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Determination of selected chlorobiphenyl congeners in fish oil with capillary gaschromatography (BCR interlaboratory study 4/1985)

L.G.M. Th. Tuinstra and A.H. Roos

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1. Introduction

The Community Bureau of Reference organized a collaborative interlaboratory project to improve the analytical protocol for some selected chlorobiphenyl (CB) congeners within European Community (EC). A series of test procedures were prescribed to optimize the gas chromatographic conditions for splitless- and on-column injection and interlaboratory studies with standards and cleaned eel-fat extracts were carried out. In the meeting on CB analysis organized in IJmuiden, The Netherlands, 3-5 December 1984, where the results of the third interlaboratory study 3/1984. (Report 84.93 dd. 1984-11-05 and report 84.98 dd. 1984-11-23.) were discussed, it was decided to organize a study in which the following excercises should be carried out:

a) determination of the linear range per CB congener
b) determination of seven selected CB congeners in a practice sample eel-fat
c) determination of seven selected CB congeners in four unknown fish oil samples with a naturally low and high CB contamination.

The results of this study are reported below.

2. Participants

A complete list of the 13 participating laboratories is given in Annex 1.

3. Interlaboratory study

3.1 Description of samples

The participating laboratories received a set of the following samples:

Seven sealed ampoules standard solution of respectively CB 28, 52, 101, 118, 138, 153 and 180, each 5 µg/ml in iso-octane for identification and quantification purposes (code A-G).

Two sealed ampoules internal standard solution of respectively 1,2,3,4-tetrachloronaphtalene (TCN), 5 µg/ml and dichlorobenzyl tetradecane ether (DCBE-C14), 50 µg/ml in iso-octane (code H and I) and a sealed ampoule blanc solvent iso-octane (code J).

For each ampoule the weight was given to control loss of weight after receipt of the samples.
The antioxidant BHT was added in a content of 100 mg/kg to the practice sample and the unknown samples of fish oil.
The unknown samples fish oil were prepared by mixing of a Baltic herring oil supplied by L. Reutergardh from the National Swedish Environmental Protection Board and from cod liver oils respectively from the North Sea and of unknown origin supplied by M.A.T. Kerkhoff from the Netherlands Institute for Fishery Investigations.
During the study it appeared that toxaphene was also present in the samples.
A practice sample eel containing on fat basis 0,035 mg/kg CB 28, 0,10 mg/kg CB 52, 0,14 mg/kg CB 101, 0,17 mg/kg CB 118, 0,30 mg/kg CB 138, 0,32 mg/kg CB 153 and 0,14 mg/kg CB 180 (code K).
Four unknown samples of fish oil, naturally contaminated respectively with a low and high CB contamination (code 1-4).

3.2 Description of study

The participating laboratories were instructed to check first the weight of the sealed ampoules. By way of an acknowledgement of receipt new ampoules could be asked in case ampoules were not received in good condition (e.g. more than 1% weight difference).
They were instructed to use the optimum gas chromatographic conditions achieved in preceeding studies.
The linear range for each CB congener had to be determined with the laboratory own CB standard in the range 0-500 pg.
Standard mixtures with a low and high concentration had to be prepared within the determined linear range with the ampoules A-G for the final quantification. The samples had to be analyzed in the linear range between both standards by interpolation.
The analysis of the practice sample and fish oil samples could be carried out with the own laboratory procedure. First the practice sample had to be analyzed. In case the results obtained were differing more than 20% from the indicated content of the chlorobiphenyls, contact should be made with the organizing laboratory. When no problems were met, analysis of the samples 1-4, together with a blanc and a recovery experiment with standard solution, could start. Analysis were carried out on all samples at the same time. Results had to be reported on enclosed forms.

4. Results and discussion

Eleven of the thirteen participating laboratories completed the total study. Laboratory 9 only completed the determination of the linear range per CB congener, due to technical problems. Laboratory 13 had no equipment available to do the work in time.

The gaschromatographic conditions used are presented in table 1. All participants used a Ni 63 electron capture detector. From these results it can be concluded that optimum settings were used by all laboratories for this study.

In our opinion, particularly with complicated environmental samples, a visual inspection of the chromatogram is necessary, also to check computer results. The chromatograms of laboratory 7 could not be examined because it was plotted at very low paper speed.

4.1 Method of analysis

A summary of the principles used in the study is given in table 2 below.

