

Report 84.93

1984-11-05

BCR-ringtest of some-individual chloro-
biphenyls in cleaned eel-fat extracts
(3/1984). Part 1. Splitless injection.
Summary of results.

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torhoofd, projektbeheer, circulatie, Afd. Org. Contaminanten/
Bestrijdingsmiddelen, deelnemers ringtest

This report has been written for information to the EC-Community
Bureau of Reference, Brussels, as described in contract
1890/1/9/246/84/9-BCR-NL (10), dated October 19, 1984.

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1. Introduction

In the meeting on PCB analyses organized in Ulm, Donau, West Germany, 11-13 April 1984, where the second ringtest 2/1983 (Report 84.27 d.d. 1984-03-26) was discussed, it was decided to organize a next ringtest. In this ringtest identification and quantification of some individual chlorobiphenyls should be carried out in the extracts of eel-fat samples (cleaned-up by saponification) using splitless injection (part 1) and/or on-column injection after optimization (part 2).

For the optimization of the on-column injection prof. D.L. Massart of the University of Brussels, Belgium had offered his co-operation to find the optimum settings by using a computer program. Results will be reported in part 2.

In this report only the results of the splitless injection are given.

2. Participants

A complete list of the 9 participating laboratories, which took part in the identification and quantification using splitless injection, is given in annex 1.

3. Description of the study

The samples of the ringtest were prepared by RIKILT. Each laboratory was supplied with a standard solution chlorobiphenyls (code A), standard solutions of internal standards (code B and C) blanc solvent isooctane (code D) and two eel-fat extracts (0.166 g/ml, code E and F) cleaned-up by saponification (method, see annex 2).

One eel-fat extract (code E) was partially a practice sample as the concentration for PCB compound 101 and 153 were given.

For the analyses of the two samples, columns with a different polarity should be used when possible.

More details with instructions concerning the materials, the method for the optimization of the gaschromatographic conditions for splitless injection, injection volume (1 µl), the procedure for the linearity test and the identification and quantification of the eel-fat extracts were given in the RIKILT letter coded 1587 d.d. 1984-06-26.

4. Results/Discussion

Nine laboratories returned results for this part of the study. Laboratory 1, 2, 4, 5, 7 and 8 used more than one column. Laboratory 1 used two columns of comparable polarity and laboratory 2 reported results on three columns.

In the interpretation of the data of the eel-fat samples we must keep in mind that for each laboratory more than one set of data can be available. Therefore, the data will be split up according to polarity of the stationary phase in the tables for the gaschromatographic conditions, resolution, linearity and quantitative results.

4.1 Gaschromatographic conditions

A summary of the gaschromatographic conditions is given in table 1. The apolar columns methylsilicone, BP 1, OV 101 and CP Sil 5 are given under the headline CP Sil 5. The more polar columns 5% phenylmethylsilicone, SE 52, SE 54, BP 5, CP Sil 7 and CP Sil 8 are given under the headline CP Sil 8. The results for the CP Sil 19 are given separately.

It is assumed that all laboratories used optimum settings for the splitless injection.

Based on the $H\bar{u}$ -curve laboratory 2 used for helium a higher linear gas velocity than other laboratories. It is striking that laboratory 9 has clear different optimum settings for the injector and detector temperature related to the settings of the other laboratories.

4.2 Resolution

A summary of the resolutions between the succeeding PCB compounds is given in table 2. Although uptill now the formula for the calculation of the resolution given in report 84.27 is used, in accordance with the literature, laboratory 4 reported that it is better to use the formula $R = 1.117 \times \Delta t / \Sigma W^{1/2}$. In spite of this the results given in table 2 can be compared with each other. In general under the given settings it can be concluded that for the given standard solution of chlorobiphenyls the resolution on columns with a polarity comparable with the CP Sil 5 column is better than for CP Sil 8 or 19 columns.

4.3 Linearity test

All laboratories, except laboratory 5, carried out the linearity test. In table 3 a summary is given of the tested range and the maximum difference of the ratio peak area/mass in the tested range for PCB 153 corrected for the internal standard TCN. In general this ratio is higher for the lower injected quantities of PCB 153. The difference in response between the highest and lowest injected quantity, covering a range of about a factor of 10, varies from 7 to 56%.

Laboratory 4 reported that, the peak heights in the samples were calculated by comparing the response of the sample with the response of the nearest standard concentration.

Laboratory 2 used the procedure by which the sample was diluted near to the standard concentration laying within the tested range for the linearity.

For the other laboratories it is not clear from the chromatograms and comments, in how far they have corrected the results for the linearity or have analysed the samples near to a diluted standard solution. We assume that the deviation in peak height in the sample and standard was as small as possible.

4.4 Blanc solvent (code D)

Interferences in the blanc solvent iso-octane with chlorobiphenyls were only observed by laboratory 7 and 9 for PCB 52.

Laboratory 7 reported an interference at the limit of detection of about 3 ng/ml and laboratory 9 reported about 4 ng/ml.

The other laboratories reported no interferences having a detection limit of 0.5 ng/ml or lower.

4.5 Eel-fat samples

The quality of the eel-fat extracts, (negative peaks, in the chromatograms), especially in sample F, left much to be desired. In sample E this problem was hardly present.

After the preparation of the samples at RIKILT we did not observe this problem because we checked the samples only on a CP Sil 7 column (see chromatogram 1 and 2). Especially with columns with a polarity comparable to CP Sil 5 this problem occurs (see chromatogram 3) and where reported by several participants and caused many problems, sometimes making analyses impossible.

Maybe the problem is due to a too long storage period at -18°C of the eel-fat of sample F (hydrolyses?).

Laboratory 7 eliminated the problem by means of a clean-up over a silica column of the extract. Only laboratory 8 using a BP 1 and BP 5 column did not observe negative peaks (extra sample clean-up?).

The results of the eel-fat extracts of the laboratories 1 (partially), 3 (partially), 5 and 9 (partially) are not used for statistical analyses and therefore not reported for the following reasons.

Laboratory 1 used two columns of comparable polarity (SE 52 and SE 54) and we made therefore a choice between the comparable results and eliminated the SE 54 results from which the resolution and linearity were not reported.

Laboratory 3 injected the samples four times a day instead of one time. As all results were comparable we only used the result of the first injection.

Laboratory 5 reported incomplete data for the eel-fat extracts and for the resolution of the succeeding PCB compounds and was therefore eliminated.

Laboratory 9 calculated the eel-fat samples, probably due to varying response (too low temperature of injector and detector?) in different ways. Only the results calculated to the standard immediately injected prior to the samples are used, as this was in agreement with the instructions.

The statistical treatment of the data is carried out according to ISO 5725 as described in report 84.27.

