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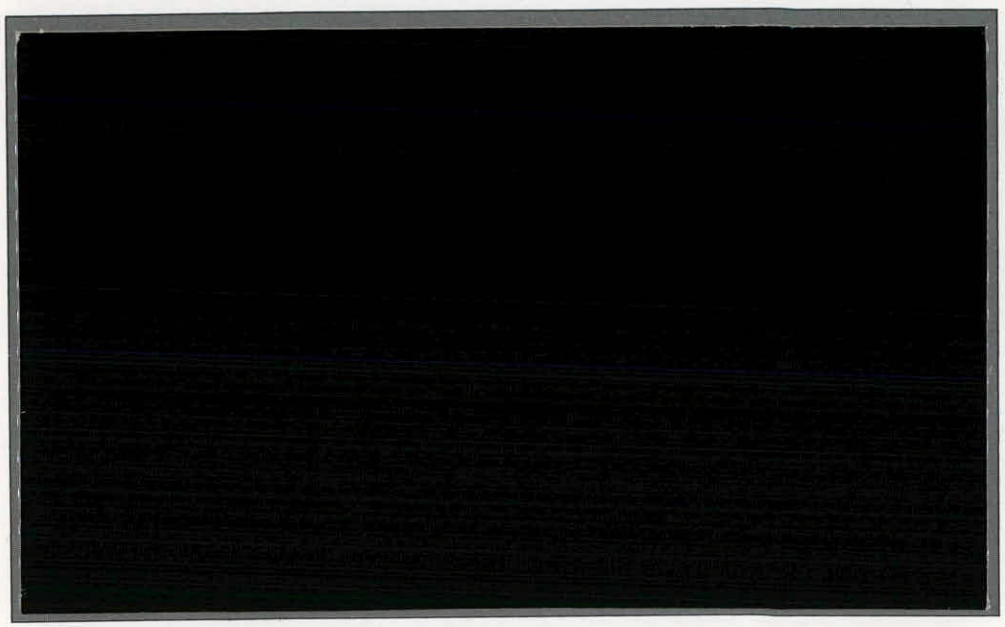

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Stability of pyrethroid pesticides in freeze-dried river sediment

LONG-TERM STABILITY STUDY

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ABSTRACT

Storage at temperatures of -20°C, room temperature and 40°C of a lyophilized natural river sediment containing the synthetic pyrethroid, permethrin, with samples analysed after approximately 50 days, 3, 6 and 12 months storage, has enabled an assessment of the stability of the pesticide in the matrix. The analysis of trace quantities of pyrethroids in complex matrices such as sediments, is currently subject to appreciable errors with a typical coefficient of variation of replicates of between 7 and 30%. However, the statistical analysis of the results shows that no significant instability (estimated at the 5% probability level) of the pesticide occurred in the samples stored at -20°C. The samples stored at 40°C showed no significant instability after 3 and 6 months storage. The t-test on values from samples stored for one year indicated no significant instability but the t-value (2.7) was close to the test limit of 2.8. The samples stored at room temperature did show signs of instability after 6 months and one year's storage. The most reliable estimate of the degradation rate was derived from the results obtained at 40°C, i.e. 10.6 ± 2.3 ng kg^{-1} d^{-1} with evidence of a decrease in the rate with decreasing storage temperature.

The sediment has also been analysed for other organic compounds and pesticides. The following compounds have been identified in the sediment stored for over one year at room temperature:

cis-permethrin
trans-permethrin
dieldrin
simazine

It is proposed that the sediment containing these various compounds will be of future use, viz in the application of the 25 kg batch dispatched to CBNM, Geel (B).

1. INTRODUCTION

The previous report, RL/T04053ml/1, on EC contract 5112/1/9/332/89/5-BCR-UK(10), described the results of the preparation and tests for homogenization of a 30 kg batch of freeze-dried river sediment, together with the measured concentration of *cis*-permethrin in the sediment after the first storage interval of 50 days. Twelve 50 g sub-samples of sediment have been stored in separate bottles at temperatures of -20°C, ≅20°C (viz room temperature) and 40°C. After the initial storage interval, no significant differences could be detected between the concentration of *cis*-permethrin in the sediments stored at different temperatures. The results indicated that if losses were occurring, then the low rates necessitated a longer-term study (reference report RL/T04053ml/1).

This report presents the results of the entire stability study, extending to a period of one year, together with analytical data on other pesticides in the sample and polyaromatic hydrocarbons (PAH's) determined by glc with mass-spectrometry. Transformation products of permethrin have also been sought in both the extracts obtained after one year's storage of the sediment and in an extract obtained in new experiments involving dichloromethane extraction without clean-up.

2. ANALYSIS

The extraction and clean-up procedures have been given in a previous report (RL/T04053ml/1) and are the same methods employed by House *et al.* (1991) for the analysis of a range of organochlorine and pyrethroid pesticides in river sediments.

At the end of the stability trial another method was used to analyse the sediment for other pesticides and trace organic compounds:

2.1 Soxhlet extraction with dichloromethane, DCM (referred to as the DCM extract method)

20 g of freeze-dried sediment was placed in a pre-extracted cellulose extraction thimble (single thickness supplied by Whatman cat. no. 2800 258) and extracted with 200 ml pesticide grade DCM for 6 h at a rate of one solvent cycle every five minutes. The resulting extract was concentrated in a Kuderna-Danish concentrator to a volume of *ca.* 10 ml and then reduced to 4 ml using a flow of dry nitrogen gas. The final extract was then analysed by gas-liquid chromatography with mass-spectrometry, GC/MS. The conditions of the analysis were similar to those described previously (report RL/T04053ml/1).

3. RESULTS OF THE STABILITY TRIAL

50.00 g (± 0.01 g) sub-samples of dry sediment were weighed on a mechanical top-pan balance (Mettler P1210) into 60 ml amber glass powder bottles. These were flushed with nitrogen before and after filling, then sealed with lined Bakelite screw caps.

The bottles were then assigned a number (BCR #) at random. Bottles were then selected at random and assigned to the different storage conditions as specified in the contract, i.e.:

(a) 3 bottles were set aside for reference-time analysis.
(b) 12 bottles were assigned to each of the storage temperatures used; 40°C, room temperature (RT) and -20°C.
(c) 10 bottles were assigned to element analysis using X-ray fluorescence.
(d) 10 samples (5 g each) were also taken from the batch and sub-sampled at

the 1 g level to assess the variation in organic content. The results of the homogeneity study have already been presented (report RL/T04053ml/1).

The results of the analysis of the samples for *cis*-permethrin at the reference time are shown in Table 1. The coefficient of variation was low, i.e. 3.6%. After ≅50 days storage, 3 samples were removed at random from each of the storage temperatures. The results of the analysis are shown in Table 2 together with the values obtained for the analysis of the blank samples. This procedure was repeated after storage intervals of 3, 6 and 12 months and the results of the analysis for *cis*-permethrin are shown in Tables 3, 4 and 5. The values of the mean concentrations at each of the storage times are summarized in Table 6 and Figure 1.

The extract, BCR Ø1 (Table 5, sediment stored at 40°C for 371 days) was also analysed using GC/MS and quantified using a single external standard (0.05 $\mu g\ ml^{-1}$) by selective-ion monitoring with ion m/z = 183. The concentration determined was 5.2 $\mu g\ kg^{-1}$ which is in reasonable agreement with the value of 5.4 $\mu g\ kg^{-1}$ obtained from the previous analysis. The chromatograms for the m/z 183 and 163 ions are shown in Figure 2.

As a further check, the extract from BCR 24 (Table 2, sediment extracted after storage of 47 days at room temperature) was re-analysed using the GC/MS method described above. The concentration was determined as 10.3 $\mu g\ kg^{-1}$ (dry weight) which is in reasonable agreement with the value of 9.6 $\mu g\ kg^{-1}$ obtained previously and 10.6 ± 1.1 $\mu g\ kg^{-1}$ obtained for the three samples BCR 12, 16 and 24 replicates stored at room temperature.

4. STATISTICAL ANALYSIS OF THE DATA

4.1 F and t-tests

The F-test was used to compare the variance in the replicates. When the variance of the replicates obtained after storage was compared with the variance at the reference time, the F-test gave significant (5% probability level) differences making it inappropriate to apply a t-test to compare the mean values. Using the analysis obtained after 50 d of storage as the reference, the majority of the replicates showed no significant differences using the F-test at the 5% probability level. The results of the calculations are shown in Table 7. The values with an asterisk show significant differences in variability as indicated by the low standard deviations obtained for these samples.

The analysis using the t-test is also shown in Table 7. Only the values with an asterisk fall outside the limit specified by the t-test ($t_{5\%}(\phi = 4) = 2.8$). The data obtained after *ca.* 372 days storage at room temperature and 40°C are close to the t-test limit. The means of the replicates obtained after 178 d and 372 d for the samples stored at -20°C show no significant difference from the mean value for the analysis after 50 d storage. The results obtained after 92 d storage do show a significant difference but this is caused by a low standard deviation obtained for this analysis and consequent failure of the F-test.

The variance of the replicates at a fixed temperature was also compared with the variance of all the results obtained at the same temperature. The results of the calculations are shown in Tables 8 and 9. The mean coefficient of variation for all the samples was between 23% and 30% (see Table 8). The analysis of the total variance revealed that the variance of the results obtained at -20°C storage led to an approximate equal division of variance within replicates and between replicates obtained at different times of storage. The results at 40°C, however, illustrate a

greater variation between replicates at different storage times than might be expected from the variation within replicates.

5. COMPARISON OF TRANSFORMATION RATES

The data obtained from the analysis of the samples stored at room temperature and 40°C for 372 days show some differences from the earlier data obtained for the samples stored for 52 days. If these differences are considered significant and caused by loss of permethrin, then the transformation rates may be computed from the results shown in Tables 1 to 5. The rates of loss evaluated at the three storage temperatures are shown in Table 10, together with the standard deviations (SD) and correlation coefficients from the regression analysis. The first-order rate constant for the transformation rates, together with the corresponding half-life are also shown in Table 10. The results illustrate an increase in the rate of loss ($\mu g\ kg^{-1}\ d^{-1}$) with increasing temperature as shown in Figure 3. The large deviations calculated for the samples stored at -20 and 20°C (viz room temperature) shown in Table 10, lead to a large uncertainty in the rates as illustrated by the low values of the correlation coefficients also shown in Table 10. However, the data from the samples stored at 40°C is better correlated and leads to a lower standard deviation on the rate, i.e. CV = 22%. The best estimate of the transformation rate is therefore 10.6 ± 2.3 ng $kg^{-1}\ d^{-1}$ at a temperature of 40°C.