Table 2. Summary analytical procedure (more details are reported in annex 2)

Lab.no.	Clean-up procedures			Column separation	
	Acid treatment	Saponification	GPC	Al_2O_3	SiO_2
1		x		x	
2				x	x
3	x	x			
4	x		x		
5				x	x
6			x		x
7				x	x
8					x
10				x	x
11		x		x	x
12	x				x

In the unknown samples 1-4 beside CBs, also toxaphene residue was present. As toxaphene can interfere the CB determination, the participants were informed between times by a telex.
Five laboratories separated the CBs from the toxaphene residue with a silica column after cleanup on a Al_2O_3 column. Laboratory 10 used in first instance the SiO_2 column.
The toxaphene residue is partially dehalogenated by the saponification procedure used in laboratory 1 and 3. The reaction product results in a peak pattern which is eluting in a smaller retention window and at shorter retention time. Now only the lower CBs are more or less influenced, depending on the resolution of the capillary column.
Laboratory 3 reported by telex that in sample 4 when the separation procedure described by Wells (to be published) was used approximately 50 percent lower results were obtained for CB 28 and 52. As the time for re-analysing of all the samples was to short, the data reported as received were used for statistical treatment.
Laboratory 4 used gelpermeation chromatography followed by a treatment with concentrated sulphuric acid. No other column separation was carried out through which the results should be strongly influenced by the toxaphene residue. This is confirmed by visual inspection of the chromatograms.

The results of the recovery experiments with the standard solution are given in table 3. Losses can be assumed, during evaporation of the extract, for the more volatile CBs 28 and 52. Too high results are probably caused by alinearity or instability of the system. As the recovery experiments were not carried out in the same matrix as the samples, only uncorrected results are reported of the samples in the tables 4, 5, 6, 9 and 10.

4.2 Linearity

In previous studies the linearity of the system was tested in the range 5-50 pg for CB 153, resulting in a moderate linearity. In this study, the range 10-500 pg for each CB congener of interest was tested. All laboratories which tested the range 10-500 pg with exception of laboratory 2, obtained a linear behaviour starting at 25 or 50 pg for all CB congeners.
Laboratory 7 only reported data for the range 0-50 pg. The plots of laboratory 7 show a non-linear behaviour below about 25 pg and a linear behaviour in the range 25-50 pg.

4.3 Identification and quantification

TCN and DCBE-C14 were used in this study as internal standards. Laboratory 3 reported a peak eluting at the same time as the TCN peak in the samples 1-4. No such peak was observed in the practice sample and corrections have been made for this peak in the samples 1-4. Laboratory 2 used beside TCN the homologous series of DCBEs C2-C16. DCBE-C7 eluted in a clearer window than TCN and is therefore more suitable as internal standard. By visual inspection of the chromatograms, also when toxaphene residue is present, this is supported. Visual inspection of the peak width of CB 28 in the chromatograms of the samples, related to the film thickness and length of the capillary column, gives the impression that for CB 28 better separation and lower data are obtained using a 25 m Sil 8 or comparable column with a 0,4 µm film or 40-50 m columns with a thin or thick film. The 25 m Sil 8 or comparable columns with a film thickness of 0,1 µm showed less separation and in general higher data for CB 28.

Another disadvantage of this type of column is that less or no separation of CB 118 and 149 is obtained then on columns with a thicker film. Laboratory 6 and 8 used more polar columns (OV 1701 and OV 17) for the analysis of the unknown samples also on which a completely separated CB 118 peak was obtained. On the OV 1701 column CB 138 was influenced by an interference.

4.4 Statistical treatment

As separation of all CB's in environmental samples is very difficult on one column, if at all possible, the participants were advised to use also a capillary with a different polarity. In the case that more columns are used the question arise which data must be used for statistical treatment. In the discussion of the data in Brest, 4-6 September 1985, it was decided to use the lowest data. From the tables 4, 5, 6, 9 and 10 containing all data, the lowest data are taken for statistical treatment.

The statistical treatment of the selected data was carried out in first instance according to ISO 5725 - Precision of test methods - Determination of repeatabiity and reproducibility by interlaboratory tests.
The definitions are reported earlier in detail in report 84.27 (BCR-ringtest of individual chlorobiphenyls (2/1983) - Summary of results) dd. 1984-03-26.