For the CP Sil 5 or comparable phases five data sets are available for both samples for all PCB compounds with exception of PCB 28, where laboratory 4 did not report results.

For the CP Sil 8 or comparable phases six data sets are available for both samples for all PCB compounds with exception of PCB 52, where laboratory 7 did not report PCB 52 due to an interference, also present in the blanc solvent.

For CP Sil 19 CB two data sets are available for both samples.

4.5.1 Eel-fat extract (code E)

The results for the PCB compounds 28, 52, 101, 118, 153, 138 and 180 used for statistical treatment are given in duplo in table 4 in ng/ml. The results have been rounded off. Although this sample was partially a practice sample, as the concentration for PCB compound 101 and 153 were known (respectively about 20 and 55 ng/ml), all data have been used for statistical treatment.

A statistical treatment was carried out with the CP Sil 5 group, the CP Sil 8 group and all data together, including CP Sil 19 CB data.

In table 5 the mean (\bar{x}), repeatability (r), reproducibility (R) and the coefficient of variation for the reproducibility are given.

For the CP Sil 5 group and CP Sil 8 group only 5 respectively 6 data sets for sample E were available. Probably rather few for a statistical treatment.

For the CP Sil 5 group Cochran outliers for PCB 28, 101 and 118 were found using TCN as internal standard. After elimination per compound of that laboratory the results are given in brackets. No Dixon outliers were obtained.

For the CP Sil 8 group no Cochran or Dixon outliers were obtained.

For all data, Cochran outliers were found for PCB 28, 52, 101 and 118 using TCN as internal standard and for PCB 52, 118, 138 and 180 using DCBE-C14 as internal standard (data only of the CP Sil 5 group). Again after elimination new results are given in brackets.

The CV(R) for the CP Sil 8 group is in general better than for the CP Sil 5 group. The mean for all data using TCN or DCBE-C14 as internal standard is equal. We may conclude that both internal standards can be used in PCB-analyses.

4.5.2 Eel-fat extract (code F)

The results for the PCB compounds 28, 52, 101, 118, 153, 138 and 180 are given in table 6. The results have been rounded off.

For all laboratories, except laboratory 7 and 8, as well as with the TCN peak as for the PCB peaks negative interferences were reported, especially with the CP Sil 5 group. For the CP Sil 8 group and the CP Sil 19 CB column only slight interferences were observed.

The results of the CP Sil 5 group, calculated with TCN, were therefore not used for statistical treatment. The results calculated with DCBE-14 are, in spite of the negative peaks with the PCB's, however used for statistical treatment.

In table 7 the mean (\bar{x}), repeatability (r), reproducibility (R) and the coefficient of variation for the reproducibility are given. For the internal standard TCN only for the CP Sil 8 group, data are reported, (6 data sets) as well as together with the CP Sil 19 data (8 data sets).

For the internal standard DCBE-C14 results for the CP Sil 5 group, the CP Sil 8 group and all data together, including CP Sil 19 are reported. For the CP Sil 5 group and CP Sil 8 group only 5 respectively 6 data sets were available. Probably rather few for a statistical treatment. For the CP Sil 5 group Cochran outliers for PCB 101 and 118 were found using DCBE-C14 as internal standard. After elimination of that laboratory results are given in brackets. No Dixon outliers were obtained. For the CP Sil 8 group no Cochran or Dixon outliers were obtained. For all data a Dixon outlier for PCB 101 and a Cochran outlier for PCB 138 were found using TCN as internal standard. A Dixon outlier for PCB 101 and Cochran outlier for PCB 118 and 153 were found using DCBE-C14 as internal standard. Again after elimination new results are given in brackets.

The CV(R) for the CP Sil 8 group is in general better than for the CP Sil 5 group. In spite of the problems encountered with this sample, acceptable results were obtained with the CP Sil 8 group.

5. Conclusions

From the reported results following can be concluded:

- a) With a standard solution of the PCB compounds 28, 52, 101, 118, 153, 138 and 180 the best resolution is obtained for CP Sil 5 or comparable phases.
- b) The linearity of PCB 153 in the tested range is in most laboratories critical. Attention should be given to this problem.
- c) The mean for all data using TCN or DCBE-C14 as internal standard is in general for all groups of data the same. Both standards can be used for quantification purposes.

d) In spite of the problems encountered with the samples (negative peaks), in particular with sample F, rather good results were obtained. For sample E (with exception of PCB 28) at the 0.1 mg/kg level a CV(R) of about 7-20% was obtained.

For sample F (with exception of PCB 28) at the 1.0 mg/kg level a CV(R) of about 10-30% was obtained, due to the negative peaks.

For PCB 28 due to interferences the CV in sample E was about 20% and in sample F about 35% respectively at the 0.05 and 0.25 mg/kg level.

e) From a point of view of CV the best results with the eel-fat extracts were obtained with CP Sil 8 or comparable phases.

Table 1. Gaschromatographic conditions BCR ringtest 3/1984. Part 1 - Splitless injection