6. ANALYSIS OF TRANSFORMATION PRODUCTS

The major transfer pathway in natural waters and sediments is reported (Hill, 1985) to be initiated by ester cleavage leading to 3-phenoxybenzyl alcohol (PBA) and further oxidation to the corresponding acid 3-phenoxybenzoic acid, PBAc. Standards of PBA and PBAc were obtained and analysed using GC/MS to give the mass-spectra illustrated in Figure 4. Analysis of the extract from sample BCR01 (1 year's storage at 40°C) revealed no trace of PBA or PBAc when the instrument was operated in its most sensitive mode (selected ion monitoring mode, SIM).

Further work was done to examine the effects of the clean-up procedure on the removal of PBA and PBAc from the extract. The alternative extraction method which employed DCM as the solvent and without clean-up (method described in §2.1) was performed on surplus sediment which had been stored at room temperature for a period of about one year. The DCM extract was analysed using GC/MS and again neither PBA (retention time 13.95 min., Figure 5) or PBAc (retention time 14.84 min., Figure 6) were detected. Hence, without further investigation, the presence of the transformation products cannot be confirmed. This may be because the compounds are present at concentrations below the limits of determination of the method, or that degradation is not occurring.

7. CONTENT OF OTHER PESTICIDES AND TRACE ORGANICS

The sampling site was located about 6 km downstream of a sewage outlet and so the sediment is likely to contain a wide variety of trace organic compounds originating from the treated sewage outfall and from diffuse sources within the catchment of the river. Previous work on the glc with electron-capture-detector (report RL/T04053m1/1) has indicated the presence of dieldrin (an organochlorine pesticide probably originating from imported wool used in the local textile industry). The DCM extraction procedure (§2), without ancillary clean-up, permits a broad spectrum of compounds to be extracted and analysed. This method was applied to the surplus sediment from the same batch used in the stability studies but stored for a period of one year at room temperature.

The extract was analysed using GC/MS in full scan mode and identified 76 peaks with many positive identifications in the NIST library including PAH's (pyrene, fluoranthene and anthracene, Figures 7, 8, 9 and retention times verified using a mixed PAH standard). The extract was also analysed using the more sensitive selective ion monitoring mode for the following pesticides: α-BHC, δ-BHC (lindane), p,p'-DDE, dieldrin, endrin, p,p'-TDE, p,p'-DDT, *cis* and *trans*-permethrin, cypermethrin, fenvalerate, deltamethrin, simazine and phosalone, i.e. a range covering organophosphorus, pyrethroid, organochlorine and triazine pesticides. The results of the analysis are shown in Table 11 and Figures 10 to 15. Quantification was done using a mixed pesticide external standard of 0.05 μg ml^{-1} with a 1 μl injection. The base-ions listed in Table 11 were used for the quantification. The results indicate substantial concentrations of *trans*-permethrin, dieldrin and simazine as well as *cis*-permethrin at a concentration similar to that determined at the beginning of the stability study.

8. CONCLUSION

The statistical analysis of the stability trial results show that no significant instability (at the 5% probability level) of *cis*-permethrin occurred in the samples stored at -20°C.

The samples stored at 40°C showed no significant instability after 3 and 6 months storage but after one year, although the t-test indicated no significant instability, the t-value (2.7) was close to a test limit of 2.8.

The samples stored at room temperature did show signs of instability after 6 months and one year's storage.

The degradation rate at 40°C was calculated as 10.6 ± 2.3 (SD) ng kg^{-1} d^{-1} with evidence of a decrease in the rate with decreasing storage temperature.

Further analysis of selected sediment extracts using GC/MS has confirmed the results of the analysis of the extracts using glc/ecd for *cis*-permethrin. It has also been demonstrated that the sediment batch dispatched to CBNB, Geel, also contains appreciable concentrations of *trans*-permethrin, dieldrin and simazine after one year's storage at room temperature.

REFERENCES

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3. I.R. Hill, 1985. Effects on non-target organisms in terrestrial and aquatic environments. In: *The pyrethroiid insecticides* (ed. J.P. Leakey), Taylor and Francis, London, pp 151.

Table 1. Results of the analysis of the sediments for *cis*-permethrin measured at the reference time.

Sample designation	Concentration of *cis*-isomer in extracts/mg dm^{-3}	*Cis*-isomer content of sediment /μg kg^{-1}
BCR 27	0.0259	10.35
BCR 29	0.0256	10.24
BCR 31	0.0242	9.68
Blank	No cis-isomer peak identified	
	Mean ± S.D.	10.09 ± 0.36

Table 2. Results of the measurement of permethrin in the sediments stored at different temperatures.

Measurement after ≅ 50 days storage.

Sample designation	Storage temperature °C	Storage interval/d	Concentration of *cis*-isomer in extracts/mg dm^{-3}	*Cis*-isomer content of sediment /μg kg^{-1}
BCR 02	40	46	0.0236	9.421
BCR 04	40	46	0.0165	7.42
BCR 21	40	46	0.0378	14.8*
Blank	No *cis*-isomer peak identified			
			Mean ± S.D.	10.55 ± 3.12
BCR 12	R.T.	47	0.0239	9.56
BCR 16	R.T.	47	0.0314	12.57
BCR 24	R.T.	47	0.0239	9.58
Blank	No *cis*-isomer peak identified			
			Mean ± S.D.	10.57 ± 1.14
BCR 06	-20	51	0.0207	8.27
BCR 26	-20	51	0.0215	8.62
BCR 46	-20	51	0.0299	11.95
Blank	No *cis*-isomer peak identified			
			Mean ± S.D.	9.61 ± 1.65

* Second component dominant, RRT of *cis*-permethrin low

Table 3. Results of the measurement of permethrin in the sediments stored at different temperatures.

Measurement after 3 months storage.

Sample designation	Storage temperature /°C	Storage interval /d	Concentration of *cis*-isomer in extract /mg dm^{-3}	*Cis*-isomer content of sediment /µg kg^{-1}
BCR 07	40	87	0.0155	6.19
BCR 20	40	87	0.0200	7.99
BCR 28	40	87	0.0198	7.92
Blank	No isomer peak identified			
			Mean ± S.D.	7.37 ± 1.02
BCR 25	R.T.	92	0.0129	5.15
BCR 44	R.T.	92	0.0233	9.32
BCR 45	R.T.	92	0.0289	11.56
Blank	No isomer peak identified			
			Mean ± S.D.	8.68 ± 3.25
BCR 35	-20	98	0.0153	6.57
BCR 36	-20	98	0.0178	6.41
BCR 37	-20	98	0.0181	6.55
Blank	No isomer peak identified			
			Mean ± S.D.	6.51 ± 0.09

Table 4. Results of the measurement of permethrin in the sediments stored at different temperatures.

Measurement after 6 months storage.

Sample designation	Storage temperature /°C	Storage interval /d	Concentration of *cis*-isomer in extract /mg dm^{-3}	*Cis*-isomer content of sediment /μg kg^{-1}
BCR 05	40	175	0.0229	9.17
BCR 30	40	175	0.0207	8.27
BCR 50	40	175	0.0202	8.08
Accidental loss of blank				
			Mean ± S.D.	8.50 ± 0.58
BCR 32	R.T.	179	0.0218	8.71
BCR 34	R.T.	179	0.0188	7.51
BCR 43	R.T.	179	0.0179	7.19
Blank	No isomer peak identified			
			Mean ± S.D.	7.80 ± 0.80
BCR 09	-20	180	0.0256	10.23
BCR 11	-20	180	0.0180	7.19
BCR 23	-20	180	0.0318	12.73
Blank	-20	180	0.0015	0.59
			Mean ± S.D.	10.05 ± 2.78

Table 5. Results of the measurement of permethrin in the sediments stored at different temperatures.

Measurement after 12 months storage.

Sample designation	Storage temperature /°C	Storage interval /d	Concentration of *cis*-isomer in extract /mg dm^{-3}	Concentration of *cis*-isomer in the sediment /µg kg^{-1} (dry weight)
BCR 01	40	371	0.0140	5.58
BCR 15	40	371	0.0075	4.24
BCR 47	40	371	0.0157	6.29
Blank	40	371	0.0029	1.17
			Mean ± S.D.	5.37 ± 1.04
BCR 08	R.T.	372	0.0199	8.78
BCR 38	R.T.	372	0.0123	4.93
BCR 41	R.T.	372	0.0167	6.68
Blank	R.T.	372	0.0035	1.39
			Mean ± S.D.	6.80 ± 1.93
BCR 18	-20	374	0.0085	3.58
BCR 42	-20	374	0.0197	7.88
BCR 49	-20	374	0.0159	6.36
Blank	-20	374	0.0011	0.44
			Mean ± S.D.	5.94 ± 2.18

Table 6. Summary of stability study results. Mean concentration of *cis*-permethrin in sediment/$\mu g\ kg^{-1}$ (dry weight).

Storage interval /d	Storage temperature /°C 40	RT	-20
0	10.09	10.09	10.09
48	10.55	10.57	9.61
92	7.37	8.68	6.51
178	8.50	7.80	10.05
372	5.37	6.80	5.94

Table 7. Results of significance tests on the variation in the concentration of permethrin in the sediment during storage. The significance of variation is measured against the 48 d analysis.

Temperature /°C	Time /d Test	92	178	372
40	F	9.4	29.0*	9.0
	t	1.7	1.1	2.7
RT	F	8.1	2.0	2.9
	t	1.0	3.4*	2.9*
-20	F	336*	2.8	1.8
	t	3.3*	0.2	2.3

Footnote:

$F_{5\%}(2,2) = 19$

$t_{5\%}(\emptyset = 4) = 2.8$

*: test failed at the 5% probability level, i.e. significant difference in variance (F-test) or mean (t-test) compared with the result obtained after ≅50 days storage.