Visual inspection of the data showed for some CB congeners in the samples two deviating values, which where not marked as outlier or straggler with the Dixon's test as this test searchs for one outlying value. In these cases the Grubbs test offers the possibility to search for outlying values (Grubbs. F.E., Procedures for Detecting Outlying Observations in Samples, Technometrics, Vol. 11, no. 1 (1969) 1-21). The outlying values in the tables 4, 5, 6, 9 and 10 are marked with a letter. C for a Cochran outlier, D for a Dixon outlier and G for a Grubbs outlier.

The results of the unknown fish oil samples 1 and 3 respectively 2 and 4, which were blind duplicates, were combined for statistical treatment.

4.5 Practice sample

The original data are presented in table 4. The results of laboratory 4 for CB 28 and 52 are given between brackets and were not used for statistical treatment. As reported due to band broadening during the cleanup procedure losses of CB 28 and 52 occured with the practice sample. In the clean-up of the unknown samples, this was not a problem. At the end of the table the mean and coefficient of reproducibility (CV(R)) are given for the selected data. By visual inspection of the TCN and DCBE data two high values for CB 28 were observed. With the Dixon and Cochran test no stragglers or outliers were found.
With the Grubbs' test for two outliers the CB 28 data of laboratory 2 and 7 were marked as outliers. The results after elimination of the outliers are also given in table 4. The results obtained with TCN or DCBE as internal standard are comparable and show a CV(R) ranging from 11 to 23%.

The CB level in the practice sample varied from 100-200 µg/kg eel-fat. This means about 20-40 µg/kg on eel as such. In relation to the maximum residue limits (MRLs) in the Netherlands which vary from 200-500 µg/kg product for the individual CBs on eel as such, a good result is obtained when we take into account that different methods of analysis have been used; of coarse the content was approximately known.
With the same sample in the Netherlands by 11 laboratories, all using the same cleanup procedure (saponification), a CV(R) ranging from 23 to 27 percent was obtained (L.G.M.Th. Tuinstra e.a. J.AOAC 4 (1985) 756-759). In this BCR study, a lower CV(R) is obtained. In other words the effect of the optimization in preceeding studies was very useful.

4.6 Fish oil sample 1 and 3

The original data of sample 1 are presented in table 5 and for sample 3 in table 6.
Laboratory 5 reported in the first instance data on a Sil 8 and 19 column. Due to problems, with the separation on the silica column, new data were given only obtained on the Sil 8 column and only these are reported.

The results of the statistical evaluation of the TCN-data are given in table 7a. With the Cochran and Dixon's test Cochran outliers were found for laboratory 2 (CB 28) and laboratory 4 (CB 101). The results after elimination of these outliers are given in table 7b.

The results of the statistical evaluation of the DCBE-C14 data are given in table 8a. With the Cochran and Dixon's test a Dixon outliers was found for laboratory 4 (CB 138) and Dixon stragglers for laboratory 4 (CB 118) and laboratory 5 (CB 138). A Cochran outlier was found for laboratory 2 (CB 28). The results after elimination of the outliers are given in table 8b.
With the Grubbs' test outliers were found for laboratory 4 (CB 118 and 138) and stragglers for laboratory 4 (CB 101) and laboratory 5 (CB 180). The results after elumination of the Cochran and Grubbs' outliers is given in table 8c.

The results obtained with TCN or DCBE as internal standard are in general comparable and show a CV(R) ranging from 36 to 55 percent for the lower CBs 28, 52 and 101. For the higher CBs a CV(R) ranging from 15 to 30 percent was obtained. The higher CV's for the lower CBs are explicable by unsufficient separation of CBs and interference with toxaphene residues. The level in this sample varied from 50-200 µg/kg fish oil and is far below the MRLs for eel as such.

4.7 Fish oil sample 2 and 4

The original data of sample 2 are presented in table 9 and for sample 4 in table 10.

The results of the statistical evaluation of the TCN and DCBE data are given respectively in table 11a and 12a. With the Cochran and Dixon test only a Dixon straggler for laboratory 11 (CB 180) was found.

With the Grubbs' test, for TCN data, outliers were found for laboratory 4 (CB 101, 118) and laboratory 11 (CB 101, 153 and 180) and a straggler for laboratory 11 (CB 118). For the DCBE data outliers were found for laboratory 4 (CB 101, 118 and 153) and laboratory 11 (CB 101, 118, 153 and 180). The results after elimination of the Grubbs' outliers are given in table 11b respectively 12b.