| Lab. no. | Apparatus (type) | Detector (type) | Column phase | Length (m) | I.d. (mm) | Film thickness (µm) | Linear gas velocity (cm/sec) | Gas (type) | Temp. injector (°C) | Temp. detector (°C) | Temp. programme | Closing time splitter (min) |
|---------------------------|------------------|-----------------|---------------------------------|------------|-----------|---------------------|------------------------------|------------|---------------------|---------------------|-----------------------------|-----------------------------|
| a) CP S11 5 or comparable | | | | | | | | | | | | |
| 2 | Carlo Erba 4160 | Ni 63 | CP S11 5 CB | 25 | 0.32 | 0.44 | 33 | He | 250 | 330 | 4 min 90°C-10°C/min-220°C | 3 |
| 4 | HP 5730 A | Ni 63 | methylsili- con CB | 50 | 0.2 | 0.11 | 22 | n.r. | 250 | 300 | 3 min 100°C-2,5°C/min-250°C | 0.5 |
| 5 | PB 430 | Ni 63 | CP S11 5 CB | 25 | 0.24 | 0.13 | 22 | He | 240 | 305 | 4 min 90°C-10°C/min-220°C | 1 |
| 6 | Carlo Erba Mega | Ni 63 | CP S11 5 CB | 50 | 0.22 | 0.14 | 36 | H2 | 275 | 275 | 4 min 90°C-10°C/min-220°C | 00 |
| 8 | HP 5880 | Ni 63 | BP 1 CB | 25 | 0.22 | 0.25 | 25 | He | 270 | 320 | 4 min 90°C-10°C/min-220°C | 1.5 |
| 9 | HP 5880 A | Ni 63 | OV 101 | 25 | 0.22 | n.r. | 25 | He | 200 | 250 | 4 min 90°C-10°C/min-220°C | 1 |
| b) CP S11 8 or comparable | | | | | | | | | | | | |
| 1 | Carlo Erba 4160 | Ni 63 | SE 52 CB | 25 | 0.25 | 0.20 | 32 | H2 | 240 | 250 | 4 min 90°C-10°C/min-220°C | 1 |
| 1 | Carlo Erba 4160 | Ni 63 | SE 54 CB | 25 | 0.25 | 0.20 | 36 | H2 | 240 | 250 | 4 min 90°C-10°C/min-220°C | 1 |
| 2 | Tracor 550 | Ni 63 | CP S11 7 | 25 | 0.25 | 0.45 | 35 | He | 240 | 330 | 4 min 90°C-10°C/min-220°C | 3 |
| 3 | HP 5880 A | Ni 63 | SE 54 CB | 40 | 0.30-0.32 | 0.17 | 34 | H2 | 280 | 320 | 4 min 90°C-10°C/min-220°C | n.r. |
| 4 | HP 5710 A | Ni 63 | 5% phenyl methylsili- con | 50 | 0.2 | 0.11 | 24 | n.r. | 250 | 300 | 3 min 100°C- 4°C/min-260°C | 0.5 |
| 5 | PB 430 | Ni 63 | CP S11 8 CB | 25 | 0.22 | 0.11 | 22 | He | 240 | 305 | 4 min 90°C-10°C/min-220°C | 1 |
| 7 | PB 419 | Ni 63 | CP S11 8 CB | 25 | 0.23 | 0.42 | 25 | He | 240 | 305 | 6 min 90°C-10°C/min-220°C | 5 |
| 8 | HP 5880 | Ni 63 | BP 5 CB | 25 | 0.2 | 0.25 | 25 | He | 270 | 320 | 4 min 90°C-10°C/min-220°C | 1.5 |
| c) CP S11 19 columns | | | | | | | | | | | | |
| 2 | PB 430 | Ni 63 | CP S11 19 CB | 25 | 0.23 | 0.19 | 36 | He | 250 | 330 | 4 min 90°C-10°C/min-230°C | 3 |
| 7 | PB 419 | Ni 63 | CP S11 19 CB | 25 | 0.23 | 0.21 | 25 | He | 240 | 305 | 6 min 90°C-10°C/min-220°C | 5 |

n.r. = not reported

Table 2. Resolution *) between the tested PCB congeners

| Lab. no. | Column phase | R 28/52 | R 52/101 | R 101/118 | R 118/153 | R 153/138 | R 138/180 |
|---|-------------------------|---------|----------|--------------|-----------|-----------|-----------|
| a) <u>Polarity comparable with CP Sil 5</u> | | | | | | | |
| 2 | CP Sil 5 CB | 9.2 | 24.1 | 17.7 | 7.4 | 7.4 | 22.0 |
| 4 | methyl-silicon CB | 13.0 | 32.0 | 22.3 | 9.9 | 9.2 | 27.2 |
| 5 | CP Sil 5 CB | 12.9 | 30.0 | 22.0 | 9.7 | 9.4 | 30.4 |
| 6 | CP Sil 5 CB | 12.1 | 36.1 | 25.3 | 10.7 | 11.4 | 33.1 |
| 8 | BP 1 CB | 11.2 | 21.7 | 16.2 | 7.0 | 7.3 | 25.0 |
| 9 | OV 101 | 9.1 | 24.5 | 18.5 | 8.0 | 8.0 | 9.8 |
| mean | | 11.2 | 28.1 | 20.3 | 8.8 | 8.8 | 24.6 |
| b) <u>Polarity comparable with CP Sil 8</u> | | | | | | | |
| 1 | SE 52 CB | 5.7 | 16.4 | 13.5 | 5.4 | 7.1 | 19.0 |
| 1 | SE 54 CB | | | not reported | | | |
| 2 | CP Sil 7 | 8.3 | 21.7 | 16.5 | 6.2 | 7.5 | 19.5 |
| 3 | SE 54 CB | 7.1 | 18.9 | 15.0 | 6.1 | 7.9 | 19.9 |
| 4 | 5% phenyl-methylsilicon | 13.1 | 31.6 | 22.3 | 7.4 | 9.6 | 22.9 |
| 5 | CP Sil 8 CB | | | not reported | | | |
| 7 | CP Sil 8 CB | 10.4 | 28.6 | 22.0 | 8.1 | 10.6 | 26.0 |
| 8 | BP 5 CB | 4.1 | 12.6 | 9.9 | 4.2 | 4.7 | 15.0 |
| mean | | 8.1 | 21.6 | 16.5 | 6.2 | 7.9 | 20.4 |
| c) <u>CP Sil 19</u> | | | | | | | |
| 2 | CP Sil 19 CB | 9.4 | 21.4 | 18.0 | 3.9 | 8.9 | 17.7 |
| 7 | CP Sil 19 CB | 9.5 | 25.4 | 21.4 | 4.9 | 12.0 | 23.8 |
| mean | | 9.4 | 23.4 | 19.7 | 4.4 | 10.4 | 20.9 |

*) Resolution defined as $R = \frac{t_r^2 - t_r^1}{W_1 \ 1/2 + W_2 \ 1/2}$

Table 3. Results of the linearity test for PCB 153, corrected for the internal standard TCN

| Lab. no. | Column phases | Injected mass range (pg) | max. diff. of the ratio peak area/mass in the tested range (%) |
|----------|---------------|--------------------------|--|
|----------|---------------|--------------------------|--|

a) Polarity comparable with CP Sil 5

| | | | |
|---|-------------------|----------------|------|
| 2 | CP Sil 5 CB | 5 - 40 | 21 |
| 4 | methyl-silicon CB | 16.67 - 166.67 | 56 |
| 5 | CP Sil 5 CB | n.r. | n.r. |
| 6 | CP Sil 5 CB | 8 - 82 | 37 |
| 8 | BP 1 CB | 3.75 - 37.5 | 34 |
| 9 | OV 101 | 6 - 36 | 12 |

b) Polarity comparable with CP Sil 8

| | | | |
|---|-------------------------|----------------|------|
| 1 | SE 52 CB | 5 - 50 | 16 |
| 1 | SE 54 CB | n.r. | n.r. |
| 2 | CP Sil 7 | 5 - 40 | 15 |
| 3 | SE 54 CB | 5 - 50 | 30 |
| 4 | 5% phenyl-methylsilicon | 16.67 - 166.67 | 50 |
| 5 | CP Sil 8 CB | n.r. | n.r. |
| 7 | CP Sil 8 CB | 5 - 50 | 7 |
| 8 | BP 5 CB | n.r. | n.r. |

c) CP Sil 19

| | | | |
|---|--------------|--------|------|
| 2 | CP Sil 19 CB | 5 - 40 | 37 |
| 7 | CP Sil 19 CB | n.r. | n.r. |