Table 8. Analysis of variance.

Temperature /°C	$\hat{\sigma}_w^2$	$\hat{\sigma}_s^2$	$\hat{\sigma}_t^2$	SD	CV%
40	0.72	2.78	3.50	1.9	23
RT	3.61	1.25	4.86	2.2	25
-20	3.34	3.05	6.39	2.5	30

Table 9. Percentage of total variance.

Temperature /°C	Within replicates	Between replicates measured at different times
40	20.5	79.5
RT	74.3	25.7
-20	52.2	47.8

Key

$\hat{\sigma}_w^2$ Average variance of replicates within the samples measured at the same time.

$\hat{\sigma}_s^2$ Average variance of different samples.

$\hat{\sigma}_t^2$ Estimated total variance of all measurements.

$$\hat{\sigma}_t^2 = \hat{\sigma}_s^2 + \hat{\sigma}_w^2$$

SD Standard deviation ($\hat{\sigma}_t^2$)

CV Coefficient of variation ($\hat{\sigma}_t^2/\bar{x}_t$) where $\bar{x}$ is the mean of all the determinations at one temperature.

Table 10. Results of the analysis of the concentration changes with time at the three storage temperatures.

Temperature /°C	Rate /μg $kg^{-1}d^{-1}$	SD /μg $kg^{-1}d^{-1}$	Correlation coefficient	First-order rate constant /d^{-1}	Half-life /d
-20	0.00905	0.00432	0.50	0.00132	527
*ca.*20	0.00982	0.00349	0.62	0.00121	575
40	0.01055	0.00229	0.80	0.00149	466

Table 11. Concentration of pesticides in the freeze-dried sediment after storage for one year at room temperature determined using the DCM extraction method without clean-up (see §2). Quantification using GC/MS.

Pesticide	Base ion / m/z	Concentration /μg kg^{-1} (dry sediment)
cis- permethrin	183	10.3
trans-permethrin	183	5.2
dieldrin	263	8.2
simazine	201	6.2

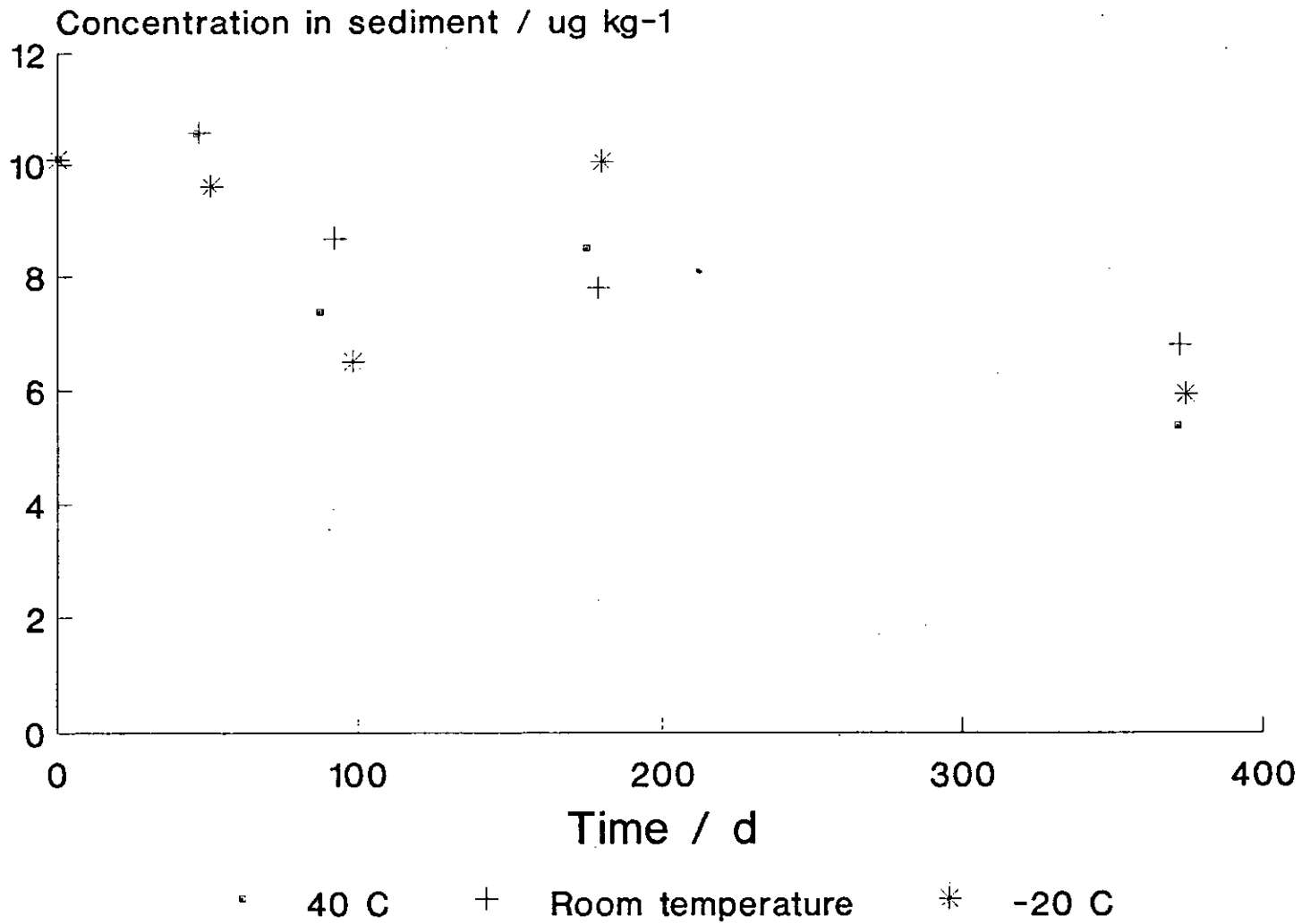


FIGURE 1 Results of the measurement of the cis-permethrin concentration in stored sediment.

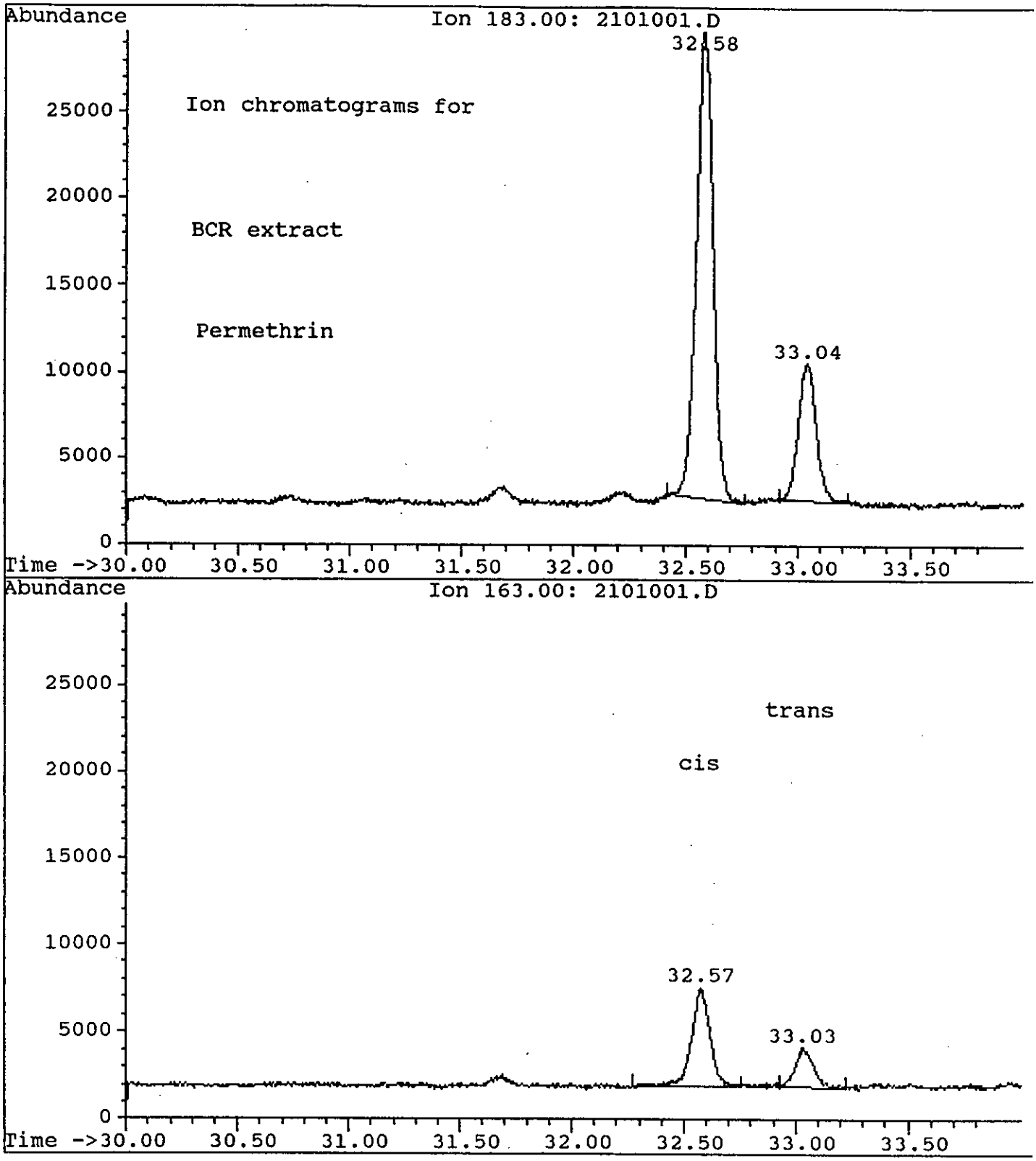


FIGURE 2 Chromatograms for permethrin obtained from the BCR24 extract (47 days storage at room temperature)