The results obtained with TCN or DCBE as internal standard are comparable, with exception of CB 101, 118 and 138. The CV(R) ranged from 6 to 32 percent and is in most cases between 10 and 20 percent.

The level in this samples varied between 100 and 1000 µg/kg on fat basis. This means about 20 to 200 µg/kg on eel as such. In relation to the MRLs in the Netherlands (range 200 to 500 µg/kg on eel as such) an acceptable CV(R) is obtained.

The CV(r) obtained in sample 2 and 4 is about 10 percent and is comparable with the CV(r) obtained in sample 1 and 3.

5. Conclusions

a) The results of the linear range finding test show in general a better linearity starting at 25 or 50 pg injected mass. This is a higher quantity than usily used in the laboratories. Therefore in any case the linear range should be tested and samples have to be analyzed in this range.

b) The results of the study are in view of the results of inter-calibration exercises obtained so far and taking into account that different methods analytical procedures have been used quite acceptable.

In the practice sample eel-fat a CV(R) ranging from 11 to 23 percent was obtained. For most CBs the CV(R) was in the order of 15 percent (100-200 µg/kg level per CB congener on fat basis).

In sample 1 and 3 which were blind duplicates a CV(R) ranging from 15 to 55 percent was obtained. For most CBs the CV(R) was in the order of 30 percent (50-200 µg/kg level per CB congener on fat basis).

In sample 2 and 4 which were blind duplicates a CV(R) ranging from 6 to 32 percent was obtained. For most CBs the CV was in the order of 15 percent (100-1000 µg/kg level per CB congener on fat basis).

c) The higer CVs obtained for the lower CBs are explicable by unsufficient separation of CBs and interference with toxaphene residues.

Table 1 Gaschromatographic conditions PCB interlaboratory study (BCR 4/1985)

Lab. no.	Apparatus (type)	Column phase	Length (m)	I.d. (mm)	Filmthickness (µm)	Linear gas velocity (cm/sec)	Gas (type)	Injector temperature (°C)	Temperature programme	Type of injection
1	Tracor 550	Sil 7	25	0,25	0,45	35	He	230	4 min 100°C-10°C/min-230°C	splitless
2	Varian 3700	Sil 5 CB	25	0,22	0,12	40	H2	-	1 min 120°C- 3°C/min-260°C	on-column
	Varian 3700	Sil 8 CB	25	0,22	0,12	40	H2	-	1 min 120°C- 3°C/min-260°C	on-column
3	HP 5880 A	SE 54 CB	40	0,30-0,32	0,17	36	H2	280	4 min 90°C-10°C/min-220°C	splitless
4	PB 427	HP Ultra 2	19	0,31	0,52	25-30	He	240	4 min 90°C-10°C/min-220°C	splitless
	PB 427	Sil 5 CB	25	0,23	0,13	25-30	He	-	4 min 130°C- 6°C/min-220°C	on-column
5	PE 8320	Sil 8 CB	25	0,24	0,44	25	He	270	3 min 90°C-30°C/min-30 min 215°C-5°C/min-225°C	splitless
	PE 8320	Sil 19 CB	25	0,23	0,21	25	He	270	3 min 90°C-30°C/min-30 min 215°C-5°C/min-225°C	splitless
6	Carlo Erba	SE 54 CB	50	0,20	0,33	38	H2	250	5 min 90°C-30°C/min-140°C-3°C/min-240°C	splitless
	Carlo Erba	OV 1701 CB	60	0,25	0,25	39	H2	270	3 min 90°C-30°C/min-140°C-3°C/min-260°C	splitless
7	HP 5880	Sil 5 CB	40	0,22	0,13	35	H2	280	4 min 90°C-10°C/min-220°C	splitless
8	HP 5710	SE 52 CB	50	0,2	0,11	26a)	He	250	4 min 90°C- 4°C/min-240°C	splitless
	HP 5730	OV 17	30	0,25	0,25	61	He	250	4 min 90°C- 2°C/min-260°C	splitless
9	Carlo Erba 4160	Sil 7 CB	25	0,32	0,25	35	n.r.	-	130°C-10°C/min-280°C	on-column
10	Carlo Erba 4160	SE 54	24	0,32	n.r.	24	He	-	40 s 110°C-10°C/min-3 min 160°C-3°C/min-255°C	on-column
11	PB 430	Sil 8	25	0,22	0,11	24	He	260	2 min 90°C-10°C/min-7 min 250°C-10°C/min-275°C	splitless
12	HP 5880	DB 1	30	0,26	0,25	32	He	270	4 min 90°C-10°C/min-220°C	splitless
	HP 5880	DB 5	30	0,25	0,25	31	He	270	4 min 90°C-10°C/min-220°C	splitless

a) = determinated at initial temperature
n.r. = not reported

Table 3 Results recovery experiments with standard solution (%)

