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Contributions to the analysis of organic xenobiotics in fish

I. A method for simultaneous determination of triazines, acetamides and lipophilic compounds in fish using GC/MS

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Abstract. For the residue-analysis of fish samples, a method has been developed to separate the medium polar triazines and acetamides together with lipophilic xenobiotics (organochlorine pesticides, PCBs and octachlorostyrene) from fish tissue. The detection limits of the analytes are mostly within the ppt-range (ng/kg fillet) and vary between 40 and 1050 ng/kg fillet of fish. Their recoveries range from 75 to 108%, spiked at 1.5 µg/kg. The substances are extracted with a mixture of petroleum ether/ethylacetate (2:1) using a soxhlet apparatus. Subsequently, the lipids in the sample extracts are reduced to 0.4% by gel chromatography. A 5 g silica gel clean-up separates the analytes according to their polarity. First, the lipophilic compounds elute with n-hexane/ethylacetate (91:9) and afterwards the more polar compounds with n-hexane/ethylacetate (1:1). Finally, the organic extracts are concentrated to 150–300 µL and the compounds are detected and quantified by GC/MS.

1 Introduction

Fish monitoring in Germany mainly includes lipophilic compounds. For the determination of lipophilic compounds e.g. chlorinated pesticides, PCBs in fish a lot of methods were reported [1–24]. The detection limits were calculated in the lower ppb-range using an ECD-detector.

There exists a lack of information about fish contamination with medium-polar compounds like triazines and acetamides. These herbicides are spread ubiquitously and can even be detected in the water of mountain lakes of Switzerland up to heights of 2446 m [25]. They are obviously transported across long distances in the atmosphere. Only few methods were described to determine triazines and acetamides in fish tissue [26–31]. Their detection limits were reported within the upper ppb (µg/kg fillet) and the lower ppm-range (mg/kg fillet) and therefore will be too high for normally expected concentrations in fish [32]. The methods in literature were mostly based on a liquid/liquid extraction. For example, an acetone (acetonitrile)/water phase was extracted by dichloromethane or chloroform. The main problem of the clean-up procedure is caused by the removal of the lipids, because of their similar polarities compared to the analytes. Gel permeation chromatography (GPC) has been sucessfully employed in residue analysis for separation for small medium-polar compounds up to a molecular weight of 400 amu from macromolecular weight interferents like lipids (600–1500 amu) with similar polarities [33–40]. GPC primarily separates molecules by size, but nevertheless, adsorption phenomena caused by specific interactions with the compounds can also be observed.

The intention of the present study was the development of a multimethod for the simultaneous separation and determination of lipophilic and medium-polar compounds in fish by GC/MS with detection limits at the ppt-level (ng/kg fillet).

2 Experimental

2.1 Apparatus and materials

- Gas chromatograph (HP 5890) equipped with MS-detector (HP 5970B), on-column injector and fused silica capillary column (SE-54-CB, 50 m × 0.25 mm id, film thickness: 0.33 µm/CS-Chromatographie Service GmbH; Langerwehe Germany) coupled with a retention gap (fused silica, phenyl-sil deactivated, 4 m × 0.32 mm/CS-Chromatographie Service)
- Automated Gel permeation chromatograph (GPCA-A, Bender & Hobein/Munich, Germany)
- Rotary evaporator (Büchi RE 111)
- Soxhlet extractor (250 mL), Glass fibre thimbles (33 × 205 mm/Schleicher & Schuell) extracted with petroleum ether/ethylacetate (2:1) for 6 h
- Muffle furnace MR 170 (Heraeus/Hanau Germany), Oven (AEG/Competence 550 B)
- Milli-Q_{PLUS} apparatus for purified water
- Chromatographic columns (300 mm × 10 mm id) with teflon stopcock

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- Agate mortar (10 cm id) with pestle
- Glass funnel (90 mm id)
- Folded filters (90 mm id), extracted with petroleum ether/ethylacetate (2:1) for 6 h
- Peak bottom flask 100 mL (specially produced)
- 5 mL vial with 450 µL micro insert (CS-Chromatographie-Service/Langerwehe)
- Glass wool silanized (Serva) extracted with petroleum ether/ethylacetate (2:1) for 6 h

All glass materials were rinsed with acetone and Milli-Q water and were subsequently heated for 15 h at 500 °C in the oven (AEG/Competence 550 B) or in the muffle furnace.