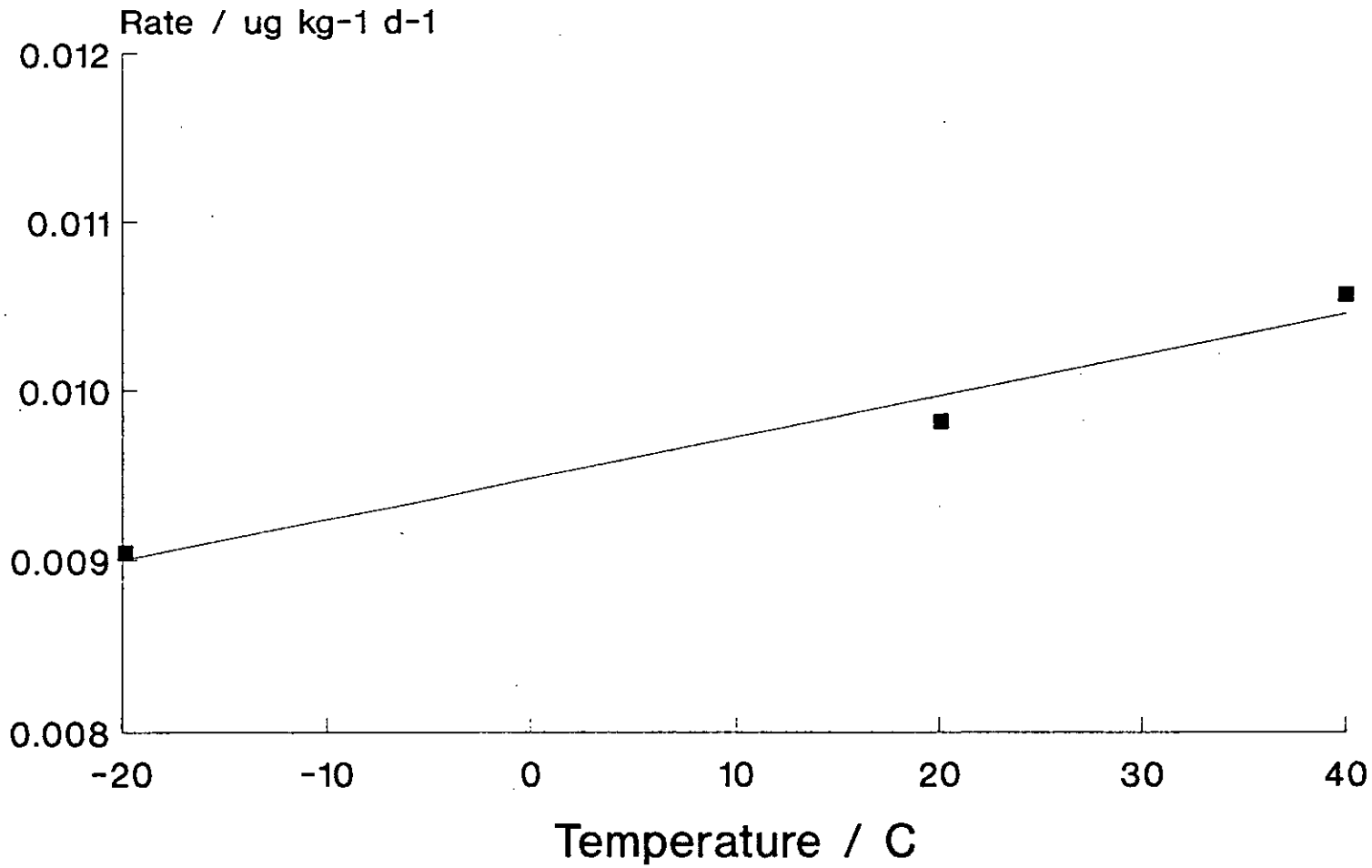
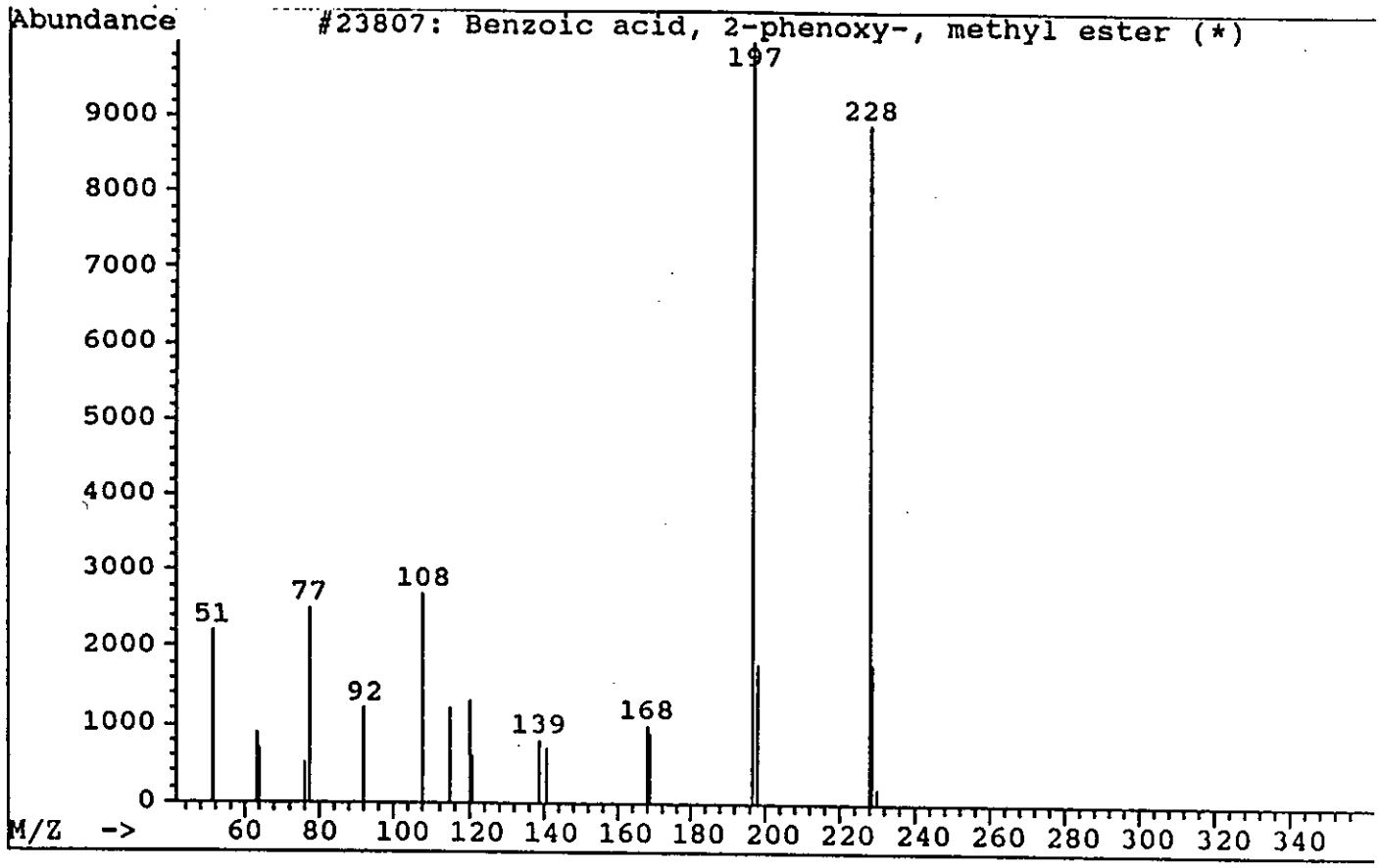


FIGURE 3 Variation in the transformation rate at different storage temperatures.



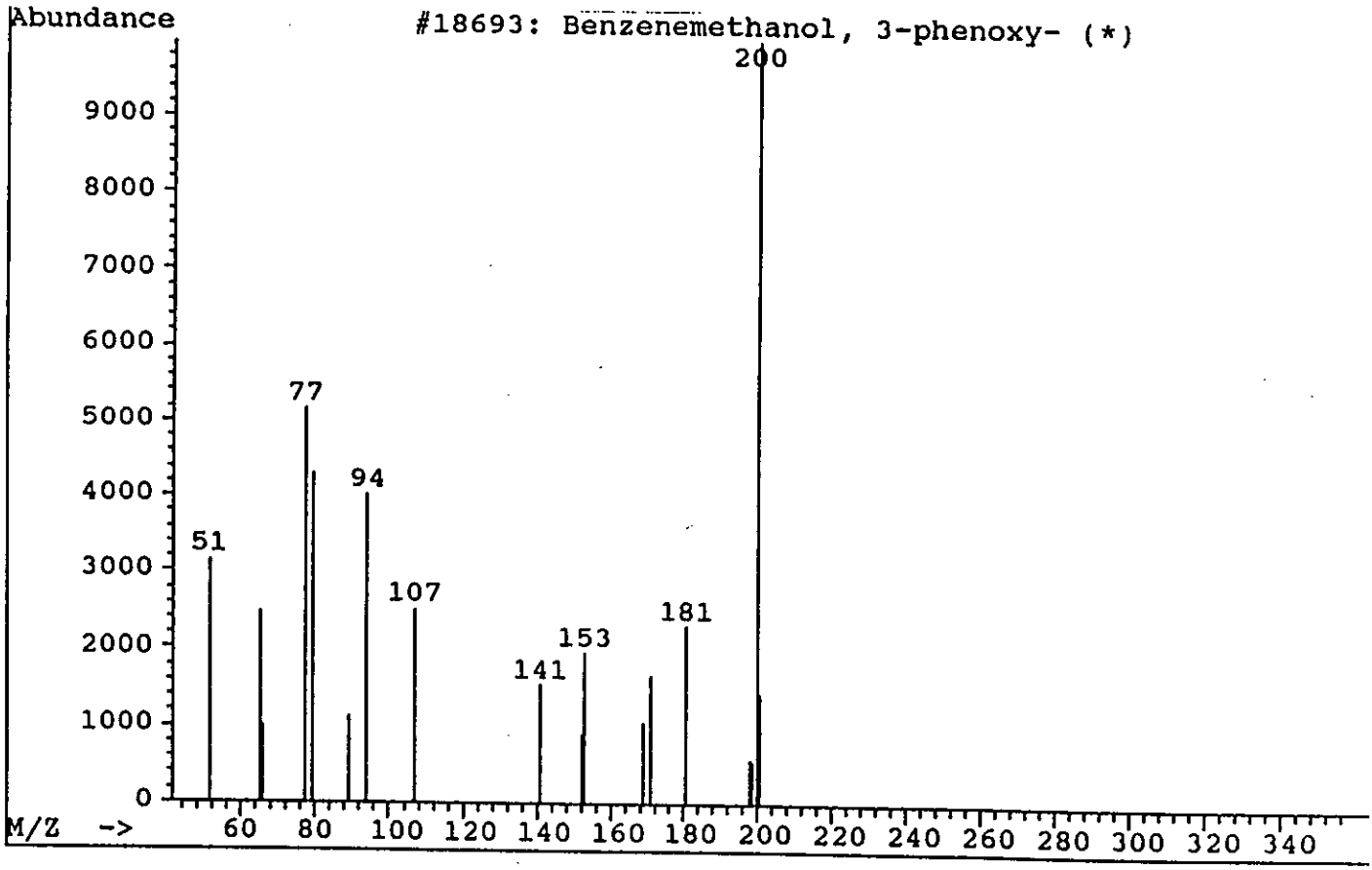


FIGURE 4 Mass-spectrograms for the transformation products of permethrin.