Lab. no.	Column phase	Type of injection	Internal standard TCN							Internal standard DCBE-C14						
			CB 28	CB 52	CB 101	CB 118	CB 153	CB 138	CB 180	CB 28	CB 52	CB 101	CB 118	CB 153	CB 138	CB 180
1	Sil 7	splitless	78	82	91	92	96	92	91	80	84	93	94	98	94	93
2	Sil 5 CB	on-column	91	96	121	112	133	109	142	96	101	128	119	140	116	149
	Sil 8 CB	on-column	96	98	117	113	131	110	127	94	94	115	110	128	107	123
3	SE 54 CB	splitless	89	97	96	95	94	99	91	94	99	102	99	98	102	95
4	HP Ultra 2 CB	splitless	128	90	93	90	85	86	78	n.r.	88	111	99	106	98	98
	Sil 5 CB	on-column	n.r.	81	87	96	95	104	101	107	96	77	92	84	86	77
5	Sil 8 CB	splitless	73	69	88	98	106	98	106	69	58	81	91	105	92	106
	Sil 19 CB	splitless	52	69	73	82	89	84	88	51	65	76	86	100	88	102
6	SE 54 CB	splitless	94	93	101	98	102	101	102	101	100	109	106	111	109	110
7	Sil 5 CB	splitless	101	100	104	107	104	104	105	90	90	93	96	93	93	94
8	SE 52 CB	splitless	105	104	102	104	101	104	105	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
10	SE 54	on-column	105	99	105	99	93	99	98	105	98	106	99	93	99	99
11	Sil 8 CB	splitless	110	114	110	121	115	121	121	89	95	94	107	103	107	108
12	DB-1 CB	splitless	78	85	92	96	98	97	98	76	82	89	93	95	93	94
	DB-5 CB	splitless	90	94	97	98	100	98	100	92	96	99	100	102	101	103

n.r. = not reported

Table 4 Results practice sample K (µg/kg eel-f

Lab. no.	Column phase	Type of injection	Internal standard TCN							Internal standard DCBE-C14						
			CB 28	CB 52	CB 101	CB 118	CB 153	CB 138	CB 180	CB 28	CB 52	CB 101	CB 118	CB 153	CB 138	CB 180
1	Sil 7	splitless	26	79	126	138	333	284	126	26	79	127	139	334	284	127
2	Sil 5 CB	on-column	100G	112	180	239	350	348	139	103	114	176	233	341	339	136
	Sil 8 CB	on-column	113	185	172	149	357	352	133	97G	159	150	130	346	306	115
3	SE 54 CB	splitless	34	105	128	133	288	263	125	33	101	123	128	272	251	121
4	HP Ultra 2 CB	splitless	(10)	(76)	165	240	331	300	188	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
	Sil 5 CB	on-column	(23)	(73)	140	182	342	188	131	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
5	Sil 8 CB	splitless	27	92	194	193	426	398	174	24	81	168	168	378	356	150
	Sil 19 CB	splitless	27	104	135	162	366	383	169	23	89	119	147	346	355	158
6	SE 54 CB	splitless	30	96	154	148	371	290	149	27	135	186	177	463	358	178
7	Sil 5 CB	splitless	71G	108	131	172	323	299	134	69G	104	127	169	320	298	131
8	SE 52 CB	splitless	23	93	112	118	358	287	117	30	114	125	133	367	297	129
	OV 17 CB	splitless	23	119	91	102	364	238	127	22	107	83	95	311	214	110
10	SE 54	on-column	42	83	141	148	306	323	144	41	81	140	146	302	319	142
11	Sil 8 CB	splitless	n.r.	n.r.	n.r.	214	426	369	188	n.r.	n.r.	n.r.	182	371	292	170
12	DB-1 CB	splitless	39	87	124	185	318	290	131	41	91	130	192	331	302	137
	DB-5 CB	splitless	32	88	135	140	338	313	138	32	87	134	139	345	314	141
mean (selected data)			43(31)	95	134	153	343	298	141	41[29]	99	132	145	339	298	138
CV(R) (%)			60[20]	12	16	19	11	19	15	62[23]	19	21	18	15	14	16

