.4	−27.7
	SD (‰ VPDB)	0.5	0.9	1.0	0.1	0.2	0.3	0.3	0.7	0.2	0.1	0.1	0.1
3	δ^{13}C-values (‰ VPDB)	−32.1	−29.7	−25.6	−28.6	−27.7	−30.1	−27.7	−27.1	−27.5	−29.0	nd	−28.0
	SD (‰ VPDB)	1.1	0.5	0.3	0.3	0.1	0.1	0.2	0.1	0.1	1.5		0.3
4	δ^{13}C-values (‰ VPDB)	−29.1	−28.9	−25.7	nd	−27.4	nd	−27.9	−27.7	−27.1	−27.9	−30.6	−27.8
	SD (‰ VPDB)	0.9	0.8	0.7		0.7		1.3	0.5	1.6	1.2	0.3	1.6
5	δ^{13}C-values (‰ VPDB)	−29.1	−27.4	−26.1	−28.9	−27.8	−29.7	−27.61	−27.0	−27.4	−26.8	nd	−27.8
	SD (‰ VPDB)	1.5	0.3	1.4	0.8	0.7	0.1	0.1	0.1	0.1	0.4		0.3

Table 3. δ^{13}C-values of compounds exclusively detected in the dichloromethane extracts of the recovery experiments.

Recovery experiments		2,4,7,9-Tetramethyl-5-decyne-4,7-diol	Tri-*n*-butyl-phosphate	Tris (2-chloroethyl-phosphate)
Original	δ^{13}C-values (‰ VPDB)	−24.4	−28.1	−29.6
	SD (‰ VPDB)	0.4	0.4	0.4
1	δ^{13}C-values (‰ VPDB)	−21.2	−26.9	−28.9
	SD (‰ VPDB)	0.7	1.8	0.3
2	δ^{13}C-values (‰ VPDB)	−21.2	−26.5	−30.4
	SD (‰ VPDB)	0.3	1.1	0.4
3	δ^{13}C-values (‰ VPDB)	−22.3	−27.6	−27.9
	SD (‰ VPDB)	0.8	0.7	0.4
4	δ^{13}C-values (‰ VPDB)	−24.4	nd	−28.3
	SD (‰ VPDB)	0.7		1.3
5	δ^{13}C-values (‰ VPDB)	−24.6	−26.0	−30.9
	SD (‰ VPDB)	0.4	1.3	0.7

Table 4. δ^{13}C-values of compounds detected in the pentane as well as in dichloromethane extracts of the recovery experiments.

Recovery experiments		2-(2-Ethoxybutoxy)-ethyl acetate		Dimethylphthalate	
Original	δ^{13}C-values (‰ VPDB)	−27.1		−26.5	
	SD (‰ VPDB)	0.2		0.3	
		Pentane	DCM	Pentane	DCM
1	δ^{13}C-values (‰ VPDB)	−27.2	−27.6	−26.5	−24.9
	SD (‰ VPDB)	0.5	0.7	0.3	0.5
2	δ^{13}C-values (‰ VPDB)	−27.9	−26.1	−26.8	−26.2
	SD (‰ VPDB)	0.1	0.1	0.3	0.1
3	δ^{13}C-values (‰ VPDB)	−27.4	−26.6	−27.1	−25.2
	SD (‰ VPDB)	0.1	0.2	0.7	0.4
4	δ^{13}C-values (‰ VPDB)	nd	−27.5	−27.4	−26.5
	SD (‰ VPDB)		2.0	0.8	0.4
5	δ^{13}C-values (‰ VPDB)	−27.6	−25.8	−26.7	−25.6
	SD (‰ VPDB)	1.7	0.3	0.1	0.3

consequently, also the recovery rates of the insufficiently separated musk fragrances galaxolide and tonalide are very similar, and therefore, more accurate carbon isotope ratios were measured for these combined compounds as described above.

Two compounds were detected in both extracts. The δ^{13}C-values of dimethylphthalate and 2-(2-ethoxybutoxy)ethyl acetate analysed in the pentane and the dichloromethane extracts varied slightly. Generally, a slight enrichment of the heavier isotope was observed for the compounds in the more polar dichloromethane extracts. These observations suggest only a minor isotopic fractionation during the extraction procedures. However, this assumption is limited to a quite balanced distribution of the compounds.