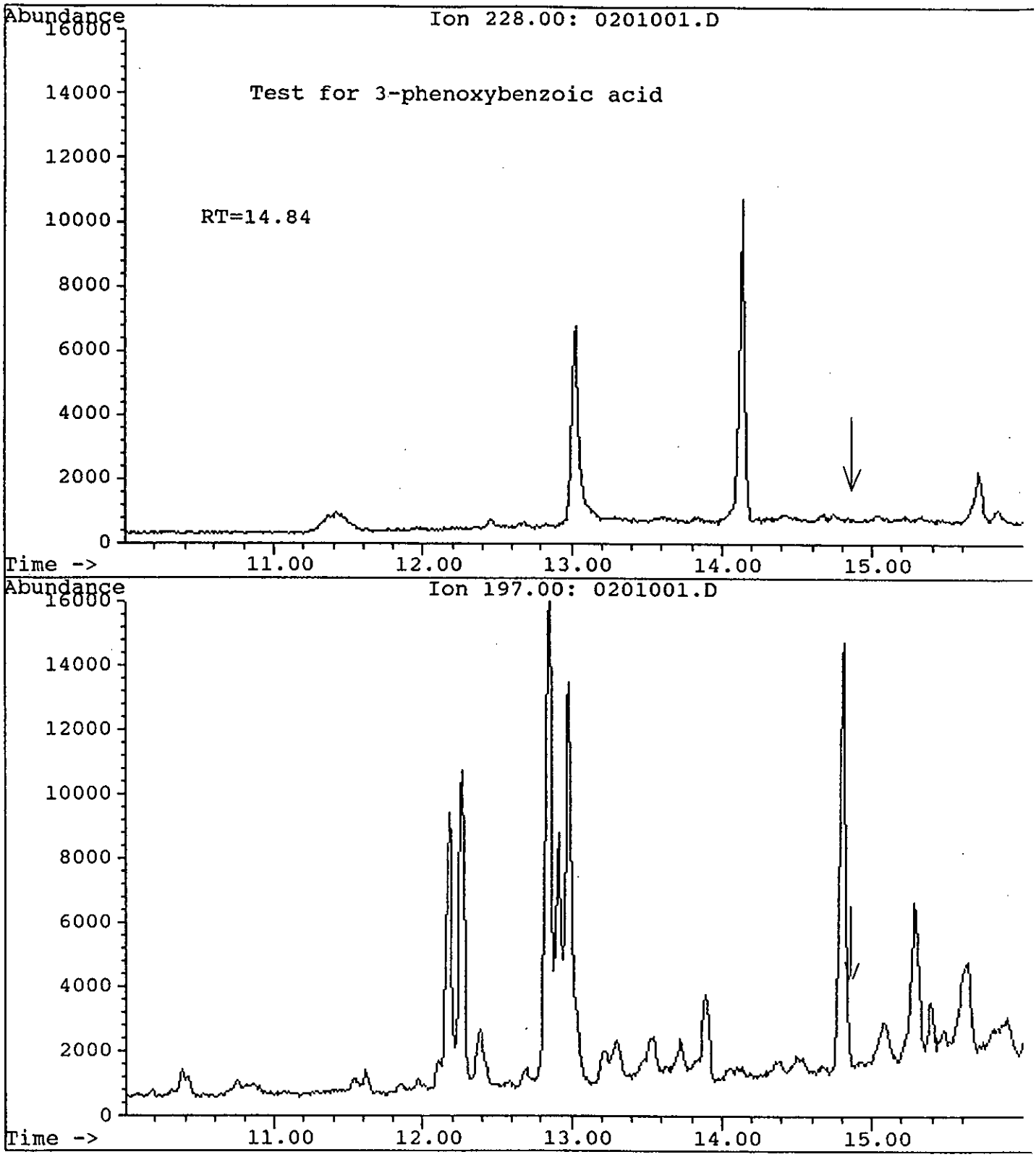


FIGURE 5 Ion-chromatograms for the identification of 3-phenoxybenzoic acid in the sediment stored at room temperature for 1 year.

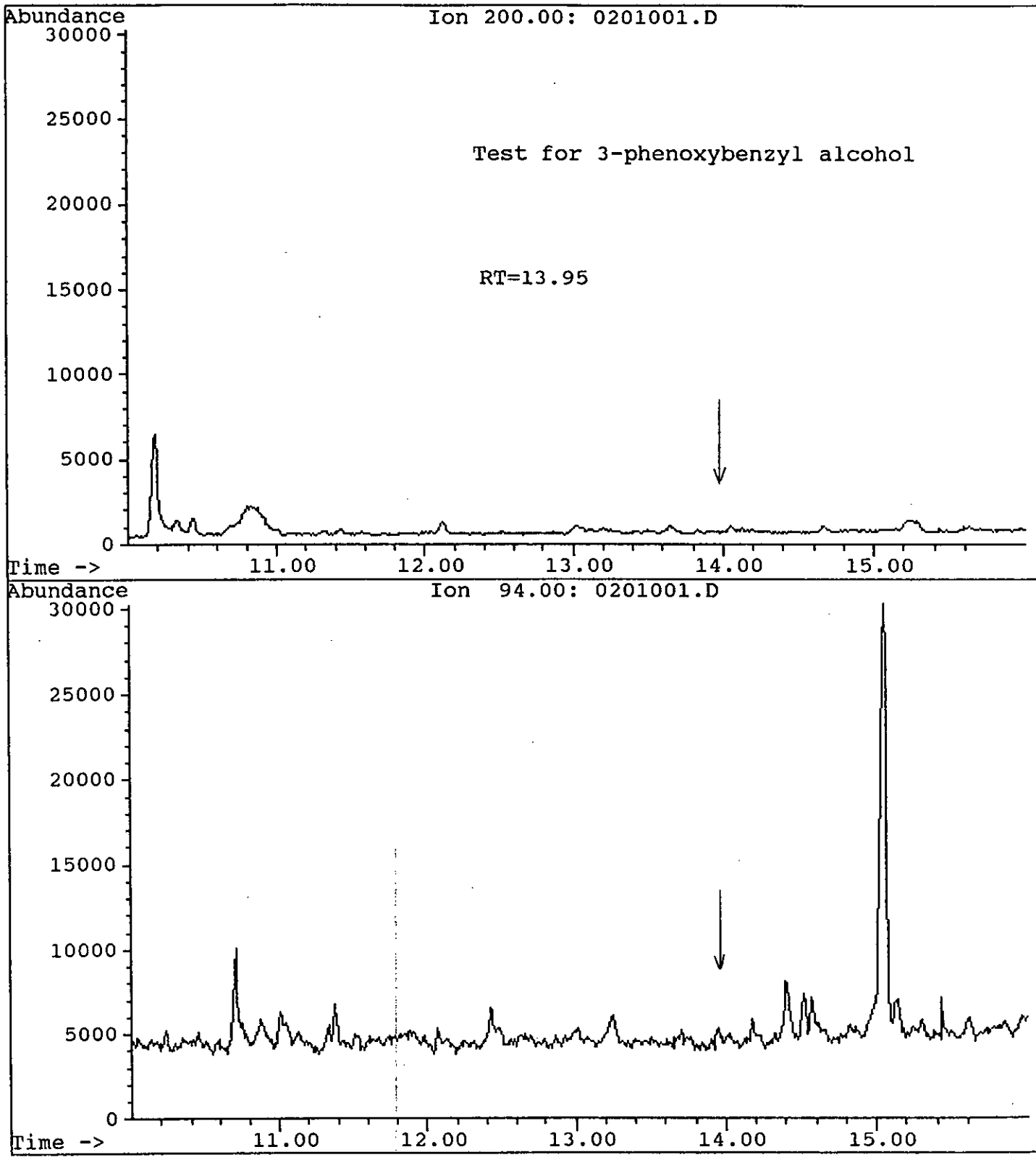


FIGURE 6 Ion-chromatograms for the identification of 3-phenoxybenzyl alcohol in the sediment stored at room temperature for 1 year.