2.2 Reagents

- Cyclohexane, n-hexane, ethylacetate, methanol, acetone, isooctane, petroleum ether (b.p.: 40–60 °C) and toluene (Nanograde, Promochem)
- n-dodecane-GC (Merck 9658 Darmstadt, Germany)
- Sodium sulphate anhydrous (Merck, Art.-Nr. 6649), heated for 15 h at 650 °C
- Silica gel 60, 70–230 mesh (Merck, Art.-Nr. 7734); deactivation with 1.5% water: Silica gel is heated at 130 °C for 6 h and after cooling down Milli-Q water is added, so that the content amount to 1.5% by weight. After 2 h of shaking the silica gel is stored in a well-closed vessel.
- Sea sand purified by acid (Merck, Art.-Nr. 7712), heated for 15 h at 650 °C
- Bio Beads SX-3, 200–400 mesh (Bio-Rad Laboratories GmbH, Munich, Germany)

Triazines, acetamides
- *Reference standards*: Simazine, Propazine, Terbuthylazine, Desethylterbuthylazine, Prometryn, Atrazine, Metolachlor, Metazachlor
- *Internal standards:* 2,4-Dibromoaniline (Aldrich), Atrazine-D_5 (Promochem)

Lipophilic substances
- *Reference standards*: Pesticide Mix XIII with HCH isomers, Cyclodiene insecticides, Hexachlorobenzene, DDT-, DDE-, DDD-isomers, Methoxychlor, PCB congeners 28, 52, 101, 138, 153, 180 and Mirex; Octachlorostyrene, Dicofol, Quintozene (Promochem)
- *Internal standards*: 2,4-Dibromoaniline (Aldrich), ε-HCH, Mirex (Promochem)

2.3 Preparation of halibut fish oil

Fillet of halibut is homogenized in a mixer, dried by grinding into powder with sodium sulphate (anhydrous) and is extracted in a Soxhlet-apparatus with petroleum ether/ethylacetate (2:1) for 6 h. After evaporation of the solvents by a rotary evaporator the fish oil remains.

2.4 Preparation of silica gel columns

A plug of silanized glass wool is tamped down in the bottom of the chromatographic glass column, Na_2SO_4 to a segment height of approx. 1 cm, 5 g silica gel (deactivated with 1.5% water) and once more Na_2SO_4 to a segment height of approximately 1 cm are successively filled in the chromatographic column (300 mm × 10 mm i.d.). Before use, the silica gel column has to be rinsed with 100 mL of n-hexane [30].

2.5 Gel permeation chromatography

2.5.1 Preparation of gelchromatographic columns

The automated gel permeations chromatograph GPCA-A is equipped with two 600 mm × 25 mm id chromatographic columns.

The columns have been slurry-packed with 70 g Bio-Beads SX-3, presoaked for 24 h in cyclohexane/acetone (3:1). The adjustable column plungers are inserted in a way that the height of the gel bed results to 37 cm. Before use, the columns have to be pre-eluted with about 2 L of the solvent mixture to remove pollutants of the styrene-divenylbenzene copolymer.

2.5.2 Gel chromatographic conditions

The sample dissolved in cyclohexane/acetone (3:1) is injected into the 5 mL sample loop of the GPC and the elution is started automatically by computer. In all experiments the elution flow rate of the solvent mixture has been 4.4 mL/min. The cutoff point between the dumped (first) and the collected (second) fraction has to be determined accurately to avoid loss of analytes as well as a higher amount of lipids in the sample fractions.

2.5.3 Determination of the elution ranges

a) A mixture of 2 µg of each compound dissolved in 5 mL cyclohexane/acetone (3:1) is injected into GPC. The column effluent is collected in 8.8 mL steps (2 min) from 0–260 mL. After concentration of the fractions by a gentle nitrogen stream to 0.5 mL the eluted compounds are determined by GC/MS.
b) Halibut fish oil (750–1000 mg) dissolved in 5 mL cyclohexane/acetone (3:1) is loaded into the GPC. The column effluent is collected in 8.8 mL (2 min) steps from 0–200 mL. The solvent of each fraction is evaporated and the remaining lipids are weighed, respectively.

