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INTERNATIONAL COUNCIL FOR THE
EXPLORATION OF THE SEA

CALCULATION OF THE PCB CONTENT

IN FISH SAMPLES

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1. ## Introduction

   The gaschromatographic determination of PCBs in fish samples is mostly
   carried out by injecting the sample extract on to a packed column and compa-
   ring the sample with a technical Aroclor 1254 or 1260 mixture.
   In the literature several procedures are given for quantitation of the PCBs.
   Sometimes the total peak area of the sample is compared with the total peak
   area of an Aroclor mixture. Sometimes only a few similar peaks in the sample
   and the Aroclor mixture are used for quantitation. Especially with this last
   method, it is to be expected that results from a ringtest (collaborative
   interlaboratory test) will differ considerably because the gaschroma-
   tographic conditions (stationary phase) differ among the participants and
   because not all the participants will use the same peaks for quantitation
   purposes.
   In the last proposal for a ringtest organized by ICES 1979/1980 an attempt
   was made to standardize the conditions of gaschromatographic analysis and
   the peaks used for quantitation.
   Recently in the literature more and more authors have noticed the poor match
   between the peak patterns from the sample and the technical standard mix-
   ture, so that quantitation is questionable. It is better to speak of an
   estimation. When only one kind of commodities is analysed within one labora-
   tory then of course this estimation is good enough. When several different
   species are analysed with different peak patterns and when it is necessary
   to correlate the different species with each other (food chain), then the
   problems arise.
   This report will show different results on the basis of two different homo-
   genized fish samples caught in the same area.
   The factors influencing the results are the calculation procedure used and
   the gaschromatographic conditions. This will be elucidated with samples from
   the Joint Monitoring Programme. Also a method which, in principle, will
   overcome these problems will be proposed.

2. Experimental

2.1 Samples

Pike-perch and eel were caught at the end of 1979 in the Yssel lake and, after homogenisation, were used for the determinations.

2.2 Method of analysis

Weigh 5.0 g of the homogenized fish sample into a flat-bottomed flask, add 20 ml of alcoholic KOH and saponify on a waterbath at 70°C at least 30 min. Add a few drops of water and mix. If the soap solution turns turbid continue with saponification. Cool mixture and pour into separator over glass wool. Add 30 ml pentane, 20 ml H_2O and shake 30 sec. Transfer pentane layer into a separator. Extract the water layer three times with 15 ml portions pentane. The combined pentane fractions are washed several times with water until the water is neutral. The pentane layer should be clear. Pass pentane through anhydrous Na_2SO_4 and concentrate carefully to 2 ml on a sand bath (50°C) with a slow stream of nitrogen.

Especially lower chlorinated biphenyls are volatile and may evaporate.

Cleanup

Transfer 2.0 g basic alumina (deactivated with 5% water) to chromatographic tubing. Prewash with 5 ml pentane, discard prewash and place a measuring glass under the column. Transfer the sample extract (2 ml) quantitatively to the column, rinsing with 2 ml pentane and elute with 8 ml pentane. Concentrate the eluate to about 1 ml. Add 1.0 ml of internal standard solution and dilute tot 5.0 ml with iso-octane.

Determination

a) 5 ul of the extract are injected on a packed column 3% OV1 on Chromosorb WHP, temperature 200°C, length 1.80 m, mobile phase Ar/CH_4.

b) 5 ul of the extract are splitless injected into the capillary. Detailed information see Journal of High Resolution chromatography and chromatography communications 2 (1979) 723-728, authors L.G.M.Th. Tuinstra and W.A. Traag.

3. Results on packed column

In figure 1, 1 ng of Aroclor 1254 is injected on the OV1 column. In figure 2 the chromatogram of the pike-perch extract is shown, corresponding to 5 mg of the original sample. In figure 3 the extract of 0.25 mg of eel sample is chromatographed . In these three figures several corresponding peaks are numbered.

3.1 The first calculation procedure is based on peak height measurement for all clearly visible PCB peaks. The heights from the sample are compared with the corresponding peak in the Aroclor 1254 standard.

In table 1 the single results for each peak are given as the mean PCB content. From this table it is clearly visible that when one does not use all peaks, but only two or three peaks the final result will strongly depend on the choice of those two or three peaks.

3.2 The second calculation procedure uses only three peaks, i.e. peak number VIII, IX and XI (in accordance with the ICES proposal). The results are also included at the end of table 1.

4. Results on capillary column

In figure 4 the chromatogram of 1 ng Aroclor 1254 is shown. In figure 5 the extract of the pike-perch is shown corresponding to 5 mg of the original sample. In figure 6 the extract of 0.25 mg eel is shown. The indicated peak numbers correspond with the numbers in table 2. This table specifies the composition and the concentration of the standard mixture of chlorobiphenyls, shown in figure 7.

4.1 The first procedure for the calculation uses the total PCB peak area of the fish extract and compares this with the total PCB peak area of Aroclor 1254 from figure 4. For pike-perch the PCB content is 0.34 mg/kg and for eel the PCB content is 2.1 mg/kg on a product basis.

4.2 In the second procedure only six peaks in the samples are compared to the six similar peaks in Aroclor 1254. Each individual peak results in a "PCB content", but all six results are summed and the mean is calculated and reported as the mean PCB content. The six peaks in the samples have retention times corresponding with the following individual chlorinated biphenyls: 2,5-2'5'; 2,3,6-2'5'; 2,4,5-2'5'; 2,3,4-2'4'5'; 2,3,4,5-2'4'5' and 2,3,4,5-2'3'4'.

In the chromatograms the numbers of these peaks (6, A, 11, 22, 26 and 27) are encircled. These six peaks are present in the samples and in Aroclor 1254 and, for the greater part, separated from other chlorinated biphenyls. The peak width at half height indicates that there are no important interferences.

In table 3 the results per peak are shown for both samples (expressed as Aroclor 1254). Notice that in this calculation procedure for the pike-perch the highest "individual PCB content" is four times as high as the lowest results. In the eel this is "only" a factor of 2.5.