With respect to the different shifts of δ^{13}C-values in the recovery procedure, it should be noted that a satisfactory determination of carbon isotope ratios depends not only on the analytical methods applied but also on the individual substances analysed. Therefore, to evaluate the quality of compound-specific isotope analyses of riverine contaminants, measurements of field samples have to be accompanied by recovery experiments of individual substances.

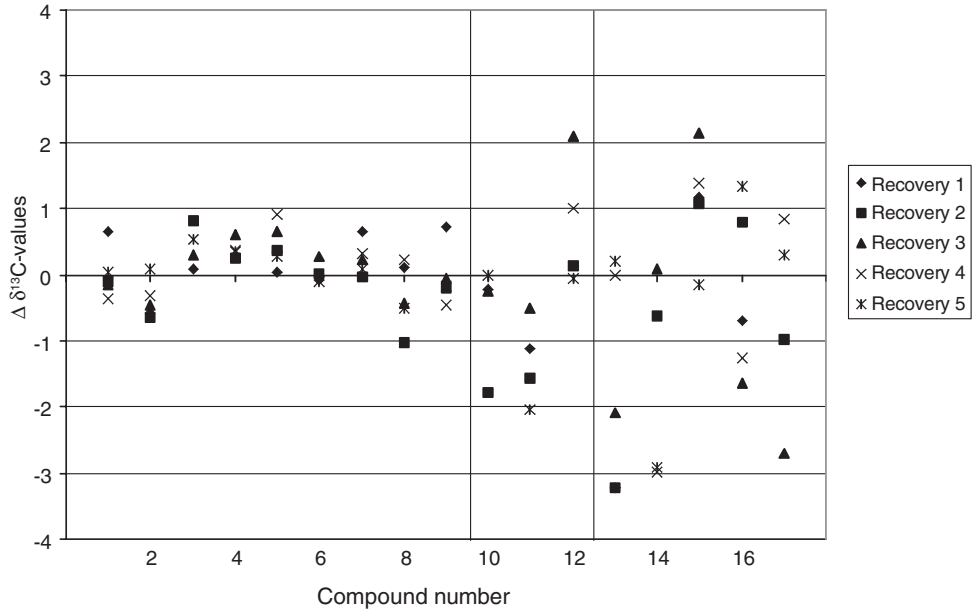

Figure 3. Differences of carbon isotope ratios measured after application of the recovery procedure. Compound numbers are: (1) galaxolide/tonalide (coeluting); (2) celestolide; (3) 2-(2-ethoxybutoxy)ethylacetate; (4) 2,2,4-trimethyl-1,3-pentandioldi-*iso*-butyrate; (5) dimethylphthalate; (6) diethylphthalate; (7) di-*iso*-butylphthalate; (8) di-*sec*-butylphthalate; (9) di-*n*-butylphthalate; (10) pantolide; (11) tri-*n*-butylphosphate; (12) bis(2-ethylhexyl)phthalate (DEHP); (13) 2,4,7,9-tetramethyl-5-decyne-4,7-diol; (14) hexachlorobutadiene; (15) cashmerane/ionol; (16) tris(2-chloroethyl)phosphate; (17) *N*,*N*-diethyltoluamide (DEET).

Furthermore, the matrix within the extracts might also affect the carbon stable isotope ratios. However, further purification or fractionation applied in order to minimize matrix effects is critical due to the low to very low amounts of the contaminants. A significant loss of numerous individual substances during further preparation steps and associated an isotopic fractionation has to be assumed. In particular, during stable carbon isotope ratio analysis of a wide range of chemically different contaminants, these adverse effects can appear. On the contrary, such an approach might be helpful in analysing only a preselected and small set of pollutants of similar chemical and physical properties, for which the preparation can be optimized in terms of concentration and isotope shifts.

3.3. *Compound-specific carbon isotope analyses of Rhine water samples*

Compound-specific carbon isotope analyses were applied to eight extracts of Rhine river water. Comparison of the chromatograms from GC/MS and GC/irmMS analyses shows a good correlation of the individual peaks as illustrated in figure 4.