2.6 Preparation of purified fish oil (separation of low molecular weight compounds from halibut fish oil)

1000 mg of halibut fish oil is dissolved in cyclohexane/acetone (3:1) and injected into the GPC. From 0–20 min (88 mL) the first fraction is collected, while the effluent of 20–60 min is discarded. The solvent mixture of the first fraction is entirely evaporated by a rotary evaporator. Thus, the remaining lipids are free of low molecular weight compounds.

2.7 Gas chromatography/mass spectrometry: operating conditions

Carrier gas: helium, Column head pressure: 100 kPa, Temperature of the transfer line (direct interface): 270 °C, Ion source: EI mode, Electron energy: 70 eV

Triazines, Acetamides. Injection volume: 3–4 µL, Temperature program: 70 °C (4 min), 70 °C/min to 140 °C (1 min), 50 °C/min to 190 °C (1 min), 1.1 °C/min to 220 °C (1 min), 7.5 °C/min to 230 °C (0.5 min), 70 °C/min to 270 °C (4.3 min). Run time: 45 min.

Lipophilic compounds. Injection volume, 3–4 µL, Temperature program: 90 °C (4 min), 70 °C/min to 140 °C (1 min), 50 °C/min to 180 °C (1 min), 1.3 °C/min to 200 °C (1 min), 1.2 °C/min to 225 °C (1 min), 2 °C/min to 250 °C (1 min), 6.5 °C/min to 270 °C (7.7 min). Run time: 70 min.

2.8 Method

2.8.1 Sample preparation

The fish is divided into the fractions fillet (fish-muscle, cleaned from skin and bones), intestines (the total content of the body cavity e.g. heart, kidneys, bile, liver) and a third one including head, skin, bones, and fins.

The fractions are homogenized in a mixer and are dried by grinding with anhydrous sodium sulphate and sea sand in an agate mortar. About 40 g sodium sulphate and 3 g sea sand are necessary for 10 g fish tissue.

2.8.2 Soxhlet extraction

The fine fish powder is filled in the glass fibre thimble, spiked with the internal standard Atrazine-D_5 and extracted with petroleum ether/ethylacetate (2:1) in the Soxhlet-apparatus for 6 h. Subsequently, the Soxhlet extract is dried by addition of 10 g sodium sulphate and after 30 min filtered through folded filters.

2.8.3 Determination of the lipid content and reduction of the extract volume

The Soxhlet extract is concentrated from about 350 mL to 5 mL by a rotary evaporator (40 °C/75 → 30 kPa) and transferred to a 20 mL vial, weighed before. Subsequently, the solvent is evaporated by a nitrogen stream until a constant weight results. Now, it is possible to calculate the extractable lipid portion of the fish fraction. If a constant weight up to 0.5 g cannot be obtained, the evaporation is stopped to avoid loss of substances with low vapour pressure. In this case, 10 mL of cyclohexane is added to the extract and the volume is reduced to 5 mL, in order to remove entirely the ethylacetate. Acetone is added to form cyclohexane/acetone (3:1), which can directly be injected into the GPC. In order to determine the amount of lipids the first fraction has to be collected.

2.8.4 Gel chromatographic separation

The gel chromatographic conditions are described in chapter 2.5.2

Fractions

First fraction (lipids):	0–28 min (0–123 mL)
Second fraction (analytes):	28–52 min (123–229 mL)
Wash	52–60 min (229–264 mL)

The volume of the second fraction is reduced by a rotary evaporator. After the acetone is completely removed, about 30 mL of n-hexane is added to accelerate the evaporation of the cyclohexane. Finally, the sample extract of about 3 mL is poured into a 5 mL vial and concentrated to 1 mL by a gentle nitrogen stream.