The mean of all six " individual" results (0.41 and 2.4 mg/kg) should be compared with the results in paragraph 4.1 (e.g. 0.34 and 2.1 mg/kg).

4.3 In this procedure every peak in the sample is compared with the corresponding individual chlorinated biphenyls from the standard mixture in figure 7 and table 2, ar far as these standards are available.

In table 4, the sample content of each individual chlorinated biphenyl is given. Up till now identification is only based on agreement of retention times. Identifications of 2,4,6- compounds are questionable. In table 4 these compounds are marked with an asterisk (*). Peaks in the sample for which no standard chlorinated biphenyls were available are not quantified. So the total summed up result of table 4 for each sample (0.094 and 2.4 mg/kg) is an underestimation of the true total PCB content. However, at this moment we have available 40 individual chlorinated biphenyls (not all are part of the standard mixture of table 2 and figure 7). We know the response factor of all these compounds for our electron capture detector. So we can estimate (by interpolation) for unknown compounds a response factor and estimate their contribution to the true PCB content. In our opinion the extra contri-bution of unknown individual chlorinated biphenyls in fish samples amounts to an average of 20-40%, dependent on the fish specimen, from the already determined content of identified chlorinated biphenyls.

5. Practical application

As part of the work for the Joint Monitoring Programme in the North Atlantic area a lot of fish samples were analysed in our institute. Sampling and final results based on determination of individual chlorinated biphenyls are reported elsewhere.

In this paper we will report the results of those samples of which the quantification of the PCB content is carried out by comparing six peaks in the samples with the similar six peaks in Aroclor 1254 resulting in a mean PCB content of the sample. This result will be compared with the summed up results of the individual chlorinated biphenyls.

Furthermore, we will, by using the results for each of the six peaks, compare the peak pattern in the several fish species with the peak pattern in Aroclor 1254.

5.1 All analyses have been carried out according to paragraph 2.2. The peaks used for the calculation are given in paragraph 4.2.

In tables 5-8 sample code, fat content and PCB contens are given.

Results shown include: livers of cod in table 5, livers of flounder in table 6, shrimp in table 7 and mussels in table 8.

In the next to the last column of all these tables, the mean PCB value is calculated from the six peaks (6, A, 11, 22, 26 and 27) and compared in the last column with the summed up results of all individual chlorinated biphenyls (as reported elsewhere).

The samples with a high fat content in table 5 and 6 show a considerable difference between the two procedures for the calculation. Even remembering the fact that the summed up result of all individuals is about 20-40% too low does not explain the differences.

The samples with a low fat content (table 7 and 8) show a good agreement between the two calculation procedures.

5.2 If we assume that the peak pattern of Aroclor 1254 corresponds very well with the peak pattern in fish then it is correct to neglect the fact that the species have been caught in different places and to neglect the fact the absolute content differs among species.

Under this assumption we calculated the mean concentration for the peaks 6, A, 11, 22, 26 and 27 for all four species. For easier comparison the relative mean concentrations are shown in the table 9 related to the relative concentrations in Aroclor 1254.

Especially from this table it is clear that there exists no peak pattern match with Aroclor 1254 and the assumption made above is not correct.

6. Conclusion

The above shows clearly that at this time there exist no one method which determines the true total PCB content. Not only because all kinds of results can be produced depending on the procedure for calculation, but also because there exists no clear relationship with Aroclor 1254, and therefore making Aroclor 1254 not suitable as a standard mixture for comparison between different species. The only correct procedure is the one where every individual chlorinated biphenyl is determined. Though, up till now not all individual compounds are known or available it is to be expected that future work will identify these compounds.

Due to new developments in capillary column technology the separation even will improve in near future.

The apparent drawback that the determination seems to be complex and time consuming may be overcome by focussing attention on a few compounds, especially when these compounds are interesting from a toxicological point of view.

Wageningen, 1980-04-09.

Table 1.   The PCB content in fish samples, calculated with use of all peak
heights compared to Aroclor 1254 (mg/kg on product basis).

PCB content (mg/kg)

peak number	pike-perch	eel
I	0.23	4.9
II	0.42	7.0
III	0.69	14.9
IV	0.27	3.3
V	0.18	1.8
VI	0.15	3.0
VII	0.11	2.3
VIII	0.16	2.2
IX	0.21	2.7
X	0.16	2.0
XI	0.17	2.4
XII	0.19	2.9
XIII	0.11	1.8
XIV	0.34	4.2
mean PCB content	0.24	4.0

PCB content when only using three peaks (VIII, IX and XI in accordance with the
ICES proposal).

VIII	0.16	2.2
IX	0.21	2.7
XI	0.17	2.4
mean PCB content	0.18	2.4

tabel 1

Table 2. Composition of standard mixture of individual chlorinated biphenyls.

peak number	structure	concentration ug/ml	peak number	structure	concentration ug/ml
1	2	2	16	2,4,5-2'4'6'	0.2
2	4	2	17	2,3,5,6-2'5'	0.2
3	2-2'	2	18	2,3,6-2'4'5'	0.2
4	2,4	0.2	19	2,3,4-2'4'6'	0.2
5	4-4'	1	20	2,4,5-2'4'5'	0.2
6	2,5-2'5'	0.2	21	2,3,4,5-2'5'	0.2
7	2,4-2'5'	0.2	22	2,3,4-2'4'5'	0.2
8	2,3-2'5'	0.2	F	2,3,5,6-2'4'5'	0.2
9	2,4,6-2'5'	0.2	23	2,3,4-2'3'4'	0.2
9.1	2,5-3'4'	0.2	24	2,3,4,5,6-2'5'	0.2
A	2,3,6-2'5'	0.2	25	2,3,5,6-2'3'5'6'	0.2
10	2,4,6-2'4'6'	0.2	26	2,3,4,5-2'4'5'	0.2
11	2,4,5-2'5'	0.2	27	2,3,4,5-2'3'4'	0.2
12	2,4,6-3'4'	0.2	28	2,3,4,5-2'3'5'6'	0.2
13	2,4,5-2'3'	0.2	29	2,3,4,5-2'3'4'5'	0.2
14	2,3,4-2'5'	0.2	30	2,3,4,5,6-2'3'4'5'	0.2
15	2,3,6-2'3'6'	0.2			

(1 ml of this solution is diluted to 20 ml with iso-octane. 5 ul is splitless injected).