For the carbon isotope analyses, a set of contaminants were selected considering the following criteria: (1) the substances were abundant in the river water extracts as revealed by GC/MS analyses; (2) the determination of the stable carbon isotope ratios was expected to be successful as indicated by the results presented above; and (3) reference material had to be available in order to verify the identification of individual compounds in case of uncertain gas-chromatographic correlations with

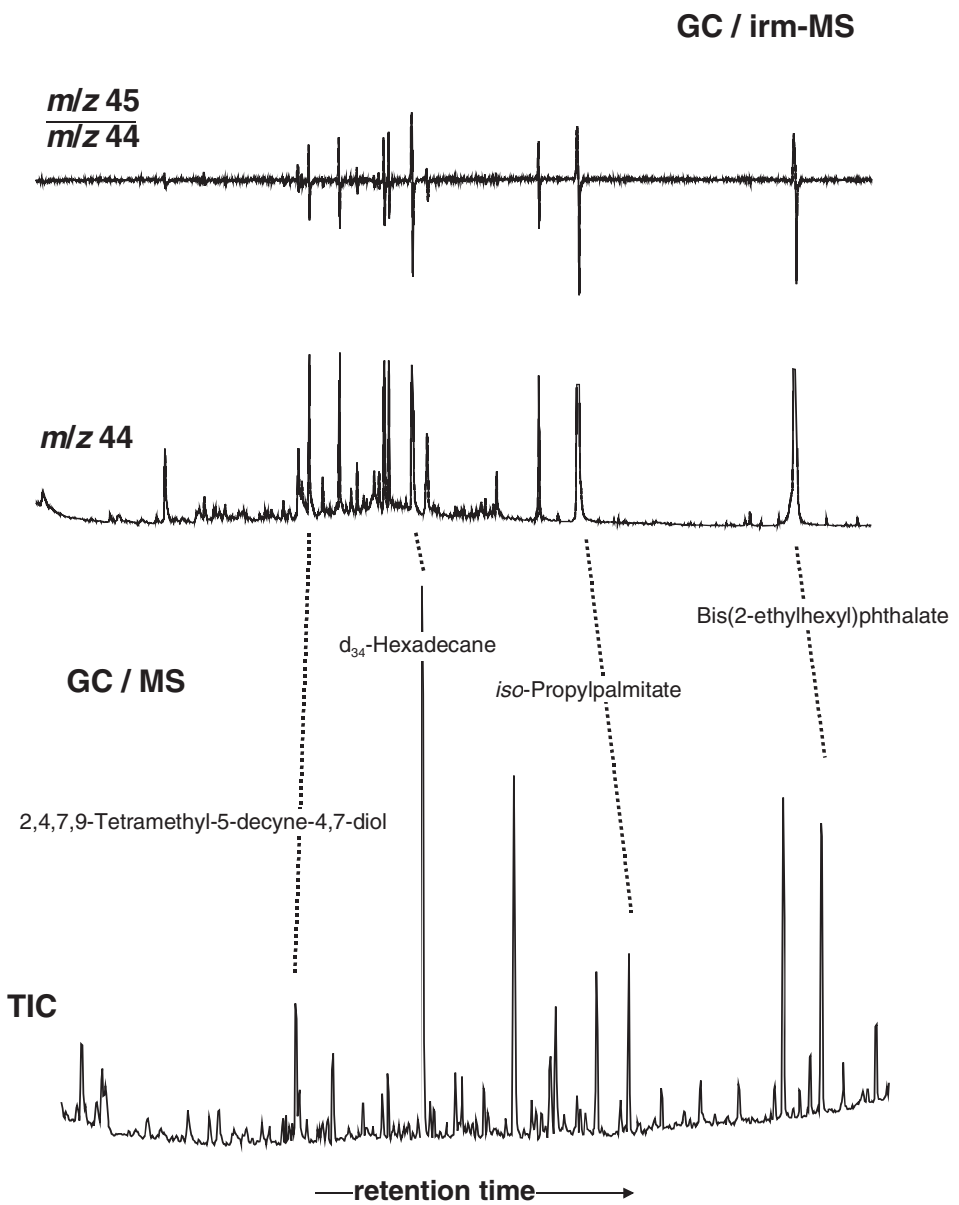

Figure 4. GC-irmMS chromatogram obtained from the pentane extract of water sample R2. Selected contaminants, which were identified and isotopically analysed, are marked.