2.8.5 Silica gel clean-up

The concentrated GPC extract is quantitatively transferred to the top of the prepared 5 g silica gel column. Then, the effluent solution is collected in special peak bottom flasks. First, the lipophilic compounds elute with 55 mL of n-hexane/ethylacetate (91:9) and then the medium-polar compounds (triazines, acetamides) with 70 mL of n-hexane/ethylacetate (1:1). Subsequently, the volume of the fractions are concentrated to 2 mL on a rotary evaporator (40 °C/38 → 30 kPa) and after addition of 10 µL of dodecane to 350 µL by a gentle nitrogen stream. Now, the extract is transferred to a 450 µL micro insert, which is filled with 100 µL of toluene for the first fraction and with 300 µL of toluene for the second fraction and is reduced to the volume of the added toluene.

The compounds are detected and quantified by GC/MS in selected ion monitoring (SIM) mode after addition of 100 ng of the internal standards 2,4-dibromoaniline and mirex (only for the first fraction) dissolved in toluene.

2.9 Determination of recoveries and detection limits

A) Purified halibut fish oil has been spiked both with the internal standard Atrazine-D_5 and analytes. The investigation follows the previously described method.

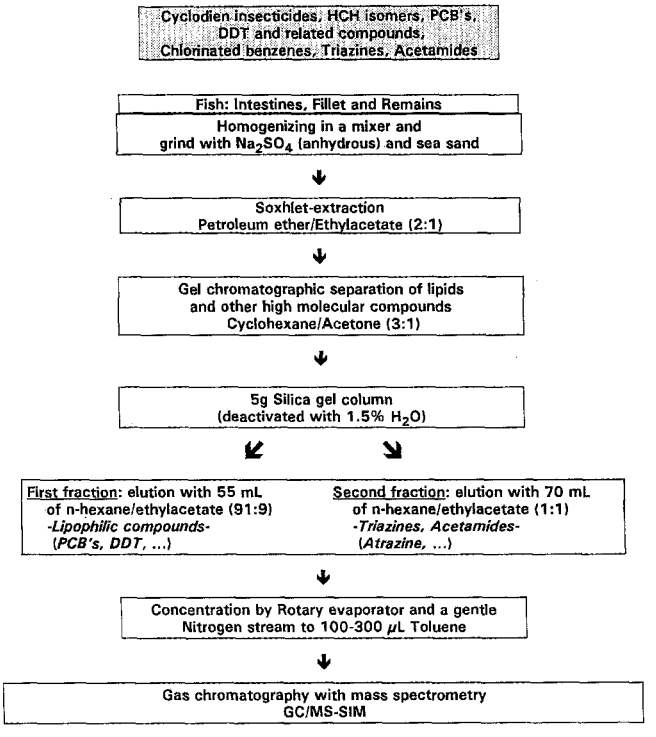

Fig. 1. Scheme for determination of triazines, acetamides and lipophilic compounds in fish

Table 1. Elution ranges in gel chromatography (70 g Bio-Beads SX-3); solvent mixture: cyclohexane/acetone (3 : 1); flow rate: 4.4 mL/min; injection: 2 µg substance or 750–1000 mg halibut fish oil in 5 mL solvent mixture

Compound	Elution volume [mL]	Compound	Elution volume [mL]
Polychlorinated biphenyls	135–195	HCH iosmers	132–195
PCB-28	135–195	α-HCH	135–185
PCB-52	135–185	β-HCH	132–175
PCB-101	135–185	γ-HCH	132–195
PCB-138	135–185	δ-HCH	132–175
PCB-153	135–185	ε-HCH	145–195
PCB-180	135–185		
DDT and related compounds	132–185	Cyclodiene insecticides	132–195
o,p′-DDT	135–175	Dieldrin	135–195
p,p′-DDT	135–175	Endrin	135–195
o,p′-DDE	132–185	Aldrin	132–175
p,p′-DDE	132–185	Isodrin	132–175
o,p′-DDD	132–185	c-Heptachloroepoxide	132–185
p,p′-DDD	132–185	t-Heptachloroepoxide	132–185
Dicofol	132–170	Oxychlordane	132–175
Methoxychlor	132–185	c-Chlordane	132–170
		t-Chlordane	132–170
Chlorinated benzenes	135–210	Heptachlor	132–170
Quintocene	135–210	Octachlorostyrene	132–155
Hexachlorobenzene	135–210		
Triazines	110–185	Acetamides	123–205
Atrazine	120–170	Metolachlor	123–195
Simazine	132–185	Metazachlor	160–205
Propazine	110–160		
Terbuthylazine	110–170		
Desethylterbuthylazine	123–175	Halibut fish oil	44–140
Prometryn	123–175		