Table 3. Estimation of PCB content (expressed as Aroclor 1254) using only six peaks for quantitation (mg/kg on product basis)

	PCB content (mg/kg)	
peak number	pike-perch	eel
6	0.36	3.7
A	0.37	2.1
11	0.25	1.4
22	0.22	1.6
26	0.91	3.6
27	0.35	2.0
mean PCB content	0.41	2.4

tabel 2

Table 4   The content of individual chlorinated biphenyls in pike-perch and eel (mg/kg on product basis).

peak number	structure	pike-perch	eel
1	2	0.002	0.014
4	2,4	0.002	0.080
5	4-4'	0.008	0.12
6	2,5-2'5'	0.003	0.16
7	2,4-2'5'	0.008	0.062
8	2,3-2'5'	0.002	0.084
9	2,4,6-2'5'*	0.001	0.018
9.1	2,5-3'4'	0.003	0.056
A	2,3,6-2'5'	0.007	0.18
10	2,4,6-2'4'6'*	0.004	0.10
11	2,4,5-2'5'	0.005	0.12
13	2,4,5-2'3'	0.001	0.033
14	2,3,4-2'5'	0.005	0.14
15	2,3,6-2'3'6'	0.006	0.18
17	2,3,5,6-2'5'	0.002	0.032
18	2,3,6-2'4'5'	0.006	0.26
19	2,3,4-2'4'6'*	0.006	0.22
20	2,4,5-2'4'5'	0.008	0.14
21	2,3,4,5-2'5'	0.001	0.040
22	2,3,4-2'4'5'	0.005	0.14
F	2,3,5,6-2'4'5'	0.001	0.060
23	2,3,4-2'3'4'	0.001	0.029
25	2,3,5,6-2'3'5'6'	0.001	0.021
26	2,3,4,5-2'4'5'	0.005	0.084
27	2,3,4,5-2'3'4'	0.001	0.022
total PCB content		0.094	2.4

* see paragraph 4.3

tabel 3

Table 5. <u>PCB content in livers of cod (mg/kg on product basis) calculated with</u>
<u>six peaks in the sample and compared with Aroclor 1254.</u>

Sample code	Fat content (g/kg)	PCB content peak number							Total individual
		6	A	11	22	26	27	mean	PCBs
7910-1007 (I)	608.2	4.29	2.05	4.29	9.86	18.33	10.62	8.2	3.9
7910-1007 (II)	565.8	4.00	2.60	4.16	14.86	31.67	17.50	12.5	5.1
7910-1007 (III)	609.4	2.26	2.19	2.86	10.94	23.89	15.00	9.5	3.8
7910-1007 (IV)	716.3	6.28	3.70	6.10	22.97	52.78	31.25	20.5	7.8
7910-1007 (V)	563.8	5.43	6.71	6.23	18.92	46.11	26.25	18.3	6.9
7910-1007 (VI)	482.4	5.14	2.74	5.58	18.92	31.11	21.25	14.1	6.3
7910-1007 (VII)	239.1	6.00	1.92	6.23	13.51	19.44	12.50	9.9	4.9
7910-1007 (VIII)	534.0	3.43	2.60	4.68	13.51	30.00	18.75	12.2	5.1
7910-1007 (IX)	492.4	3.43	2.60	4.80	22.97	54.44	27.50	19.3	7.2
7910-1007 (X)	786.8	4.57	3.84	5.19	11.89	17.78	10.12	8.9	4.2
	mean	4.43	3.01	4.91	15.84	32.62	19.00		

Table 6. <u>PCB content in livers of flounder (mg/kg on product basis) calculated</u>
<u>with six peaks in the sample and compared with Aroclor 1254.</u>

Sample code	Fat content (g/kg)	PCB content peak numer							Total individual
		6	A	11	22	26	27	mean	PCBs
7908-0110	182.4	0.66	0.55	0.71	2.16	4.17	2.62	1.8	1.0
7908-0310	121.4	0.51	0.60	0.43	1.76	5.44	3.38	2.0	0.80
7908-0410	163.2	1.57	1.37	1.30	2.03	6.11	4.00	2.7	1.3
7908-0710	168.3	0.69	0.68	0.87	2.03	6.11	4.25	2.4	0.97
7908-0910	182.1	1.60	1.92	1.56	3.11	9.44	6.00	3.9	1.8
	mean	1.00	1.02	0.98	2.22	6.25	4.04		

tabel 4

Table 7. PCB content in shrimps (mg/kg on product basis) calculated with six peaks in the sample and compared with Aroclor 1254.

Sample code	Fat content (g/kg)	6	A	11	22	26	27	mean	Total individual PCBs
7904-0302	5.1	0.06	0.01	0.01	0.04	0.17	0.12	0.07	0.07
7904-0902	4.2	0.09	0.03	0.03	0.07	0.28	0.12	0.10	0.07
7910-0702	9.3	<0.03	<0.01	<0.01	0.03	0.11	0.12	0.05	0.05
7910-0402	9.8	0.03	0.01	<0.01	0.03	0.17	0.12	0.06	0.06
7910-0902	7.9	0.03	0.01	0.01	0.03	0.17	0.12	0.06	0.04
7910-0302	5.8	<0.03	<0.01	<0.01	0.01	0.06	<0.12	0.03	0.05
7910-0102	5.7	<0.03	<0.01	<0.01	0.01	0.06	<0.12	0.03	0.04
	mean	0.04	0.01	0.01	0.03	0.14	0.09		

Table 8. PCB content in mussels (mg/kg on product basis) calculated with six peaks in the sample and compared with Aroclor 1254.