GC/MS analyses. The following contaminants matched the described conditions, and consequently, their δ^{13}C-values were determined in the Rhine water samples: di-*n*-butylphthalate, bis(2-ethylhexyl)phthalate, galaxolide, *iso*-propylpalmitate, 2,4,7,9-tetramethyl-5-decyne-4,7-diol and 2,2,4-trimethyl-1,3-pentandioldi-*iso*-butyrate. Furthermore, d_{34}-hexadecane was analysed as an internal standard. All results are presented in figure 5. The attribution of the compounds analysed to the individual gas chromatographic peaks by GC-irmMS measurements based on the comparison

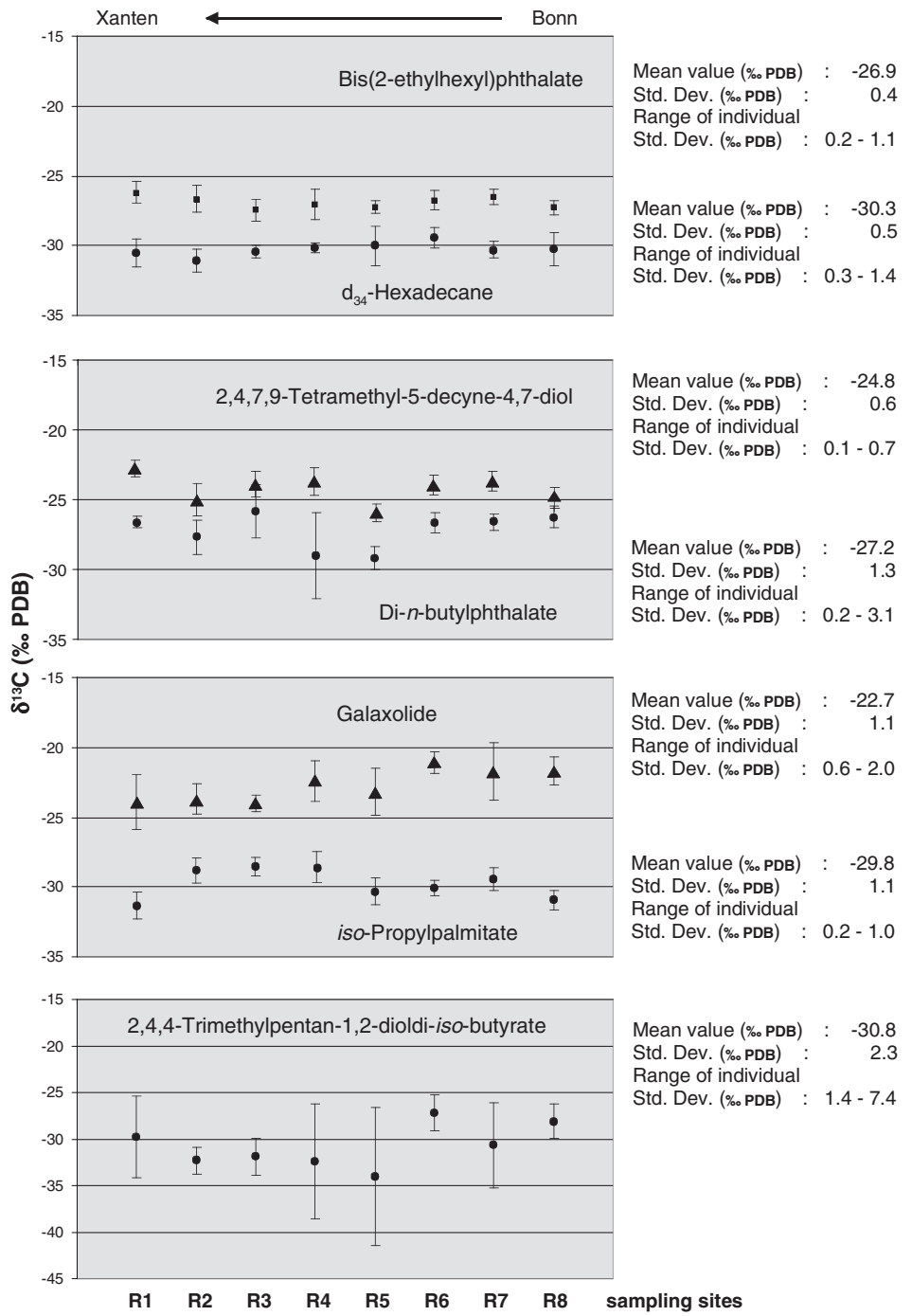

Figure 5. $\delta^{13}C$-values of selected contaminants in water samples from the Rhine river. On the right-hand side, the average value of the isotope ratios with the corresponding standard deviations obtained from the individual sampling locations is presented. (Calculation of the standard deviations was performed without consideration of the standard deviation of the individual data points.)

of the retention times with those of reference substances. In addition, the appearance or disappearance of all individual compounds in each sample was checked by GC-MS analyses.