Table 4. The chlorobiphenyl content of eel-fat sample E, duplo results (ng. l.)

| Lab. no. | Column phase | Internal standard TCN | | | | Internal standard DCBE-C14 | | | | | | | | |
|----------|----------------------------|-----------------------|--------|---------------|---------|----------------------------|---------|---------|--------|--------|---------------|---------|---------------|---------|
| | | PCB 28 | PCB 52 | PCB (PCB 101) | PCB 118 | PCB (PCB 153) | PCB 138 | PCB 180 | PCB 28 | PCB 52 | PCB (PCB 101) | PCB 118 | PCB (PCB 153) | PCB 138 |
| 2 | CP Sil 5 or comparable | 6.9 | 15 | 23 | 29 | 51 | 27 | 6.4 | 14 | 21 | 27 | 46 | 47 | 25 |
| 2 | CP Sil 5 CB | 6.7 | 15 | 23 | 31 | 51 | 25 | 5.2 | 12 | 18 | 24 | 39 | 41 | 20 |
| 4 | methylsilicon CB | n.r. | 13 | 16 | 37 | 41 | 19 | n.r. | 14 | 20 | 46 | 51 | 45 | 24 |
| 4 | | n.r. | 15 | 23 | 54 | 57 | 25 | n.r. | 17 | 26 | 59 | 63 | 52 | 27 |
| 6 | CP Sil 5 CB | 11 | 25 | 24 | 27 | 60 | 25 | 9 | 21 | 20 | 23 | 50 | 49 | 21 |
| 6 | | 8 | 17 | 23 | 26 | 54 | 23 | 8 | 17 | 23 | 26 | 54 | 58 | 23 |
| 8 | BP 1 CB | 7.3 | 16 | 25 | 33 | 58 | 23 | 8.0 | 17 | 27 | 37 | 64 | 57 | 26 |
| 8 | | 7.2 | 16 | 25 | 34 | 58 | 23 | 7.4 | 17 | 25 | 35 | 59 | 52 | 24 |
| 9 | OV 101 | 7.0 | 21 | 24 | 35 | 48 | 24 | 6.9 | 21 | 24 | 34 | 47 | 50 | 23 |
| 9 | | 7.0 | 24 | 23 | 32 | 51 | 33 | 8.6 | 29 | 28 | 40 | 63 | 76 | 41 |
| 1 | CP Sil 8 or comparable | 8.0 | 12 | 19 | 22 | 48 | 20 | 8.7 | 13 | 21 | 24 | 53 | 47 | 22 |
| 1 | SE 52 CB | 8.6 | 13 | 21 | 28 | 56 | 23 | 8.1 | 12 | 20 | 26 | 52 | 47 | 22 |
| 2 | | 6.0 | 16 | 24 | 25 | 59 | 24 | 5.2 | 14 | 21 | 21 | 51 | 44 | 20 |
| 2 | CP Sil 7 | 5.1 | 15 | 21 | 24 | 52 | 22 | 4.7 | 14 | 19 | 22 | 47 | 41 | 20 |
| 3 | | 6.5 | 16 | 24 | 27 | 57 | 24 | 6.7 | 16 | 25 | 28 | 59 | 53 | 25 |
| 3 | SE 54 CB | 6.3 | 17 | 24 | 28 | 56 | 24 | 6.2 | 16 | 24 | 28 | 55 | 49 | 24 |
| 4 | | 5.8 | 16 | 23 | 20 | 49 | 21 | 7.4 | 21 | 30 | 26 | 62 | 50 | 26 |
| 4 | 5% phenylmethyl-silicon CB | 5.4 | 16 | 23 | 29 | 51 | 23 | 6.4 | 19 | 27 | 25 | 60 | 52 | 27 |
| 7 | | 5.1 | n.r. | 25 | 24 | 58 | 24 | 4.7 | n.r. | 23 | 22 | 53 | 45 | 22 |
| 7 | CP Sil 8 CB | 5.4 | n.r. | 24 | 23 | 55 | 23 | 5.3 | n.r. | 24 | 23 | 54 | 47 | 22 |
| 8 | | 7.6 | 16 | 22 | 26 | 58 | 21 | 8.3 | 17 | 26 | 31 | 65 | 56 | 24 |
| 8 | BP 5 CB | 8.6 | 16 | 23 | 29 | 60 | 24 | 9.0 | 17 | 24 | 29 | 61 | 54 | 24 |
| 2 | | CP Sil 19 | 8.4 | 23 | 26 | 27 | 55 | 29 | 6.6 | 18 | 20 | 21 | 44 | 43 |
| 2 | CP Sil 19 CB | 8.9 | 22 | 27 | 27 | 58 | 28 | 7.3 | 18 | 22 | 22 | 48 | 43 | 23 |
| 8 | | 6.3 | 17 | 23 | 24 | 53 | 24 | 6.3 | 17 | 23 | 25 | 54 | 45 | 24 |
| 8 | CP Sil 19 CB | 6.1 | 16 | 22 | 24 | 51 | 24 | 6.3 | 17 | 22 | 25 | 53 | 44 | 24 |