Sample code	Fat content (g/kg)	6	A	11	22	26	27	mean	Total individual PCBs
7904-0503	9.1	0.09	0.08	0.09	0.14	0.11	<0.12	0.09	0.09
7904-0703 (02)	6.1	0.11	0.12	0.14	0.19	0.22	<0.12	0.14	0.12
7904-0703 (01)	5.3	0.06	0.06	0.06	0.09	0.11	<0.12	0.07	0.07
7904-0703 (03)	8.0	0.26	0.23	0.26	0.32	0.28	<0.12	0.24	0.20
7904-0903	4.5	0.11	0.10	0.08	0.08	0.06	<0.12	0.08	0.08
7910-0303	8.0	0.06	0.07	0.06	0.12	0.11	<0.12	0.08	0.08
7910-0503	14.6	0.06	0.08	0.10	0.14	0.11	<0.12	0.09	0.09
7910-0103	11.2	0.03	0.04	0.04	0.08	0.06	<0.12	0.05	0.13
7910-0703 (01)	14.8	0.09	0.10	0.10	0.16	0.11	<0.12	0.10	0.13
7910-0703 (02)	7.5	0.09	0.14	0.13	0.19	0.17	0.12	0.14	0.14
7910-0703 (03)	9.6	0.17	0.23	0.21	0.28	0.28	0.12	0.22	0.26
7910-0903	15.7	0.34	0.41	0.32	0.34	0.39	0.12	0.32	0.42
	mean	0.12	0.14	0.13	0.18	0.17	0.07		

tabel 5

Table 9. Relative mean PCB content for compound 6, A, 11, 22, 26 and 27 in the different products, expressed as Aroclor 1254 (mg/kg on product basis), related to the relative concentrations in Aroclor 1254.

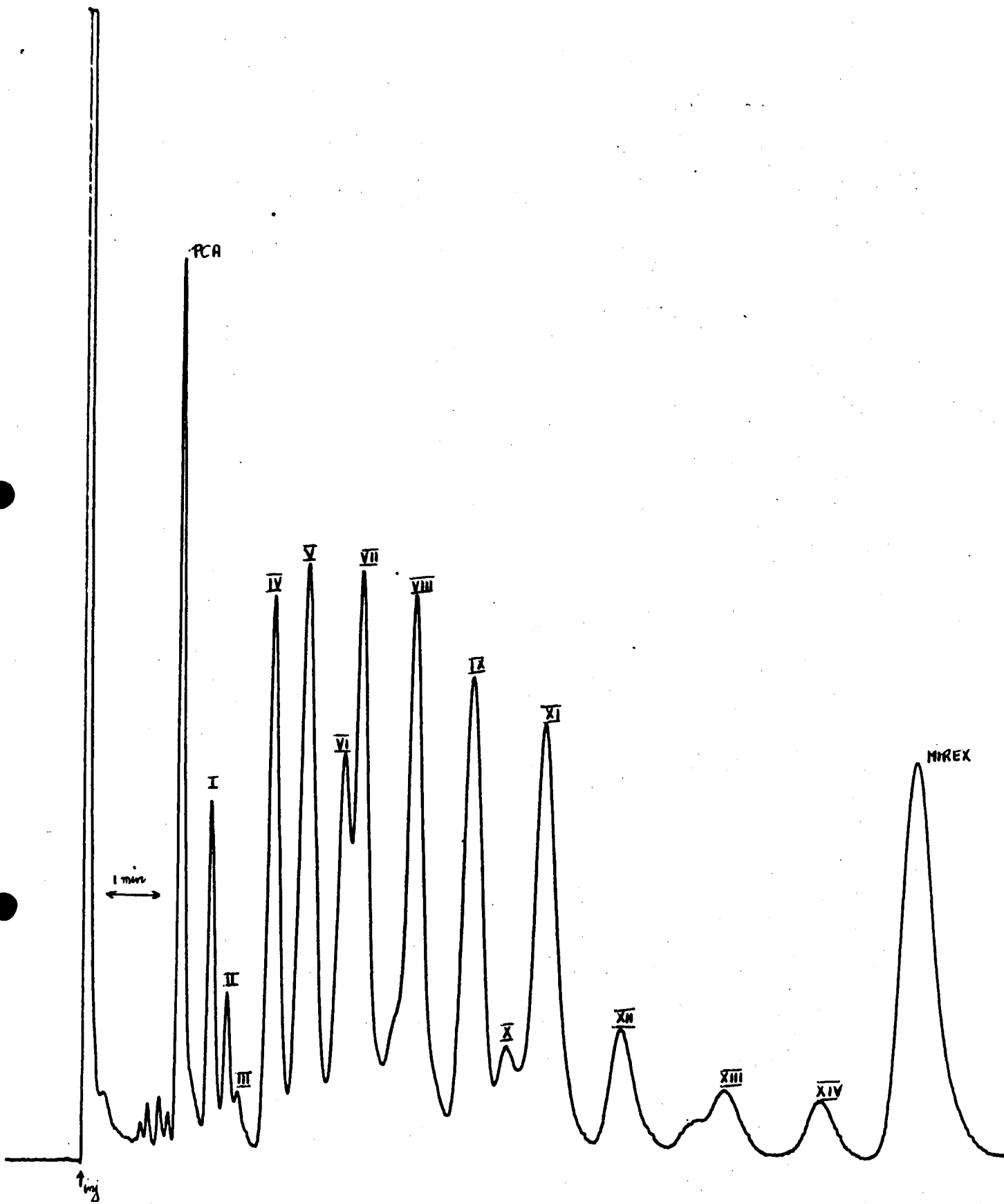| peak number products	Relative mean PCB content					
	6	A	11	22	26	27
livers of cod	0.28	0.19	0.31	1	2.06	1.20
livers of flounder	0.45	0.46	0.44	1	2.82	1.82
shrimps	1.16	0.35	0.32	1	4.71	3.32
mussels	0.69	0.78	0.75	1	0.95	0.42
Aroclor 1254	0.47	0.95	1.05	1	0.24	0.10