B) The fillet of a pike from the river Rhine has been homogenized in a mixer, divided into six portions and spiked with the internal standard Atrazine-D_5. Three portions of the fillet were additionally spiked with the analytes. Extraction, clean-up procedure and GC/MS determination have been performed as described above.

3 Results and discussion

3.1 Clean-up procedure

The completeness of the Soxhlet extraction has been proved for lipophilic and medium-polar compounds. They are all quantitatively recovered in the extract after 6 h. Primarily, the portion of 33% ethylacetate used in Soxhlet extraction improves the extraction of medium-polar triazines and acetamides.

For the lipophilic compounds in gel permeation chromatography a cutoff point at 132 mL (30 min) is sufficient. The different elution ranges are listed in Table 1. In the fraction collected the analytes are quantitatively recovered, while lipids and other high molecular weight biogenic compounds are reduced to almost 0.2%. However, the elution of Propazine and Terbuthylazine starts already at 110 mL. Hence, the cutoff point for the simultaneous clean-up procedure of lipophilic and medium-polar compounds is selected to 123 ml (28 min). It has to be accepted that 0.5% Atrazine, 5% Terbuthylazine and 10% Propazine are lost, while 0.4% of the injected lipids get into the sample fraction. A cutoff point at lower elution volume is not useful, because of the higher amount of lipids, which would create a lot of problems in the GC-MS determination. Already 97.5% of the lipids elute between 53 and 100 mL, while about 2% are found between 100 and 123 mL.

One modification of the employed gel chromatographic conditions, which have been reported by Steinwandter [9] is the increase of the amount of Bio-Beads SX-3 from 50 to 70 g. Nevertheless, a quantitative separation of lipids as Steinwandter described for rendered butter, lard and vegetable oils, cannot be observed for halibut fish oil.

After GPC clean-up, the 5 g silica gel clean-up (70–230 mesh; 1.5% water-deactivated) separates the analytes with regard to their polarity. The recoveries of lipophilic compounds in the first fraction exceed 99.5% and the medium-polar compounds in the second fraction are recovered above 99%. Highly polar coextractants remain on the silica gel column and therefore are separated. Finally, the organic extracts are gently concentrated to 150–300 µL to achieve detection limits at the ppt-range. The analytical identification and quantification of xenobiotics by GC/MS permits the determination of non-halogenated molecules and even the use of deuterium labeled Atrazine-D_5 as internal standard. In order to improve the confirmation of the analysis results 2 or even 3 internal standards have been used.

Table 2. Recoveries[a] of analytes in purified halibut fish oil

Triazines	[%]	Acetamides	[%]
Atrazine	103	Metolachlor	114
Simazine	101	Metazachlor	103
Propazine	105		
Terbuthylazine	102		
Desethylterbuthylazine	67		
Prometryn	15		
HCH isomers	**[%]**	**Chlorinated benzenes**	**[%]**
α-HCH	70	Hexachlorobenzene	65
β-HCH	75	Quintocene	81
γ-HCH	73		
δ-HCH	74	Octachlorostyrene	90
Cyclodiene insecticides	**[%]**	**DDT and related compounds**	**[%]**
Heptachlor	84	p,p′-DDT	80
Aldrin	77	o,p′-DDT	83
Isodrin	67	p,p′-DDD	78
t-Heptachloroepoxide	93	o,p′-DDD	78
c-Heptachloroepoxide	93	p,p′-DDE	84
Oxychlordane	92	o,p′-DDE	78
Endrin	91	Dicofol	74
c-Chlordane	95	Methoxychlor	85
t-Chlordane	94		
Polychlorinated biphenyls	**[%]**		
PCB-28	76		
PCB-52	86		
PCB-101	92		
PCB-138	90		
PCB-153	93		
PCB-180	90		