n.r. = not reported
 () = content approximately known

Table 5. Results of the statistical analyses of eel-fat sample E

| PCB compound | Internal standard TCN | | | Internal standard DCBE-Cl4 | | | | |
|--------------|-----------------------------|-----------|-----------|----------------------------|-----------|-----------|-----------------------|-------------|
| | x (ng/ml) | r (ng/ml) | R (ng/ml) | CV(R) (%) | x (ng/ml) | r (ng/ml) | R (ng/ml) | CV(R) (%) |
| 28 | (5 data sets) 7.6 (7.0) | 3.0 (0.3) | 4.1 (0.7) | 19.2 (3.3) | 7.4 | 2.4 | (5 data sets) 3.7 | 17.4 |
| 52 | 18 | 7.8 | 12 | 24.2 | 18 | 8.6 | 14 | 28.0 |
| 101*) | 23 (24) | 6.4 (1.4) | 7.3 (2.6) | 11.3 (3.9) | 23 | 7.7 | 9.7 | 14.8 |
| 118 | 34 (31) | 16 (3.9) | 23 (9.9) | 24.1 (11.3) | 35 | 14 | 33 | 33.6 |
| 153*) | 53 | 16 | 16 | 10.9 | 54 | 20 | 24 | 16.1 |
| 138 | 52 | 14 | 21 | 14.5 | 53 | 26 | 27 | 18.4 |
| 180 | 25 | 10 | 10 | 14.6 | 25 | 17 | 17 | 23.0 |
| 28 | (6 data sets) 6.5 | 1.3 | 3.9 | 21.2 | 6.7 | 1.3 | (6 data sets) 4.6 | 24.2 |
| 52 | 15 | 1.6 | 4.7 | 10.8 | 16 | 2.0 | 8.3 | 18.4 |
| 101*) | 23 | 3.2 | 5.0 | 7.7 | 24 | 3.6 | 9.2 | 13.8 |
| 118 | 25 | 9.3 | 8.1 | 11.3 | 25 | 2.7 | 9.3 | 12.9 |
| 153*) | 55 | 9.3 | 11 | 7.4 | 56 | 6.0 | 16 | 9.9 |
| 138 | 48 | 7.9 | 12 | 8.7 | 49 | 5.0 | 13 | 9.5 |
| 180 | 23 | 4.2 | 4.0 | 6.2 | 23 | 1.2 | 6.5 | 10.0 |
| 28 | (13 data sets) 7.0 (6.8) | 2.0 (1.0) | 4.1 (3.4) | All data 17.4 | 7.0 | 1.7 | (13 data sets) 3.9 | 19.6 |
| 52 | 17 (17) | 5.2 (2.6) | 9.9 (9.0) | 20.4 (17.4) | 17 (16) | 5.7 (3.5) | 10 (7.2) | 21.6 (15.6) |
| 101*) | 23 (23) | 4.6 (2.5) | 6.2 (4.9) | 9.5 (7.3) | 23 | 5.5 | 8.6 | 13.1 |
| 118 | 29 (27) | 12 (6.9) | 19 (11) | 23.4 (14.2) | 29 (27) | 8.6 (4.8) | 25 (15) | 30.9 (19.7) |
| 153*) | 54 | 12 | 13 | 8.5 | 54 | 13 | 19 | 12.6 |
| 138 | 50 | 10 | 17 | 12.0 | 50 (48) | 17 (8.7) | 20 (14) | 14.7 (10.4) |
| 180 | 24 | 6.9 | 8.1 | 11.9 | 24 (23) | 11 (3.8) | 11 (5.8) | 16.5 (8.8) |

n.r. = not reported
() = content approximately known

Table 6. The chlorobiphenyl content in eel-fat sample F, duplo results (ng/ml)

| Lab. no. | Column phase | Intern standard TCN | | | | | | | | Internal standard DCBE-Cl ₄ | | | | | | | | | | | | | | | | | | | | | | | | | | |
|----------|-------------------------------|---------------------|--------|---------|---------|---------|---------|---------|--------|--|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|--|--|--|--|--|--|
| | | PCB 28 | PCB 52 | PCB 101 | PCB 118 | PCB 153 | PCB 138 | PCB 180 | PCB 28 | PCB 52 | PCB 101 | PCB 118 | PCB 153 | PCB 180 | PCB 205 | PCB 200 | PCB 126 | PCB 124 | PCB 182 | PCB 240 | PCB 155 | PCB 154 | PCB 272 | PCB 261 | PCB 289 | PCB 296 | PCB 335 | PCB 288 | PCB 253 | PCB 268 | | | | | | |
| 2 | <u>CP Sil 5 or comparable</u> | 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. | 200 | 300 | 181 | 73 | | | | | | | | | | | | | | | | | | |
| 2 | CP Sil 5 CB | 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. | 193 | 306 | 181 | 126 | | | | | | | | | | | | | | | | | | |
| 4 | methylsilicon CB | n.r. | 170 | 168 | 285 | 268 | 193 | 133 | n.r. | 184 | 290 | 308 | 290 | 144 | 182 | 209 | 209 | 144 | | | | | | | | | | | | | | | | | | |
| 4 | | n.r. | 209 | 234 | 194 | 411 | 254 | 167 | n.r. | 216 | 408 | 262 | 172 | | 240 | | | | | | | | | | | | | | | | | | | | | |
| 6 | CP Sil 5 CB | 103 | 210 | 190 | 189 | 332 | 321 | 136 | 84 | 170 | 261 | 112 | | 155 | 272 | 261 | 112 | | | | | | | | | | | | | | | | | | | |
| 6 | | 106 | 189 | 207 | 202 | 344 | 380 | 137 | 80 | 144 | 289 | 103 | | 156 | 260 | 289 | 103 | | | | | | | | | | | | | | | | | | | |
| 8 | | 58 | 175 | 193 | 250 | 311 | 269 | 118 | 62 | 194 | 296 | 131 | | 213 | 342 | 296 | 131 | | | | | | | | | | | | | | | | | | | |
| 8 | BP 1 CB | 52 | 177 | 193 | 251 | 324 | 281 | 122 | 53 | 183 | 288 | 126 | | 200 | 335 | 288 | 126 | | | | | | | | | | | | | | | | | | | |
| 9 | | n.r. | n.r. | n.r. | n.r. | n.r. | n.r. | n.r. | n.r. | n.r. | n.r. | n.r. | | 31 | 260 | 253 | 117 | | | | | | | | | | | | | | | | | | | |
| 9 | OV 101 | n.r. | n.r. | n.r. | n.r. | n.r. | n.r. | n.r. | n.r. | n.r. | n.r. | n.r. | | 32 | 310 | 268 | 162 | | | | | | | | | | | | | | | | | | | |
| 1 | <u>CP Sil 8 or comparable</u> | 60 | 145 | 184 | 171 | 288 | 215 | 124 | 59 | 142 | 212 | 122 | | 181 | 282 | 212 | 122 | | | | | | | | | | | | | | | | | | | |
| 1 | SE 52 CB | 70 | 147 | 194 | 173 | 293 | 211 | 135 | 62 | 131 | 188 | 120 | | 173 | 262 | 188 | 120 | | | | | | | | | | | | | | | | | | | |
| 2 | | 36 | 176 | 210 | 188 | 357 | 311 | 138 | 30 | 147 | 262 | 116 | | 176 | 298 | 262 | 116 | | | | | | | | | | | | | | | | | | | |
| 2 | CP Sil 7 | 37 | 169 | 192 | 209 | 359 | 322 | 148 | 30 | 140 | 268 | 123 | | 159 | 282 | 268 | 123 | | | | | | | | | | | | | | | | | | | |
| 3 | | 28 | 184 | 202 | 198 | 317 | 283 | 131 | 28 | 179 | 276 | 129 | | 198 | 308 | 276 | 129 | | | | | | | | | | | | | | | | | | | |
| 3 | SE 54 CB | 30 | 196 | 204 | 208 | 316 | 284 | 131 | 27 | 179 | 286 | 120 | | 185 | 286 | 257 | 120 | | | | | | | | | | | | | | | | | | | |
| 4 | 5% phenylmethyl-silicon CB | 47 | 188 | 259 | 212 | 360 | 277 | 128 | 50 | 202 | 296 | 137 | | 277 | 385 | 296 | 137 | | | | | | | | | | | | | | | | | | | |
| 4 | | 40 | 160 | 261 | 237 | 399 | 331 | 127 | 45 | 182 | 307 | 145 | | 244 | 370 | 307 | 145 | | | | | | | | | | | | | | | | | | | |
| 7 | | 30 | n.r. | 182 | 173 | 302 | 272 | 124 | 30 | n.r. | 273 | 124 | | 183 | 303 | 273 | 124 | | | | | | | | | | | | | | | | | | | |
| 7 | CP Sil 8 CB | 29 | n.r. | 186 | 174 | 306 | 272 | 129 | 30 | n.r. | 282 | 133 | | 192 | 316 | 282 | 133 | | | | | | | | | | | | | | | | | | | |
| 8 | | 61 | 159 | 180 | 200 | 307 | 268 | 107 | 66 | 191 | 315 | 128 | | 213 | 356 | 315 | 128 | | | | | | | | | | | | | | | | | | | |
| 8 | BP 5 CB | 56 | 164 | 183 | 219 | 320 | 288 | 123 | 58 | 173 | 300 | 131 | | 192 | 356 | 300 | 131 | | | | | | | | | | | | | | | | | | | |
| 2 | <u>CP Sil 19</u> | 36 | 197 | 86 | 168 | 219 | 244 | 135 | 31 | 170 | 211 | 117 | | 77 | 190 | 211 | 117 | | | | | | | | | | | | | | | | | | | |
| 2 | CP Sil 19 CB | 37 | 199 | 90 | 168 | 187 | 246 | 116 | 33 | 177 | 219 | 103 | | 80 | 166 | 219 | 103 | | | | | | | | | | | | | | | | | | | |
| 8 | | 36 | 182 | 173 | 172 | 295 | 256 | 132 | 36 | 183 | 257 | 133 | | 173 | 296 | 257 | 133 | | | | | | | | | | | | | | | | | | | |
| 8 | CP Sil 19 CB | 37 | 191 | 181 | 176 | 307 | 264 | 140 | 36 | 187 | 259 | 137 | | 178 | 301 | 259 | 137 | | | | | | | | | | | | | | | | | | | |