tabel 6

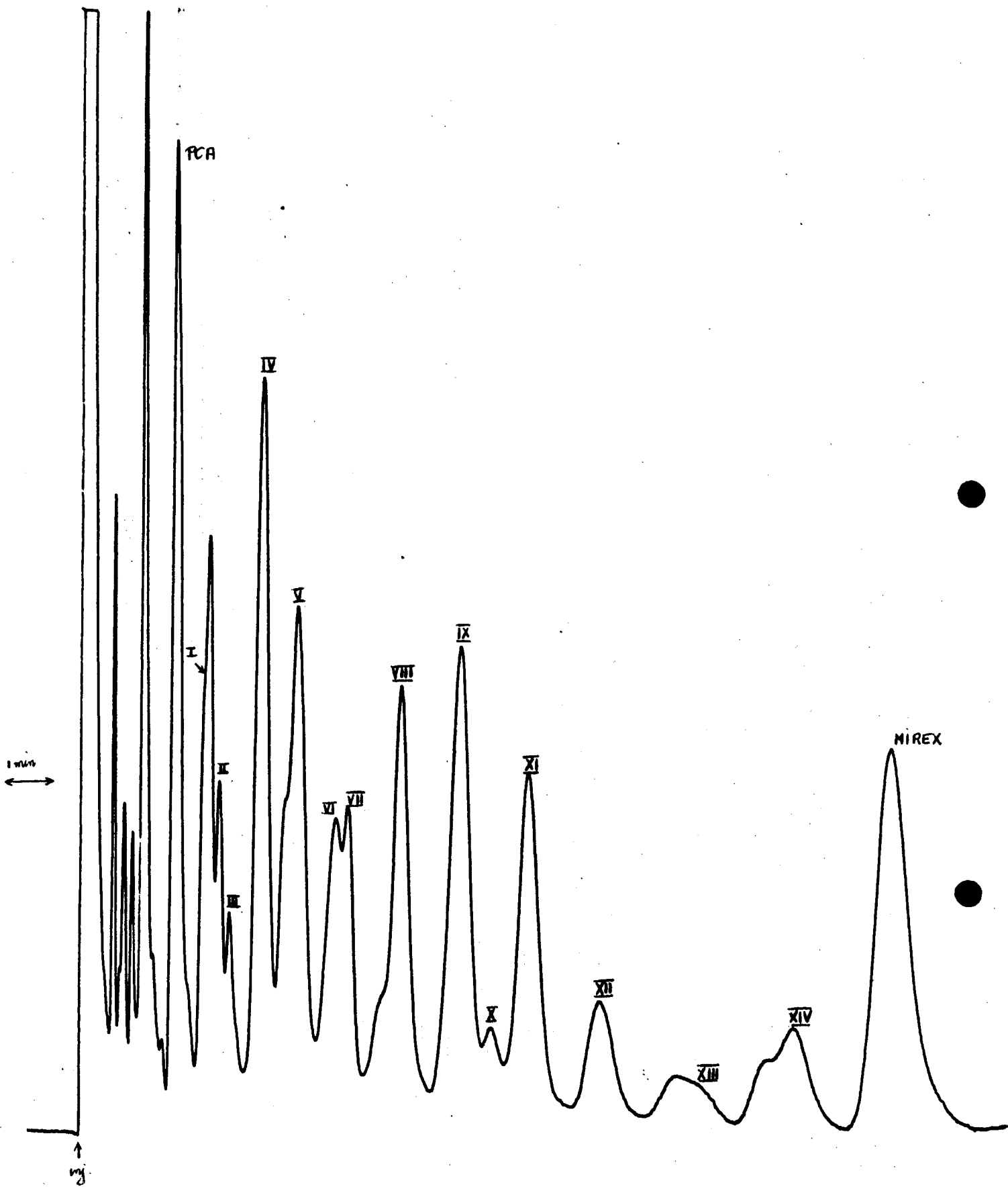fig. 1 Chromatogram of 1 ng Aroclor 1254 on a packed OV1 column.

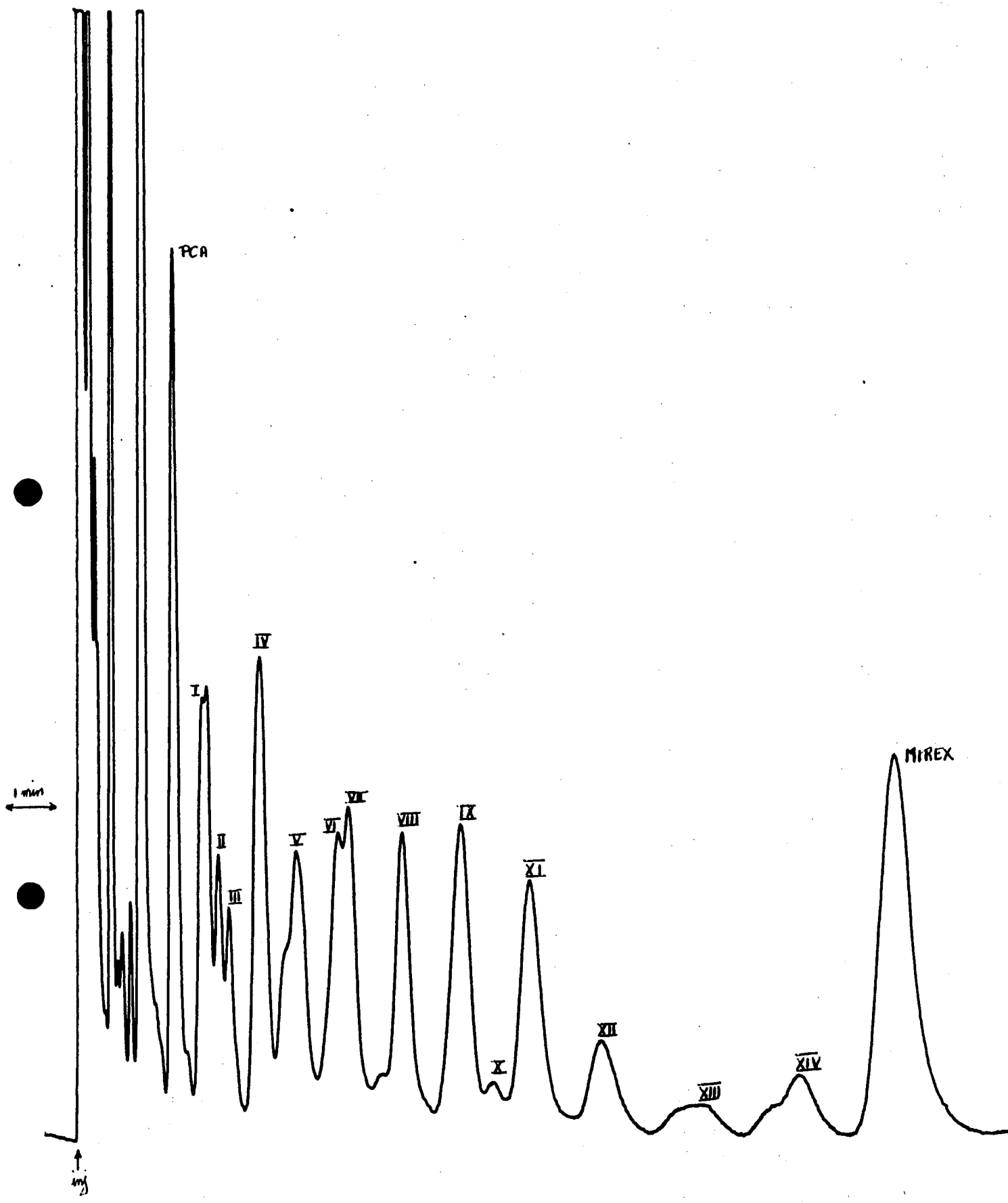fig.2 Chromatogram of 5 mg pike-perch extract on a packed OV1 column.