[a]Percent recovery (mean of n = 4), 1 g purified fish oil spiked with 100 ng substance; relative mean error: $\Delta X/X = \pm 7$–10% (P = 0.95) for lipophilic compounds; relative mean error: $\Delta X/X = \pm 8$–11% (P = 0.95) for triazines und acetamides (exception: prometryn with ± 40%)

Table 3. Recoveries[a] of analytes in the fillet of pike

Triazines	[%]	Acetamides	[%]
Atrazine	101	Metolachlor	98
Simazine	110	Metazachlor	101
Propazine	75		
Terbuthylazine	90		
Desethylterbuthylazine	79		
Prometryn	26		
HCH isomers	**[%]**	**Chlorinated benzenes**	**[%]**
α-HCH	81	Quintocene	85
Cyclodien insecticides	**[%]**	**Octachlorostyrene**	**84**
Heptachlor	98		
Aldrin	78		
Isodrin	71		
t-Heptachloroepoxide	94		
c-Heptachloroepoxide	85		
Oxychlordane	84		
Endrin	103		
t-Chlordane	84		

[a] Percent recovery (mean of n = 3), 30 g fillet of pike spiked with 100 ng substance; relative mean error: $\Delta X/X = \pm 12$–15% (P = 0.95) for lipophilic substances; relative mean error: $\Delta X/X = \pm 7$–10% (P = 0.95) for triazines und acetamides (exception: prometryn with ± 40%) The evaluation of many substances is impossible because of their high concentrations in the pike. For example, DDT with related compounds as well as PCB congeners have been detected up to 100 µg/kg fillet so that recoveries cannot be calculated

3.2 Recoveries

Fish without contaminations of PCBs and lipophilic pesticides were not available. Therefore, the recoveries of the analytes have been studied by spiking purified fish oil. In order to achieve recoveries for the total method including the soxhlet extraction fillet of a pike from the river Rhine was spiked with the lipophilic and medium-polar

compounds. This was simultaneously analysed with unspiked portions of the pike fillet.

3.2.1 Lipophilic compounds

Average recoveries of the lipophilic compounds in purified halibut fish oil mainly range from 70 to 93%, spiked with 100 µg/kg of each compound. However, values of HCB and Isodrin in the 65 to 67% range are observed. The relative mean error with 7–10% and the recoveries indicates the sufficient precision and reproducibility of the entire method. Similar recoveries of lipophilic compounds, listed in Tables 2 and 3, are obtained for the spiked pike fillet and the spiked halibut fish oil. As

Table 4. Retention time, detection ions in SIM mode and detection limits

Compounds	Retention time [min]	Detected ions [m/z]	Detection limits [ng/kg fillet]	[ng/g lipid]
HCH Isomers:				
αHCH	22.91	217, 219	280	11
β-HCH	25.23	217, 219	270	10
γ-HCH	25.54	217, 219	240	8
δ-HCH	27.76	217, 219	250	10
Cyclodiene insecticides:				
Heptachlor	32.19	272, 274	180	9
Aldrin	35.96	263, 265	380	18
Isodrin	39.34	193, 263	490	20
c-Heptachloroepoxide	40.58	353, 355	45	2
Oxychlordane	40.71	387, 389	150	12
t-Heptachloroepoxide	41.12	353, 355	320	16
t-Chlordane	43.55	373, 375	100	6
c-Chlordane	45.45	373, 375	100	6
Endrin	51.26	261, 263	170	10
Polychlorinated biphenyls:				
PCB-28	30.53	256, 258	60	2
PCB-52	34.03	290, 292	60	2
PCB-101	44.44	324, 326	60	2
PCB-153	55.20	360, 362	60	2
PCB-138	58.47	360, 362	60	2
PCB-180	65.20	394, 396	60	2
Chorinated benzenes:				
Hexachlorobenzene	23.53	284, 286	50	2
Quintocene	25.89	295, 297	490	20
Octachlorostyrene	39.80	378, 380	80	5
DDT and related compounds:				
Dicofol	37.20	250, 252	350	17
o, p′-DDE	44.05	235, 237	40	2
p, p′-DDE	48.17	235, 237	50	2
o, p′-DDD	49.35	235, 237	60	2
p, p′-DDD	53.57	235, 237	55	5
o, p′-DDT	53.86	235, 237	70	3
p, p′-DDT	57.98	235, 237	70	3
Methoxychlor	63.89	227, 274	80	4
Triazines				
Desethylterbuthylazine	20.12	186, 188	350	20
Simazine	21.96	186, 201	350	20
Atrazine	22.25	200, 215	340	20
Propazine	22.47	214, 229	530	30
Terbuthylazine	23.38	214, 229	350	20
Prometryn	29.24	184, 241	1050	60
Acetamides				
Metolachlor	32.74	162, 238	120	7
Metazachlor	36.80	133, 209	350	20
Internal standards for Triazines and Acetamides				
2,4-Dibromoaniline	16.72	249, 251		
Atrazine-D_5	22.10	205, 220		
Internal standards for lipophilic compounds				
2,4-Dibromoaniline	17.14	249, 251		
Mirex	68.00	272, 274		