n.r. = not reported

Table 7. Results of the statistical analyses of eel-fat sample F

| PCB compound | Internal standard TCN | | | Internal standard DCBE-C14 | | | | |
|--------------|-----------------------|-----------|-----------|----------------------------|-----------|-----------|----------------|-------------|
| | x (ng/ml) | r (ng/ml) | R (ng/ml) | CV(R) (%) | x (ng/ml) | r (ng/ml) | R (ng/ml) | CV(R) (%) |
| 28 | | | | CP S11 5 or comparable | | | (5 data sets) | 47.4 |
| 52 | | | | 51 | 11 | 68 | 68 | 19.9 |
| 101 | | | | 163 | 41 | 92 | 92 | 13.4 (12.5) |
| 118 | | | | 194 (190) | 54 (15) | 74 (67) | 74 (67) | 25.7 (27.2) |
| 153 | | | | 235 (230) | 98 (18) | 171 (177) | 171 (177) | 14.7 |
| 138 | | | | 308 | 115 | 128 | 128 | 18.2 |
| 180 | | | | 249 | 56 | 128 | 128 | 23.0 |
| | | | | 127 | 68 | 82 | 82 | |
| 28 | | | | CP S11 8 or comparable | | | (6 data sets) | 37.4 |
| 52 | | | | 43 | 8.1 | 45 | 45 | 15.4 |
| 101 | | | | 167 | 27 | 73 | 73 | 17.2 |
| 118 | | | | 198 | 39 | 96 | 96 | 16.2 |
| 153 | | | | 193 | 21 | 88 | 88 | 12.8 |
| 138 | | | | 315 | 36 | 114 | 114 | 14.5 |
| 180 | | | | 270 | 31 | 111 | 111 | 6.7 |
| | | | | 127 | 14 | 24 | 24 | |
| 28 | | | | All data | | | (13 data sets) | 40.1 |
| 52 | | | | 44 | 8.7 | 50 | 50 | 15.5 |
| 101 | | | | 167 | 32 | 73 | 73 | 22.8 (14.5) |
| 118 | | | | 186 (194) | 43 (44) | 120 (81) | 120 (81) | 24.3 (23.4) |
| 153 | | | | 204 (200) | 62 (18) | 140 (132) | 140 (132) | 17.6 (17.0) |
| 138 | | | | 301 (297) | 77 (42) | 150 (142) | 150 (142) | 15.6 |
| 180 | | | | 256 | 40 | 113 | 113 | 15.0 |
| | | | | 126 | 44 | 54 | 54 | |

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Afdeling Organische Contaminanten/Bestrijdingsmiddelen F 26

INTERN ANALYSEVOORSCHRIFT NR. A 182 (Engelse versie)

1e oplage (1984-06-26)

QUANTITATIVE DETERMINATION OF SIGNIFICANT INDIVIDUAL CHLOROBIPHENYLS
IN FISH OIL WITH GLASS CAPILLARY GAS CHROMATOGRAPHY

Note. The method demands experience in capillary gaschromatography. Special consideration has to be given to the problems associated with carrier gas impurities, septum and column bleeding, injection technique and to inertness problems in the low picogram range.

1. SCOPE AND FIELD OF APPLICATION

The method is applicable to fish oil and allows the determination of specified individual chlorobiphenyls at the 1-10 µg/kg level.

2. PRINCIPLE

The fish oil is saponified. Reaction products solved in pentane are washed with water, dried on sodium sulphate and cleaned on alumina.

The residues are identified and determined in the eluate by capillary gas chromatography using splitless (or cold direct) injection techniques.

3. DEFINITION

The content of significant chlorobiphenyls in fish oil: The contents of substances determined by the procedure described and usually expressed in milligrams of compound per kilogram of oil.

4. REAGENTS

4.1 n-Pentane, suitable for residue analysis. Distil, if necessary, over a Raschig column of at least 50 cm length.

4.2 i-Octane, suitable for residue analysis. Distil, if necessary, over a Raschig column of at least 50 cm length.

4.3 Glass wool, heated at 135°C for 24 h.

4.4 Sodium sulphate, anhydrous, heated at 600°C for 2 h and stored in a well sealed container.

4.5 Alumina, basic, Woelm, activity I. Deactivate with 5% (m/m) of water (4.7). Control elution pattern so that the chlorobiphenyls elute completely and dieldrin and endrin are retained on the column.