fig.3 Chromatogram of 0.25 mg eel extract on a packed OVI column.
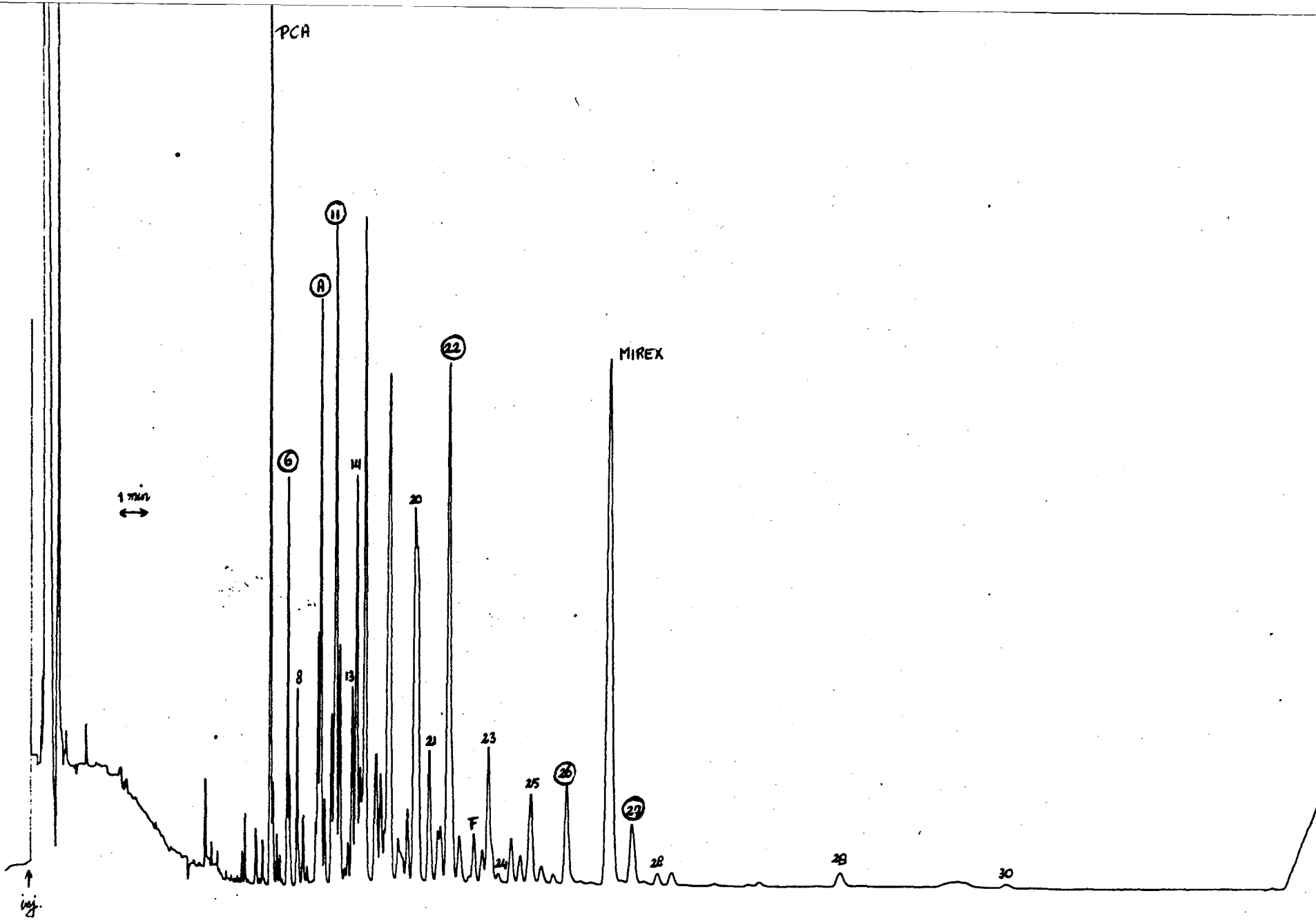
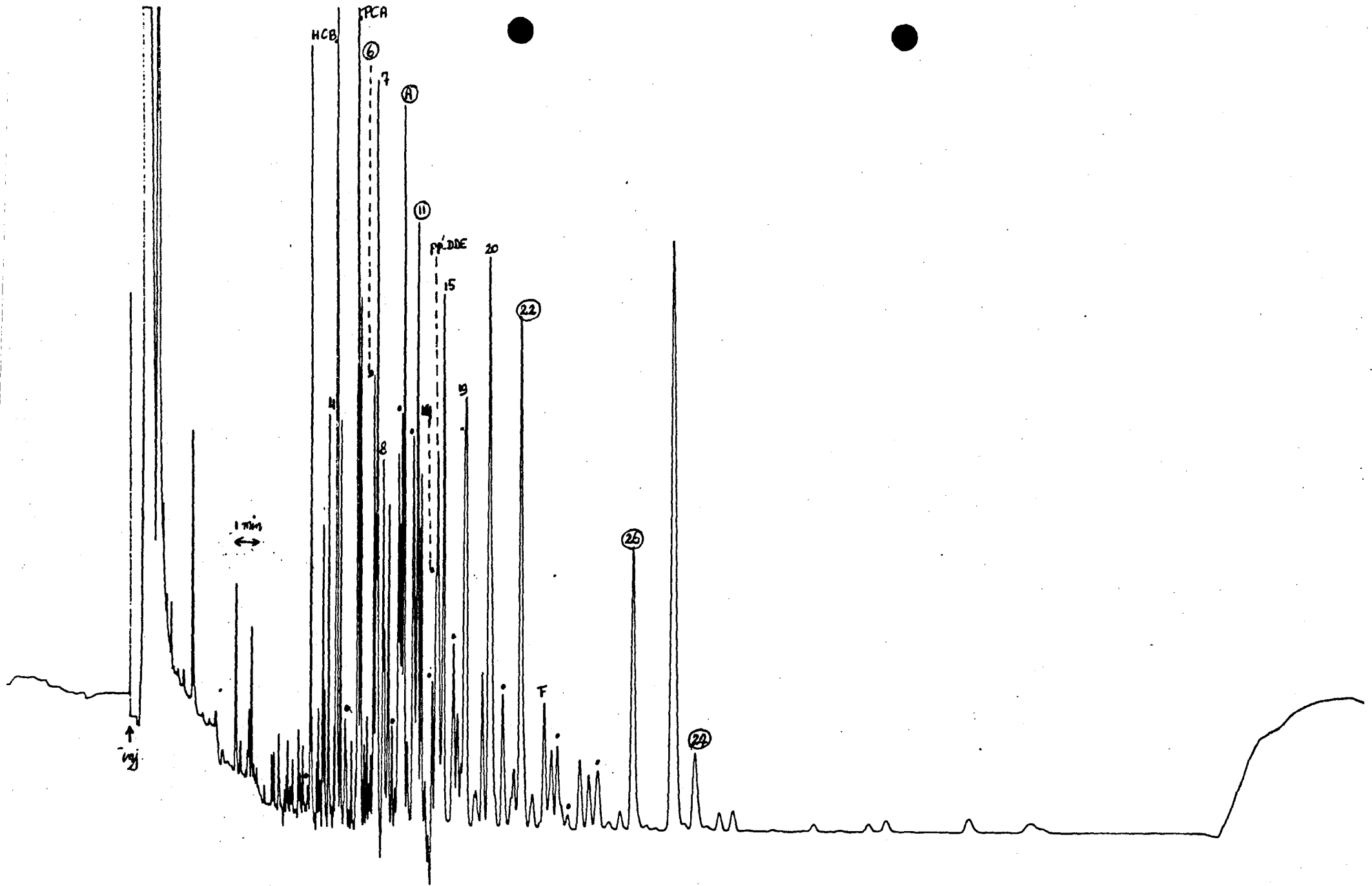fig. 4   Chromatogram of 1 ng Aroclor 1254 on a capillary CP-Sil 7 column.