( ) = not used for statistical treatment (see text)
G = Grubbs' outlier
[ ] = after elimination of two outliers according to the Grubbs'test
n.r.= not reported

Table 5 Results sample 1 (µg/kg fish oil)

Lab. no.	Column phase	Type of injection	Internal standard TCN							Internal standard DCBE-C14						
			CB 28	CB 52	CB 101	CB 118	CB 153	CB 138	CB 180	CB 28	CB 52	CB 101	CB 118	CB 153	CB 138	CB 180
1	Sil 7	splitless	25	36	83	97	116	108	41	28	42	96	112	136	127	48
2	Sil 5 CB	on-column	76C	n.r.	123	181	198	199	66	81C	n.r.	122	181	197	197	64
	Sil 8 CB	on-column	139	122	167	173	218	185	57	121	104	149	141	194	166	51
3	SE 54 CB	splitless	41	89	81	100	124	111	37	51	106	101	120	148	134	47
4	HP Ultra 2 CB	splitless	38	62	320	260	230	220	63	54	96	295	280	279	243D+G	82
	CP Sil 5 CB	on-column	48	59	127C	229	181	236	109	64	83	193	259G	236	274	156
5	Sil 8 CB	splitless	18	9	52	82	129	126	24	18	3	51	83	134	130	20
6	SE 54 CB	splitless	17	50	78	106	145	121	50	19	61	96	131	181	151	60
	OV 1701 CB	splitless	15	47	76	98	139	185	51	19	48	74	94	131	173	51
7	Sil 5 CB	splitless	19	64	88	148	190	154	59	19	62	85	143	183	148	57
8	SE 52 CB	splitless	13	50	94	161	164	176	51	15	55	87	145	148	159	49
	OV 17 CB	splitless	28	95	76	107	179	119	51	29	95	77	106	172	125	53
10	SE 54 (A)	on-column	41	60	164	126	172	193	50	40	59	162	124	170	190	50
	(B)	on column	43	66	152	115	174	178	51	42	63	150	113	171	174	50
11	Sil 8 CB	splitless	45	140	171	178	257	194	67	39	122	161	164	236	177	60
12	DB-1 CB	splitless	25	46	74	126	150	134	54	24	45	72	121	147	131	53
	DB-5 CB	splitless	20	49	77	119	157	145	56	21	49	78	122	159	146	57

C = Cochran outlier, D = Dixon outlier, G = Grubbs' outlier
n.r. = not reported

Table 6 Results sample 3 (µg/kg fish oil)

Lab. no.	Column phase	Type of injection	Internal standard TCN							Internal standard DCBE-C14						
			CB 28	CB 52	CB 101	CB 118	CB 153	CB 138	CB 180	CB 28	CB 52	CB 101	CB 118	CB 153	CB 138	CB 180
1	Sil 7	splitless	27	40	82	96	127	116	42	29	36	86	102	135	123	44
2	Sil 5 CB	on-column	67	n.r.	91	136	161	151	50	73	n.r.	93	140	164	155	51
	Sil 8 CB	on-column	37C	107	131	107	181	147	50	35C	95	121	90	167	136	46
3	SE 54 CB	splitless	45	95	80	100	123	112	34	54	108	96	114	141	130	41
4	HP Ultra 2 CB	splitless	34	71	309C	266	234	227	70	34	70	236	290G	248	295D+G	69
	Sil 5 CB	on-column	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
5	Sil 8 CB	splitless	25	25	58	93	145	127	21	23	19	52	87	139	121	13
6	SE 54 CB	splitless	18	52	83	109	142	124	48	23	66	104	138	179	156	59
	OV 1701 CB	splitless	18	51	81	108	135	191	56	20	52	78	104	128	180	56
7	Sil 5 CB	splitless	23	78	107	187	241	198	75	21	73	100	174	223	183	70
8	SE 52 CB	splitless	14	49	86	151	164	169	43	15	53	77	133	145	150	45
	OV 17 CB	splitless	28	99	89	112	157	129	55	32	108	99	123	169	148	65
10	SE 54 (A)	on-column	33	61	119	124	182	184	54	33	60	118	124	181	183	54
	(B)	on-column	80	216	132	134	198	195	55	75	202	125	127	187	185	51
11	Sil 8 CB	splitless	37	154	165	192	282	207	78	32	131	148	176	257	184	73
12	DB-1 CB	splitless	26	48	77	129	151	135	52	26	48	77	127	151	135	52
	DB-5 CB	splitless	20	50	84	119	156	144	56	21	51	87	124	160	147	56