4.6 Alcoholic potassium hydroxide solution. Dissolve 35 g of potassium hydroxide in 20 ml of water (4.7) and dilute with ethanol to 1 litre. Prepare the solution on the day of use.

4.7 Water, distilled, suitable for residue analysis.

4.8 Chlorobiphenyl standard solution

(0,1 µg/ml of each compound in 1-octane/pentane (4/1 v/v))

| | |
|------------------------------------|----------|
| 2,4,4'-Trichlorobiphenyl | (No 28) |
| 2,5,2'5'-Tetrachlorobiphenyl | (No 52) |
| 2,4,5,2'5'-Pentachlorobiphenyl | (No 101) |
| 2,4,5,2'4'5'-Hexachlorobiphenyl | (No 153) |
| 2,3,4,2'4'5'-Hexachlorobiphenyl | (No 138) |
| 2,3,4,5,2'4'5'-Heptachlorobiphenyl | (No 180) |

Note - The concentrations are for guidance only and can be adjusted to suit various requirements.

Chlorobiphenyls are commercially available from Promochem, Wesel, FRG or Analabs, USA.

4.9 Internal standard solution

Mirex (150 ng/ml) or Pentachlorobenzene (100 ng/ml) or Hexabromobenzene (400 ng/ml) in 1-Octane.

Note - The concentrations and compounds are for guidance only and can be adjusted to suit various requirements.

5. APPARATUS

5.1 Rotary evaporator with accessories or special concentrator (Kuderna-Danish).

5.2 Chromatographic columns, inner diameter 6,5-7,0 mm, with glass wool (4.3) plug.

5.3 Gas chromatography with ^{63}Ni electron capture detector and pressure controlled capillary inlet system suitable for splitless (or cold direct) injection.

5.4 Glass or fused silica capillary column, at least 25 m length, inner diameter 0,2-0,35 mm; stationary phase: SE 30, SE 54, CP Sil 5 CB, CP Sil 7 or comparable.

6. PROCEDURE

6.1 Saponification and clean-up on alumina

Weigh 1,0 g of oil into a 100 ml flask, add 20 ml of alcoholic potassium hydroxide and saponify on a waterbath for at least 45 min at 70°C inside the flask. Use a condensor on the flask. Add a few drops of water and mix. If the soap solution turns turbid proceed with saponification. Cool the solution and pour it into a separating funnel (250 ml). Add 30 ml of pentane, 20 ml of water and shake for 30 s. Transfer the pentane layer into another separating funnel. Extract the water layer 3 times with 15 ml of pentane and transfer the pentane layers to the second separating funnel. Wash the combined pentane solutions several times with 15 ml of water until neutral. The pentane solution should now be clear. Pass the pentane solution through a chromatographic column containing anhydrous sodium sulphate. Rinse the column with pentane and concentrate carefully to 2 ml at 40°C using a nitrogen flow.

Note - Especially chlorobiphenyls with lower chlorine contents are volatile and may evaporate.

Pour 2,0 g of alumina into a chromatographic column. Prewash with 5 ml of pentane, discard the eluate and place a measuring cylinder under the column. Transfer the sample solution quantitatively onto the column using a pipette, rinse with 2 ml of pentane and elute with 8 ml of pentane and concentrate to 2,0 ml. Add 1,0 ml internal standard and fill up to 10,0 ml with i-octane.

Note - The solvent of the final solution must be the same as used for the standard solution.

6.2 Gas chromatography

The gas chromatographic conditions must be thoroughly optimized to achieve a good resolution of the compounds to be analysed.

The following conditions are for guidance only and must be determined for each instrument and column used.

- | | |
|---|--|
| - carrier gas | - helium or nitrogen |
| - linear velocity of the carrier gas | - 10-15 cm/s for nitrogen, 25 cm/s for helium |
| - gas flows in the injection port e.g. septum purge | - depending on injection port design |
| - detector purge gas | - nitrogen or argon/methane 90/10 (v/v) 20-40 ml/min depending on the detector |
| - injection port temperature | - around 250°C |
| - detector temperature | - 300-350°C |
| - initial temperature | - use 90°C for i-octane |
| - initial time | - 2-5 min |
| - program rate | - 10°C/min |
| - final temperature | - 210-240°C |
| - final time | - 0-20 min, depending on final temperature |
| - cool time | - depending on instrument specifications |
| - closing time of splitter | - 20-200 s after injection, depending on injection port volume |

For splitless injection close the splitter, inject 0,5-5 μ l of the sample extract with a microliter syringe and reopen the splitter after the optimised time. For cold direct injection inject 0,5-2 μ l using a narrow bore syringe. Start the temperature programmer at the time of injection.

Note - It is advisable to run with each series of determinations a standard solution for calibration as well as a blank and a recovery determination.

6.3 Identification and quantitative determination

Identification should be carried out on the basis of relative retention data, e.g. referred to the internal standard. Peak height should be used for quantification. The recoveries of all compounds determined in the separate recovery experiment should be higher than 80%.