Comparing the δ^{13}C-values of the standard reference substances (see tables 1–4) with the data analysed in the water samples consistent values were observed for di-*n*-butylphthalate and bis(2-ethylhexyl)phthalate. In addition, the standard deviations of the water analyses ranging from 0.2 to 3.1‰ were very similar compared with the variations obtained from the recovery experiments (see table 2). This is in accordance with the quantitative data (unpublished results). Both phthalates occurred with concentrations of approximately 100–900 ng L^{-1}, which was considered to be sufficient for the determination of the carbon isotope ratios.

On the contrary the δ^{13}C-values of 2,2,4-trimethyl-1,3-pentandioldi-*iso*-butyrate in the river water samples were significantly higher than the value of the reference material. However, the high standard deviations obtained from the analyses of the river water samples with up to 7.4‰ reflect an insufficient determination of the carbon isotope ratios. This might be mainly the result of very low concentrations of 2,2,4-trimethyl-1,3-pentandioldi-*iso*-butyrate in the water samples (approximately 4–20 ng L^{-1}). Hence, no assured information can be obtained from these data.

For the interpretation of the distribution of the carbon isotope ratios within the riverine system investigated, two different variations have to be considered. If the mean values summarized from all data of all sampling locations vary with a higher deviation than their individual standard deviations as well as the variations obtained from the recovery experiments, a significant alteration of the isotopic composition of the contaminants can be inferred. These assumptions were supported by the δ^{13}C-values of d_{34}-hexadecane. As expected for the internal standard, the variation of the individual data points (standard deviation between 0.5 and 1.4‰) was in the same range as compared with the standard deviation derived from the data of all sample extracts analysed (standard deviation 0.5‰).

Significant variations are only obvious for the substances galaxolide and *iso*-propylpalmitate, because most or nearly all individual data points deviated less than the variation of the δ^{13}C-values in the longitudinal profile (standard deviation 1.1‰).

On the contrary, the variation of the individual δ^{13}C-values of bis(2-ethylhexyl)phthalate in the range of 0.2–1.1‰ did not differ significantly as compared with the variation of the data derived from all samples (standard deviation 0.4‰). Hence, no significant alteration of the composition of the stable carbon isotopes can be stated for this contaminant within the river water samples investigated.

With respect to di-*n*-butylphthalate, most of the values analysed in the individual samples are characterized by a significantly lower variation (standard deviation 0.2–1.0‰) as compared with the summarized standard deviation derived from all samples (1.3‰). However, the δ^{13}C-values analysed in extracts derived from sampling locations 5 and 6 exhibit higher standard deviations up to 3.1‰; hence, the information obtained from these data are limited.

For 2,4,7,9-tetramethyl-5-decyne-4,7-diol, the information derived from the stable carbon isotope ratios is also limited as the result of a non-perfect base line separation in a couple of samples. This is illustrated in figure 4. In the TIC, a minor peak at the right flank of 2,4,7,9-tetramethyl-5-decyne-4,7-diol was overlapped in the m/z

44 trace by a broad CO_2 peak. Hence, the variation of $\delta^{13}C$-values of 2,4,7,9-tetramethyl-5-decyne-4,7-diol can be induced by coeluting substances.

Generally, significant variations of carbon isotope ratios along the river section investigated have to be attributed either to an superimposition of several emissions sources discharging contaminants with different carbon isotope compositions or to processes within the river system modifying the $\delta^{13}C$-values of the affected substances. Those effects can be the result of degradation or transformation as well as of transfer processes within the aquatic environment.