expected, the calculation of recoveries for many compounds in the pike were impossible, because of high residues of these compounds in the pike. A small loss of compounds during the clean-up are probably created by the evaporation of the large solvent volumes. 500 mL of extracting solvents are reduced up to 100 µL.

3.2.2 Medium-polar compounds

Average recoveries of the triazines and the acetamides exceed 75% at spiking levels of 100 µg/kg for fish oil and 1.5 µg/kg for fish fillet. These results are listed in Tables 2 and 3. However, recoveries of Prometryn are observed in 15 to 26% range. Spiked fillet of pike and spiked halibut fish oil yield similar recoveries and comparable relative mean errors (8–11%, 7–10%). The internal standard Atrazine-D_5 is recovered with about 100% according to 2,4-dibromoaniline. Nevertheless, the baseline of the GC/MS-spectra for the medium-polar compounds is high compared to the lipophilic analytes. Incompletely separated lipids are probably responsible for the high baseline, but on the other hand act as effective keepers. Atrazine-D_5 is an excellent internal standard for Atrazine and other Triazines, because of its similar physical and chemical properties. The loss of Promethryn can only be explained by reactions of the analyte with coextractants of fish constituents. Thermal degradation during Soxhlet extraction, loss by GPC and silica gel clean-up can be ruled out, because of recoveries nearly 100% are observed in the single clean-up steps.

3.3 Detection limits

For the determination of detection limits two limiting factors have to be considered.

I. The amount of investigated samples
II. Only a maximum of 1 g lipids can be loaded into GPC

In fish with a high amount of lipids the detection limits of the analytes are confined by the GPC, while for fish with low lipid portions the amount of the investigated sample is decisive. Hence, the detection limits given in Table 4 have been determined with regard to 1 g fish oil and to 60 g fillet of fish. They are defined as the concentration that causes a chromatographic peak with a height almost equal to three times the standard deviation of the baseline noise [41].

The detection limits of the investigated compounds are mainly within the ppt-range and vary between 50 and 1050 ng/kg fillet of fish. With regard to 1 g fish oil the limits of detection are calculated in the range of 2 to 60 ng/g lipid.

Lipophilic compounds generally show lower detection limits, because of the smaller final volume of 100 µL compared to 300 µL for triazines and due to the lower base line in GC/MS spectra.

4 Conclusions

The developed method, summarized in Fig. 1, permits the simultaneous determination of lipophilic and medium-polar compounds in fish. Lipophilic compounds like PCBs and Cyclodiene insecticides can be analysed in all of the fractions down to the ppt-range. Even the analysis of a total fish sample can be performed. Although the method has been validated for several species of fish, this procedure is expected to be applicable to other animal tissues. Residue analysis of the lipophilic compounds in whole samples of water-snails (*Lymnaea stagnalis*) has been performed as one possible application in our laboratory without any problems and yielded limits of detection at the ppt-range. Medium-polar compounds like triazines and acetamides can also been determined in the fillet of fish at the upper ppt-range. However, in the fish intestines the detection of medium-polar compounds is impossible, because of an incomplete separation of intestinal instituents with similar polarities.

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