ID : Pyrene

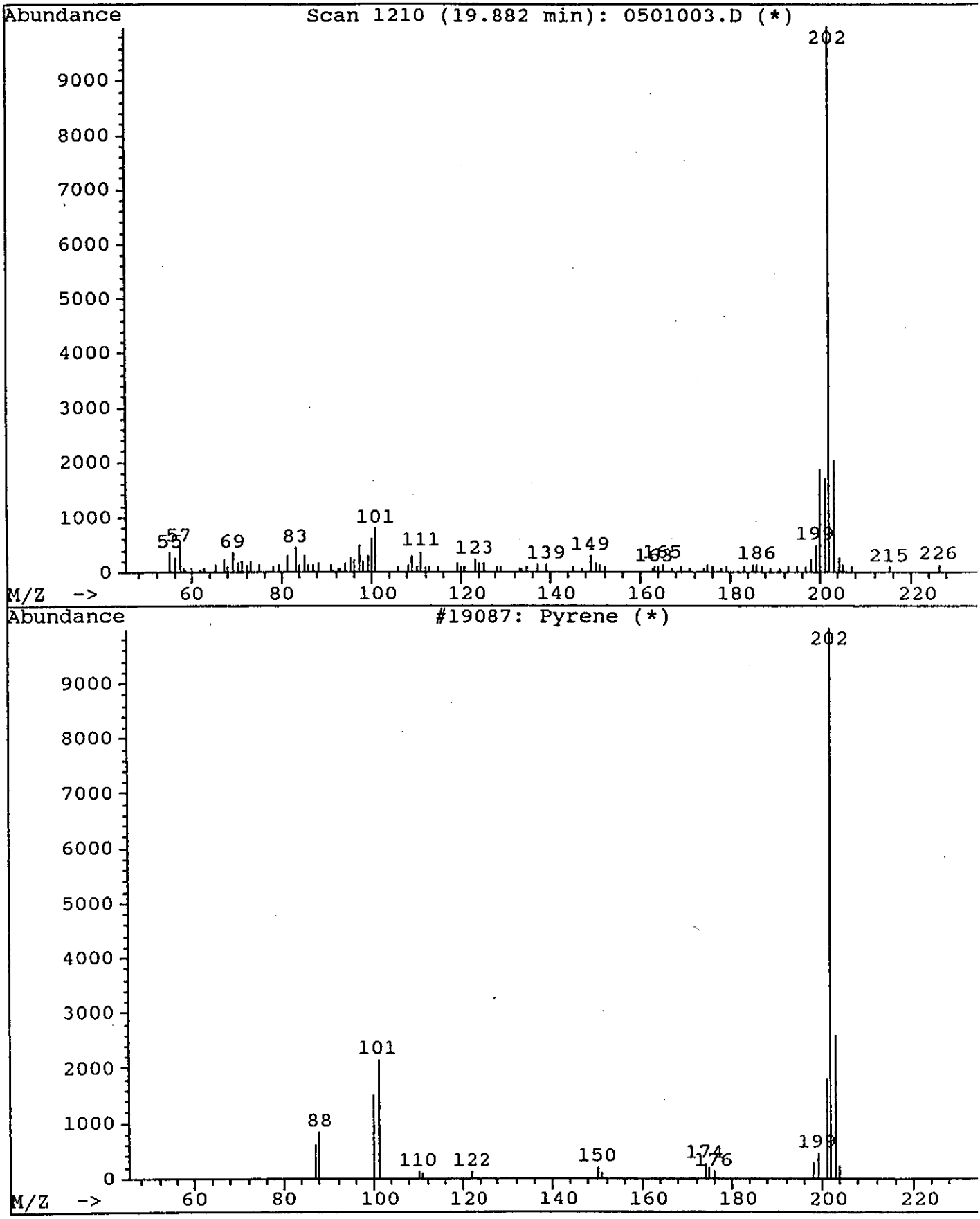
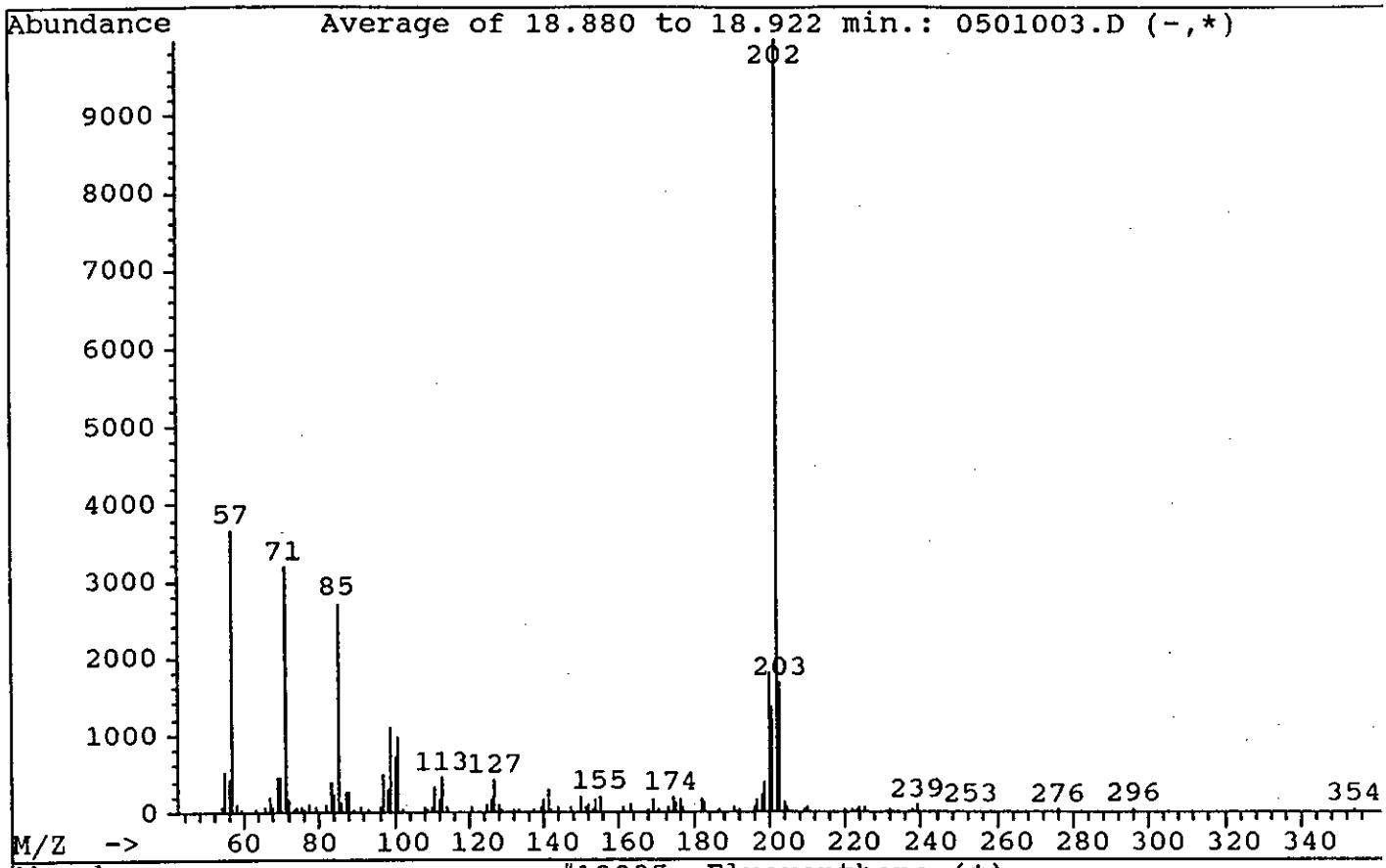


FIGURE 7 Identification of pyrene in the DCM extract.

ID : Fluoranthene



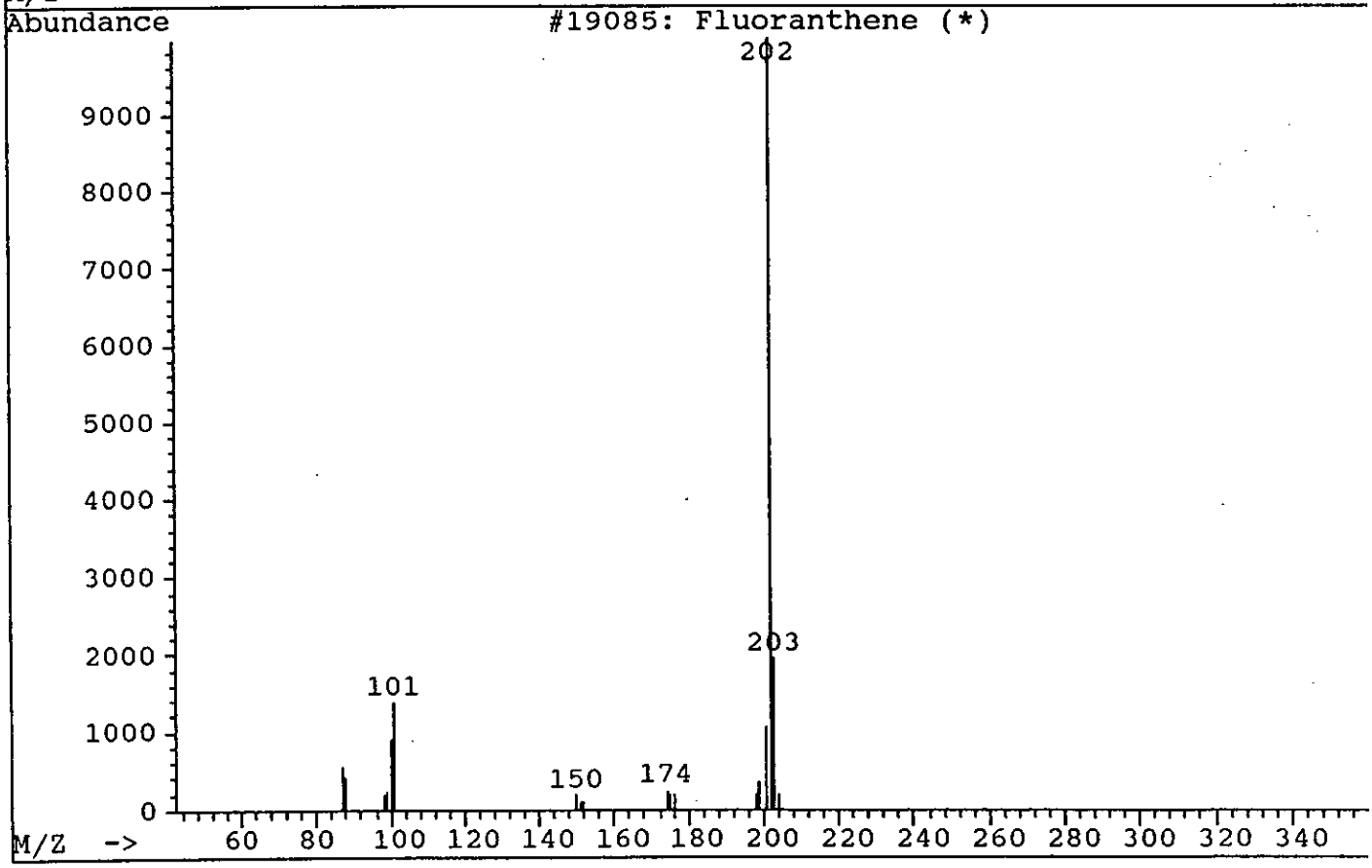


FIGURE 8 Identification of fluoranthene in the DCM extract.