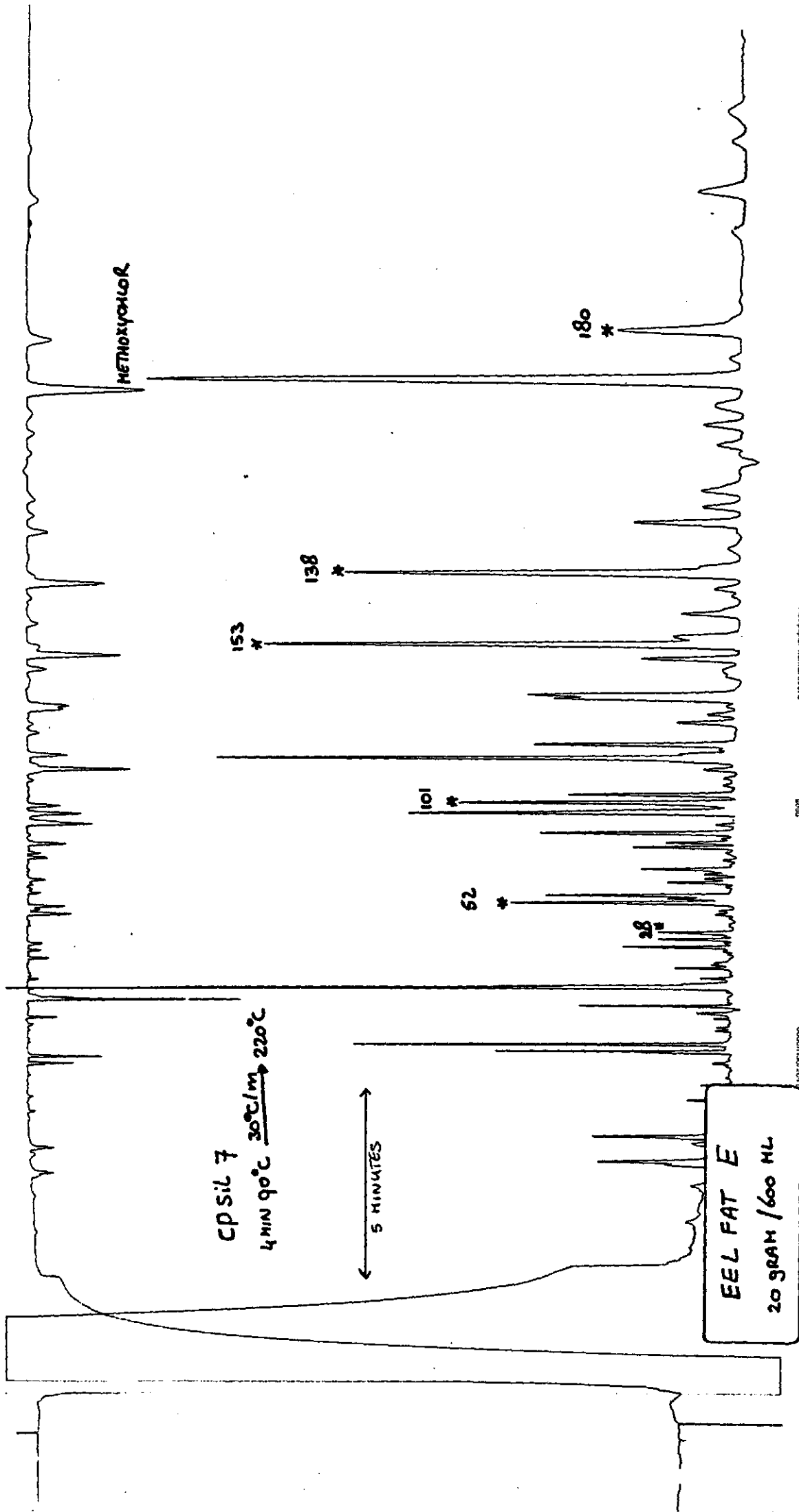
Note - It is advisable to use a second column of different polarity or another independent qualitative and quantitative information for confirmation of the results obtained.

The blank values determined in the separate blank tests should not exceed 5 μ g/kg for each compound.

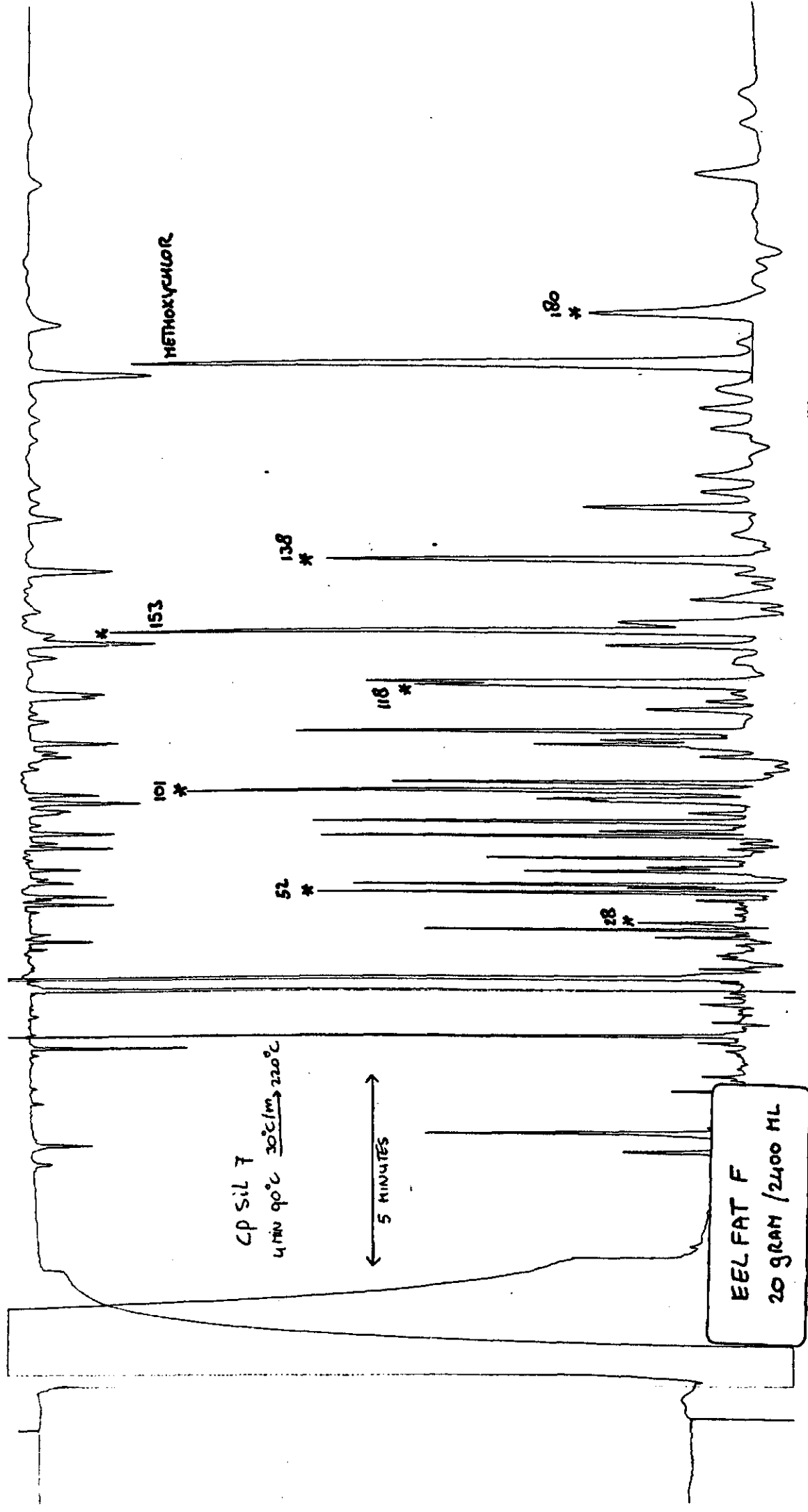
7. EXPRESSION OF RESULTS

Contents of individual compounds are calculated in milligrams per kilogram fish-oil using the internal standard method.

Chromatogram I



Chromatogram 2



12/20/68

1000

EELFAT F
20 g / 2400 ml

CP SIL 5CB
4 min 90°C 20°C/min 230°C