4. Conclusions

The presented results obtained from compound-specific carbon isotope analyses applied to standard solutions under various conditions support the following conclusions:

1. For most of the anthropogenic contaminants investigated, carbon isotope analyses were performed with an acceptable standard deviation down to amounts of approximately 5 ng absolutely applied to the gas chromatograph. These amounts correspond to concentrations in water samples at a natural abundance level of low to medium contaminated river systems. At lower amounts, standard deviations of $\delta^{13}C$-values of individual compounds increase significantly.
2. The precision as well as the sensitivity of the analytical methods depends partially on the chemical properties of the substances measured. In particular, higher chlorinated compounds exhibited $\delta^{13}C$-values with elevated standard deviations. This fact is tentatively attributed to lower carbon contents of these compounds and to interactions of halogens or tin with the oxidation catalysts.
3. Isotopic shifts or higher variations of the isotope ratios as a result of the analytical procedures applied were observed only for selected contaminants. Therefore, for accurate compound-specific carbon isotope analyses of riverine contaminants, it is recommended that supplementary recovery experiments of the individual substances be conducted.

These conclusions were confirmed by stable carbon isotope analyses performed on river water extracts derived from the Rhine River. Using a sequential extraction procedure, the determination of stable carbon isotope ratios of several riverine contaminants could be achieved by an appropriate gas-chromatographic separation. Comparing the variation of the data of the individual compounds with the deviations obtained from the recovery experiments, it was possible to differentiate contaminants with unaffected isotope ratios and substances with significant alterations of the $\delta^{13}C$-values. These significant variations reflect either multiple emission sources of different isotopic quality or environmental processes modifying the isotopic signature of the individual substances. With respect to the analyses of 2,2,4-trimethyl-1,3-pentandioldi-*iso*-butyrate, the low concentrations of riverine contaminants were pointed out as a major limitation of the analytical method.

Acknowledgements

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References

[1] M. Sano, Y. Yotsui, H. Abe, S. Sasaki, *Biomed. Mass Spectrom.*, **3**, 1 (1976).
[2] D.E. Matthews, J.M. Hayes, *Anal. Chem.*, **50**, 1465 (1978).
[3] W.A. Brand, *J. Mass Spectrom.*, **31**, 225 (1996).
[4] W. Meier-Augstein, *J. Chromatogr. A*, **842**, 351 (1999).
[5] H. Dempster, B. Sherwood Lollar, S. Feenstra, *Environ. Sci. Technol.*, **31**, 3193 (1997).
[6] N.C. Sturchio, J.L. Clausen, L.J. Heraty, L. Huang, B.D. Holt, T.A. Abrajano, *Environ. Sci. Technol.*, **32**, 3037 (1998).
[7] L. Mazeas, H. Budzinski, N. Raymond, *Org. Geochem.*, **33**, 1259 (2002).
[8] A. Stark, T.A. Abrajano Jr, J. Hellou, J.L. Metcalf-Smith, *Org. Geochem.*, **34**, 225 (2003).
[9] R.R. Harrington, S.R. Poulson, J.I. Drever, P.J.S. Colberg, E.F. Kelly, *Org. Geochem.*, **30**, 765 (1999).
[10] A. Jackson, J. Pardue, *Org. Geochem.*, **30**, 787 (1999).
[11] B. Sherwood-Lollar, G.F. Slater, J. Ahad, B. Sleep, J. Spivack, M. Brennan, P. Mackenzie, *Org. Geochem.*, **30**, 813 (1999).
[12] L. Mazeas, H. Budzinski, *Org. Geochem.*, **33**, 1253 (2002).
[13] G.F. Slater, B. Sherwood-Lollar, R. Allen King, S. O'Hannesin, *Chemosphere*, **49**, 587 (2002).
[14] P.J. Yanick, T.H. O'Donnell, S.A. Macko, Y. Qian, M.C. Kennicutt II, *Org. Geochem.*, **34**, 291 (2003).
[15] W.M. Jarman, A. Hilkert, C.E. Bacon, J.W. Collister, K. Ballschmiter, R.W. Risebrough, *Environ. Sci. Technol.*, **32**, 833 (1998).
[16] P.J. Yanick, T.H. O'Donnell, S.A. Macko, Y. Qian, M.C. Kennicutt II, *Org. Geochem.*, **34**, 239 (2003).
[17] S. Franke, S. Hildebrandt, J. Schwarzbauer, M. Link, W. Francke, *Fresenius J. Anal. Chem.*, **353**, 39 (1995).
[18] L. Dsikowitzky, J. Schwarzbauer, R. Littke, *Org. Geochem.*, **33**, 1747 (2002).
[19] L. Dsikowitzky, J. Schwarzbauer, R. Littke, *Chemosphere*, **57**, 1289 (2004).