ID : Anthracene

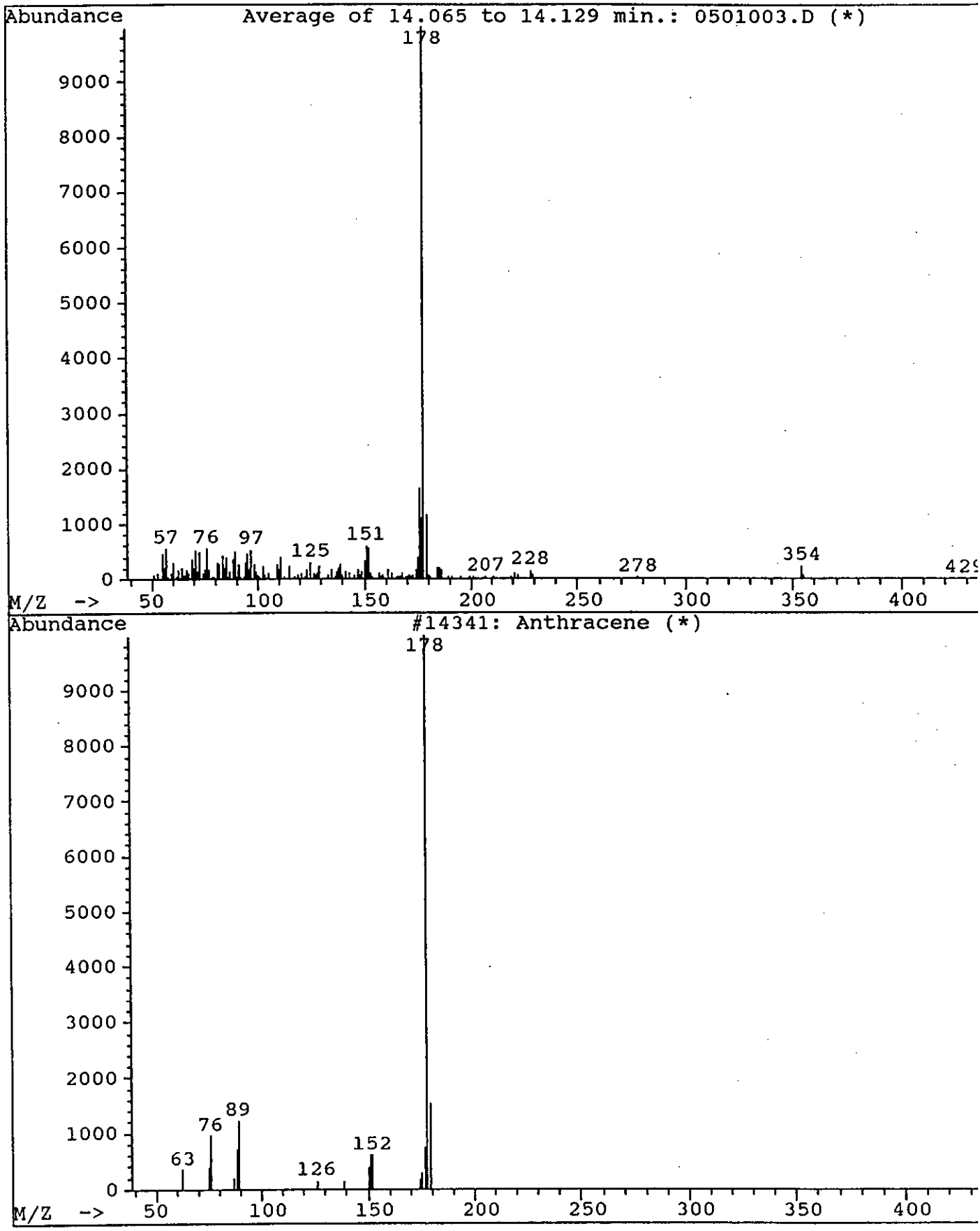


FIGURE 9 Identification of anthracene in the DCM extract.

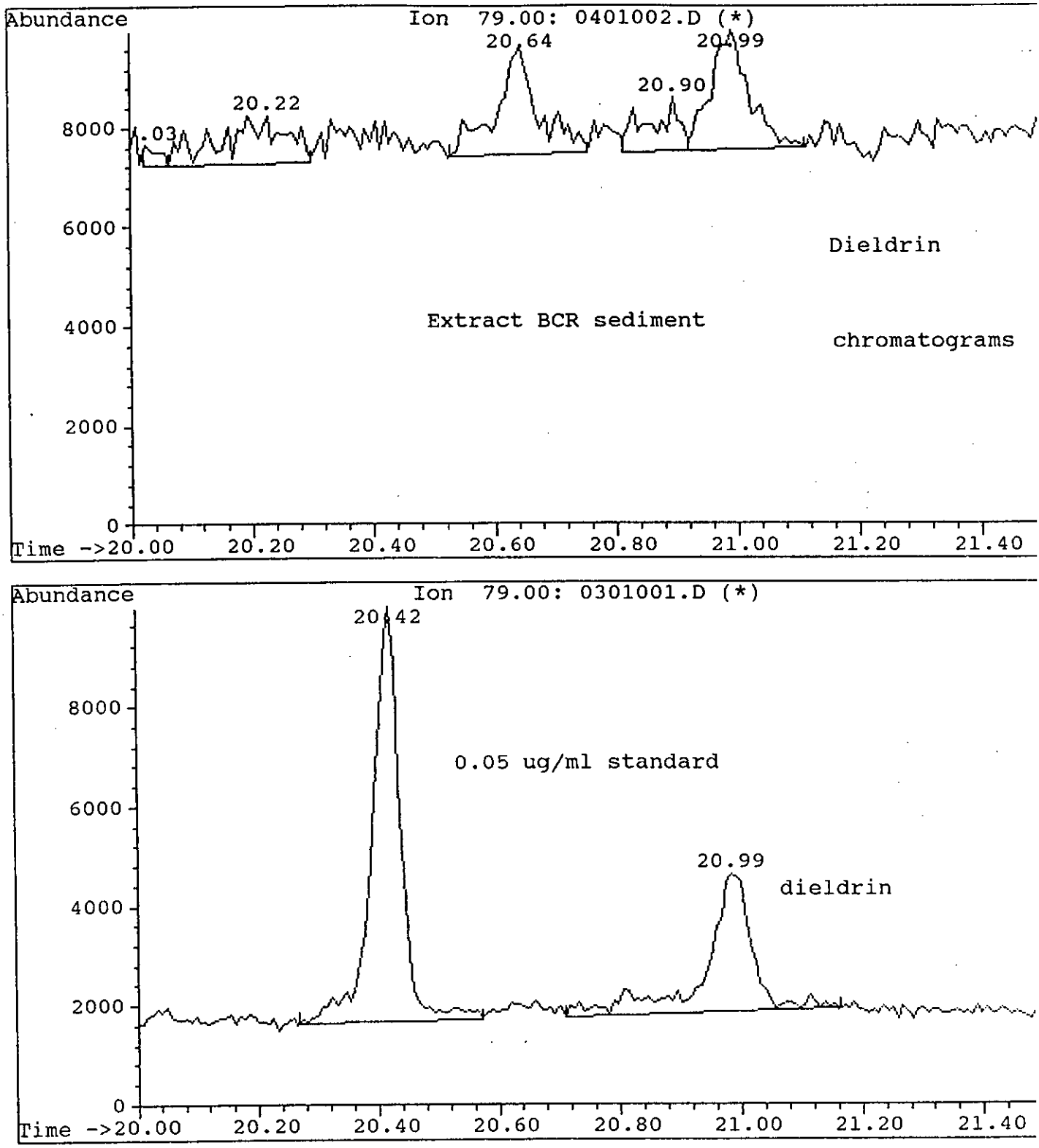


FIGURE 10 Identification of dieldrin in the DCM extract.