fig. 5 Chromatogram of 5 mg pike-perch extract on a capillary CP-Sil 7 column.
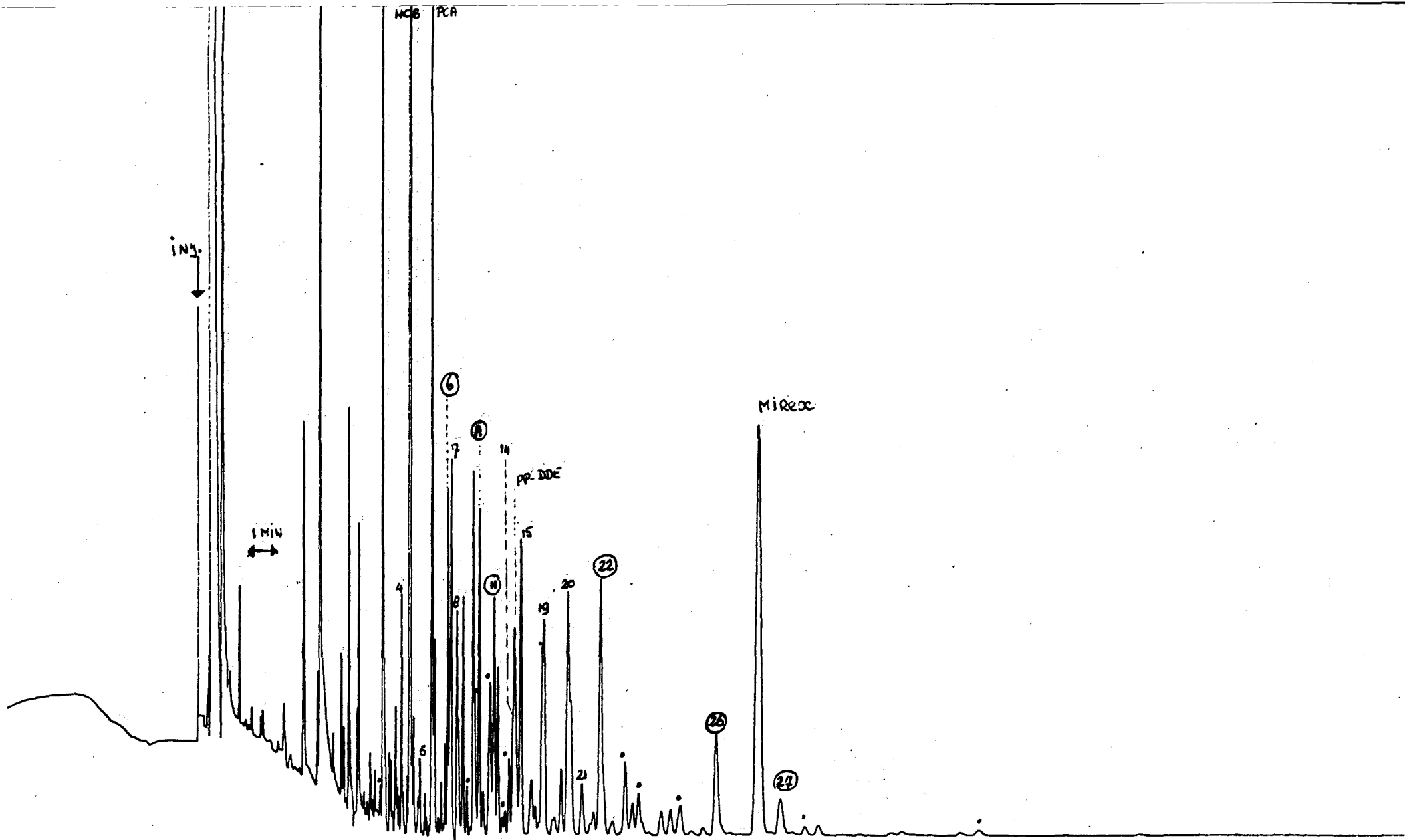
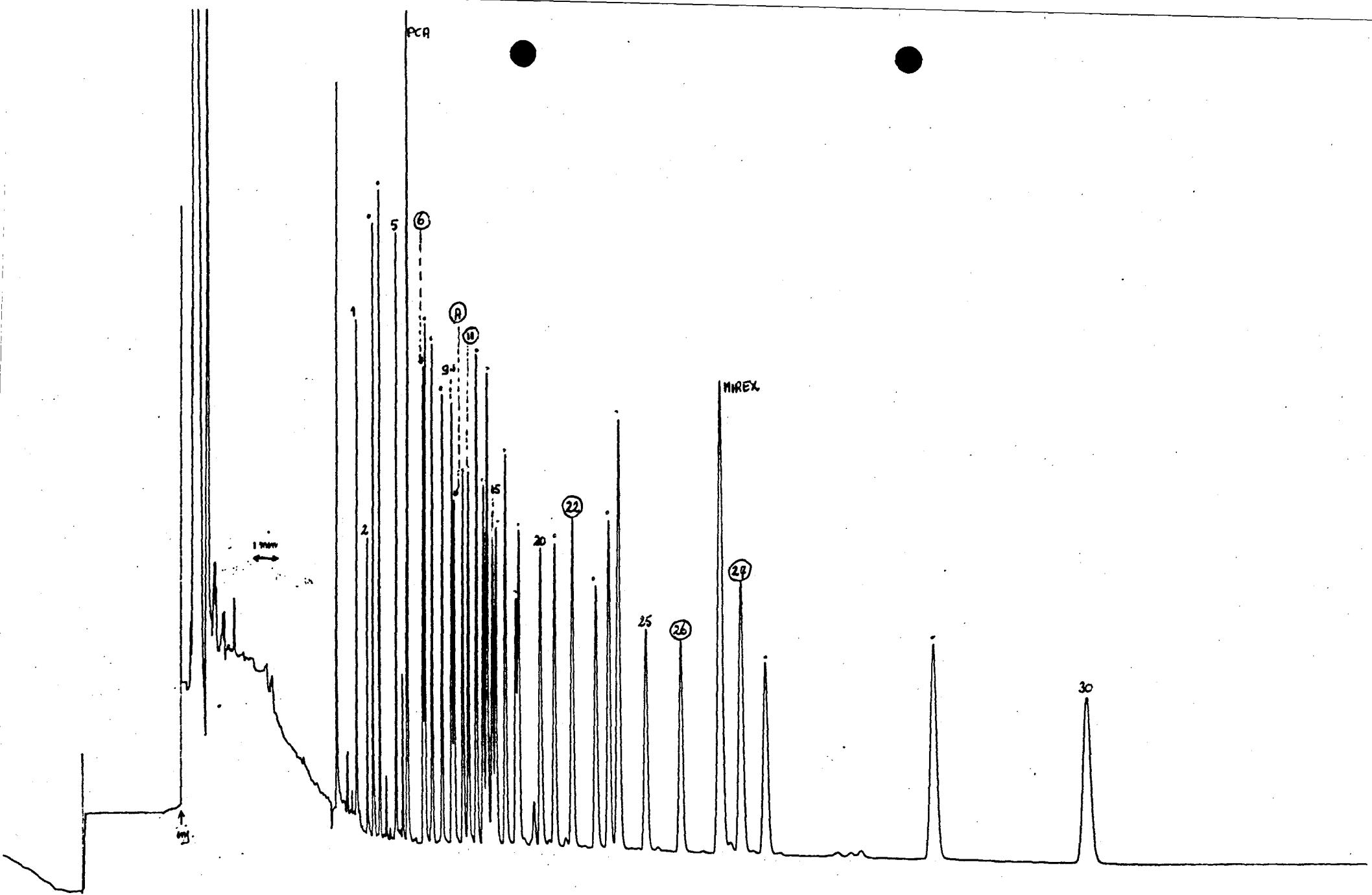fig. 6 Chromatogram of 0.25 mg eel extract on a capillary CP-Sil 7 column.

fig. 7 Chromatogram of a standard mixture individual chlorinated biphenyls on capillary CP-Sil 7 column. For composition see table 2.

VERZENDLIJST

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