C = Cochran outlier, D = Dixon outlier, G = Grubbs' outlier
n.r. = not reported

Table 7 Results after statistical evaluation of the TCN data of sample 1 and 3

a) all selected lowest data

CB	x (µg/kg)	r (µg/kg)	R (µg/kg)	CV(R) %	n
28	30	26	42	49	11
52	68	20	106	55	11
101	107	114	159	52	11
118	134	53	142	37	11
153	170	54	134	28	11
138	153	37	112	26	11
180	51	15	43	30	11

b) after elimination of Cochran and Dixon outliers (see text)

CB	x (µg/kg)	r (µg/kg)	R (µg/kg)	CV(R) %	n
28	28	9,8	31	40	10
52	68	20	106	55	11
101	96	33	197	36	10
118	134	53	142	37	11
153	170	54	134	28	11
138	153	37	112	26	11
180	51	15	43	30	11

Table 8 Results after statistical evaluation of the DCBE-C14 data of sample 1 and 3

a) all selected lowest data

CB	x (µg/kg)	r (µg/kg)	R (µg/kg)	CV(R) %	n
28	32	31	47	52	11
52	67	17	95	50	11
101	106	39	133	44	11
118	135	44	151	40	11
153	172	35	122	25	11
138	159	45	122	27	11
180	51	16	44	30	11

b) after elimination of Cochran and Dixon outliers (see text)

CB	x (µg/kg)	r (µg/kg)	R (µg/kg)	CV(R) %	n
28	29	15	36	44	10
52	67	17	95	50	11
101	106	39	133	44	11
118	135	44	151	40	11
153	172	35	122	25	11
138	148	34	164	15	10
180	51	16	44	30	11

c) after elimination of outliers according to the Cochran and Grubbs' test (see text)

CB	x (µg/kg)	r (µg/kg)	R (µg/kg)	CV(R) %	n
28	29	15	36	44	10
52	67	17	95	50	11
101	106	39	133	44	11
118	121	42	78	23	10
153	172	35	122	25	11
138	148	34	64	15	10
180	51	16	44	30	11

Table 9 Results sample 2 (µg/kg fish oil)

Lab. no.	Column phase	Type of injection	Internal standard TCN							Internal standard DCBE-C14						
			CB 28	CB 52	CB 101	CB 118	CB 153	CB 138	CB 180	CB 28	CB 52	CB 101	CB 118	CB 153	CB 138	CB 180
1	Sil 7	splitless	95	197	590	423	820	845	285	98	207	622	444	866	897	299
2	Sil 5 CB	on-column	166	275	673	732	850	945	275	179	291	685	746	865	962	280
	Sil 8 CB	on-column	167	280	565	345	986	922	230	162	266	559	328	975	911	228
3	SE 54 CB	splitless	140	277	570	435	811	842	214	146	288	595	450	816	846	225
4	HP Ultra 2 CB	splitless	162	230	890	1180G	981	1128	337	168	347	1202	1367G	1345G	1323	354
	Sil 5 CB	on-column	168	242	760G	1638	1139	1161	282	202	354	966G	1519	1489	1400	380
5	Sil 8 CB	splitless	63	178	576	399	888	987	275	59	168	572	392	887	987	266
6	SE 54 CB	splitless	89	249	591	467	856	778	302	87	256	616	484	896	811	311
	OV 1701 CB	splitless	116	266	584	456	856	1218	336	116	242	518	407	749	1062	302
7	Sil 5 CB	splitless	89	206	477	556	771	770	271	87	202	467	544	754	753	265
8	SE 52 CB	splitless	67	226	662	490	909	948	308	83	269	683	485	901	938	308
	OV 17 CB	splitless	108	341	557	497	875	790	344	112	343	562	495	911	818	336
10	SE 54 (A)	on-column	87	280	657	570	1035	1213	323	86	262	636	554	1000	1177	311
	(B)	on-column	87	248	626	536	997	1167	332	82	246	614	523	974	1144	323
11	Sil 8 CB	splitless	128	349	840G	966	1421G	1353	472G	118	322	866G	1016G	1478G	1403	452G
12	DB-1 CB	splitless	92	234	587	748	939	960	306	84	216	504	644	858	866	284
	DB-5 CB	splitless	96	237	588	468	935	967	314	98	240	604	477	954	989	318

G = Grubbs' outlier

Table 10 Results sample 4 (µg/kg fish oil)

Lab. no.	Column phase	Type of injection	Internal standard TCN							Internal standard DCBE-C14						
			CB 28	CB 52	CB 101	CB 118	CB 153	CB 138	CB 180	CB 28	CB 52	CB 101	CB 118	CB 153	CB 138	CB 180
1	Sil 7	splitless	99	205	617	470	837	952	290	99	204	614	470	835	945	288
2	Sil 5 CB	on-column	161	258	676	762	886	980	292	171	240	678	762	888	982	292
	Sil 8 CB	on-column	144	253	542	268	1002	901	243	148	253	567	349	1046	941	254
3	SE 54 CB	splitless	134	264	597	452	822	844	260	135	264	598	447	795	816	262
4	HP Ultra 2 CB	splitless	146	298	977G	1350G	1166	1465	356	132	285	814G	1291G	1107G	1194	325
	Sil 5 CB	on-column	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
5	Sil 8 CB	splitless	87	226	575	383	854	957	254	85	219	600	396	895	1005	259
6	SE 54 CB	splitless	93	244	629	488	932	885	322	115	266	649	507	946	902	341
	OV 1701 CB	splitless	88	292	577	453	845	1152	318	53	267	612	446	878	1220	304
7	Sil 5 CB	splitless	122	285	640	750	1004	1007	353	117	272	614	719	963	965	339
8	SE 52 CB	splitless	65	229	722	523	907	967	321	76	257	667	485	843	896	255
	OV 17 CB	splitless	195	430	607	552	860	882	487	123	301	389	360	699	630	333
10	SE 54 (A)	on-column	73	312	630	568	1081	1258	343	73	317	636	573	1085	1266	345
	(B)	on-column	91	304	634	543	1008	1172	319	96	310	655	563	1043	1214	330
11	Sil 8 CB	splitless	129	339	768G	757	1153G	1139	423G	140	329	846G	924G	1293G	1312	482G
12	DB-1 CB	splitless	98	226	585	750	926	935	298	88	204	496	637	832	836	272
	DB-5 CB	splitless	98	241	595	472	937	968	305	105	258	641	505	1000	1040	323

G = Grubbs' outlier
n.r. = not reported

Table 11 Results after statistical evaluation of the TCN data of sample 2 and 4

a) all selected data

CB	x (µg/kg)	r (µg/kg)	R (µg/kg)	CV(R) %	n
28	107	31	92	30	11
52	256	71	134	18	11
101	629	174	322	18	11
118	576	209	781	48	11
153	935	244	432	16	11
138	986	301	526	19	11
180	305	81	170	20	11

b) after elimination of outliers according to the Grubbs' test (see text)

CB	x (µg/kg)	r (µg/kg)	R (µg/kg)	CV(R) %	n
28	107	31	92	30	11
52	256	71	134	18	11
101	583	118	104	6,3	9
118	507	191	456	32	10
153	910	192	266	10	10
138	986	301	526	19	11
180	290	79	108	13	10

Table 12 Results after statistical evaluation of the DCBE-C14 data of sample 2 and 4

a) all selected data

CB	x (µg/kg)	r (µg/kg)	R (µg/kg)	CV(R) %	n
28	105	43	96	32	11
52	256	80	134	18	11
101	620	176	398	23	11
118	586	154	855	52	11
153	947	274	573	21	11
138	978	212	586	21	11
180	303	68	183	21	11

b) after elimination of outliers according to the Grubbs' test (see text)

CB	x (µg/kg)	r (µg/kg)	R (µg/kg)	CV(R) %	n
28	105	43	96	32	11
52	256	80	134	18	11
101	563	166	185	12	9
118	461	151	265	20	9
153	867	227	250	10	9
138	978	212	586	21	11
180	286	69	102	13	10