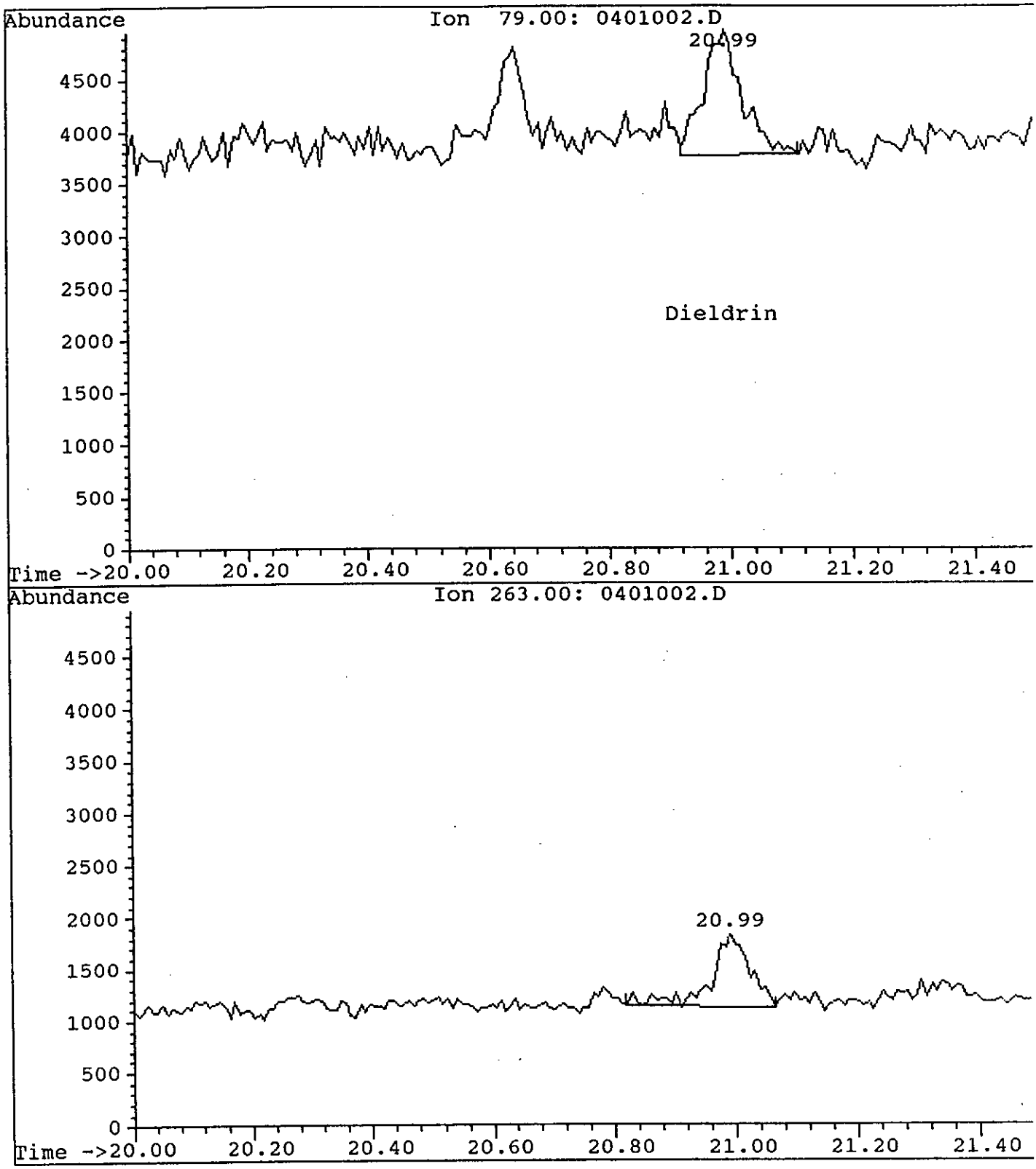


FIGURE 11 Identification of dieldrin in the DCM extract.

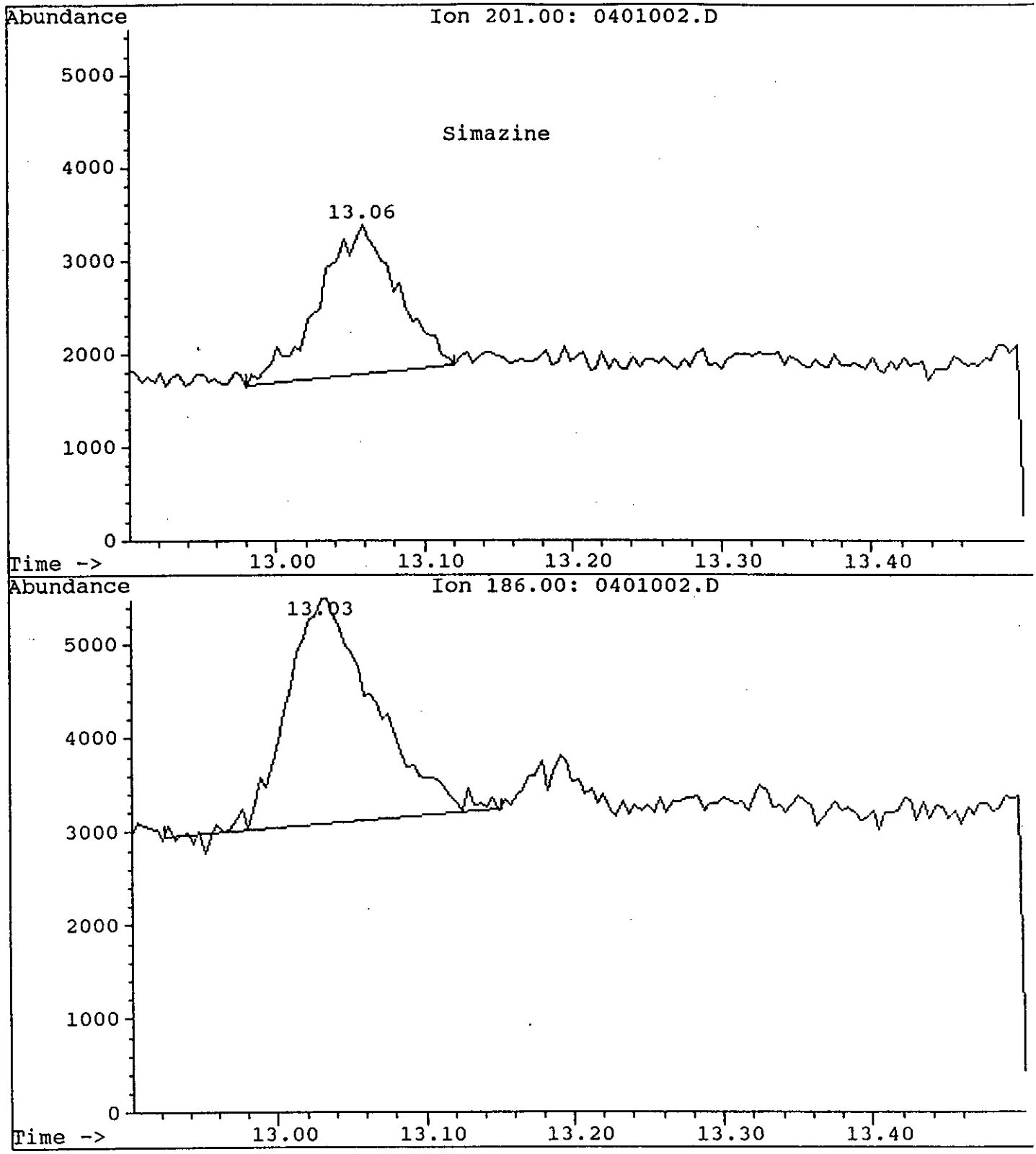


FIGURE 12 Identification of simazine in the DCM extract.

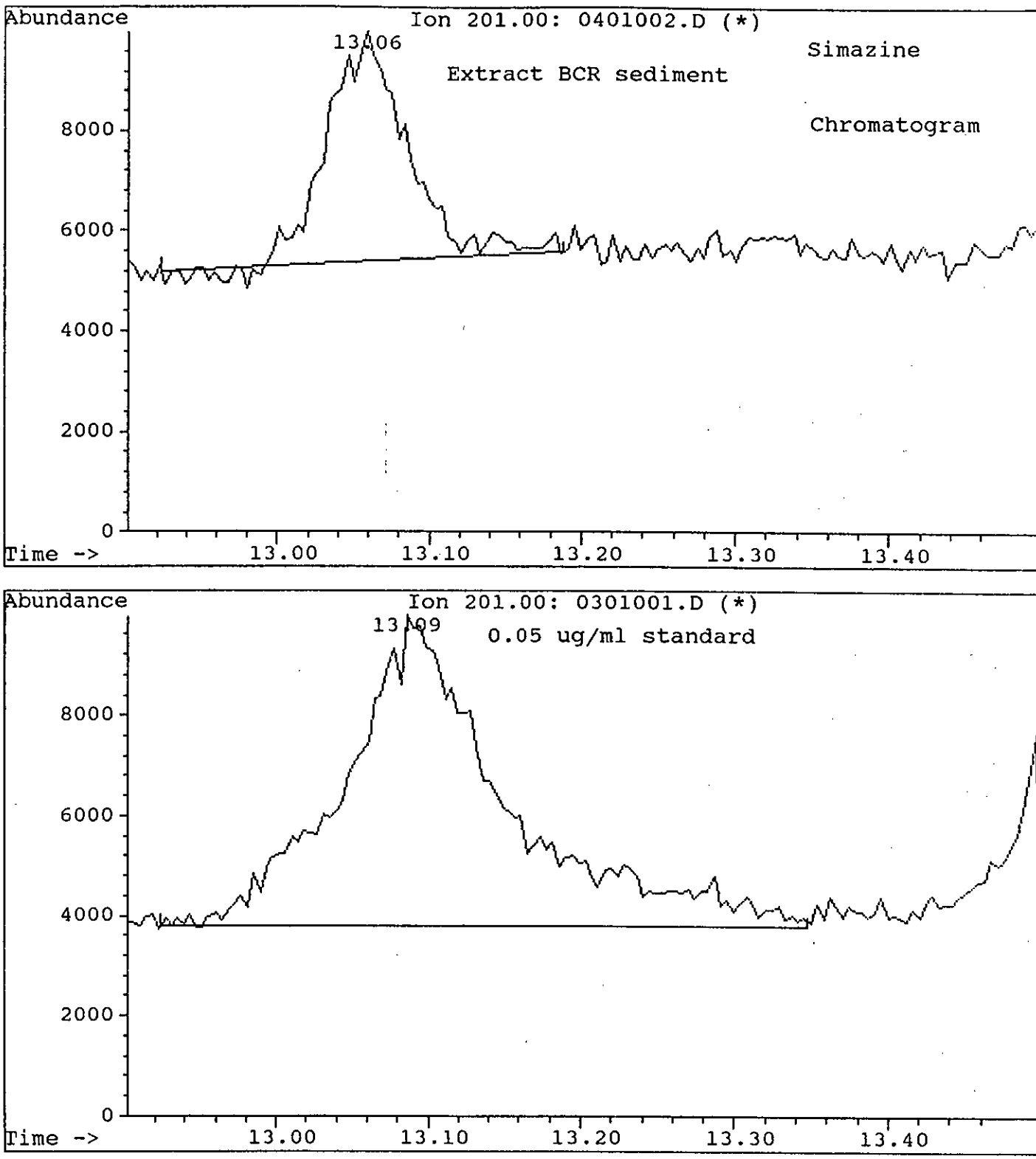


FIGURE 13 Identification of simazine in the DCM extract.

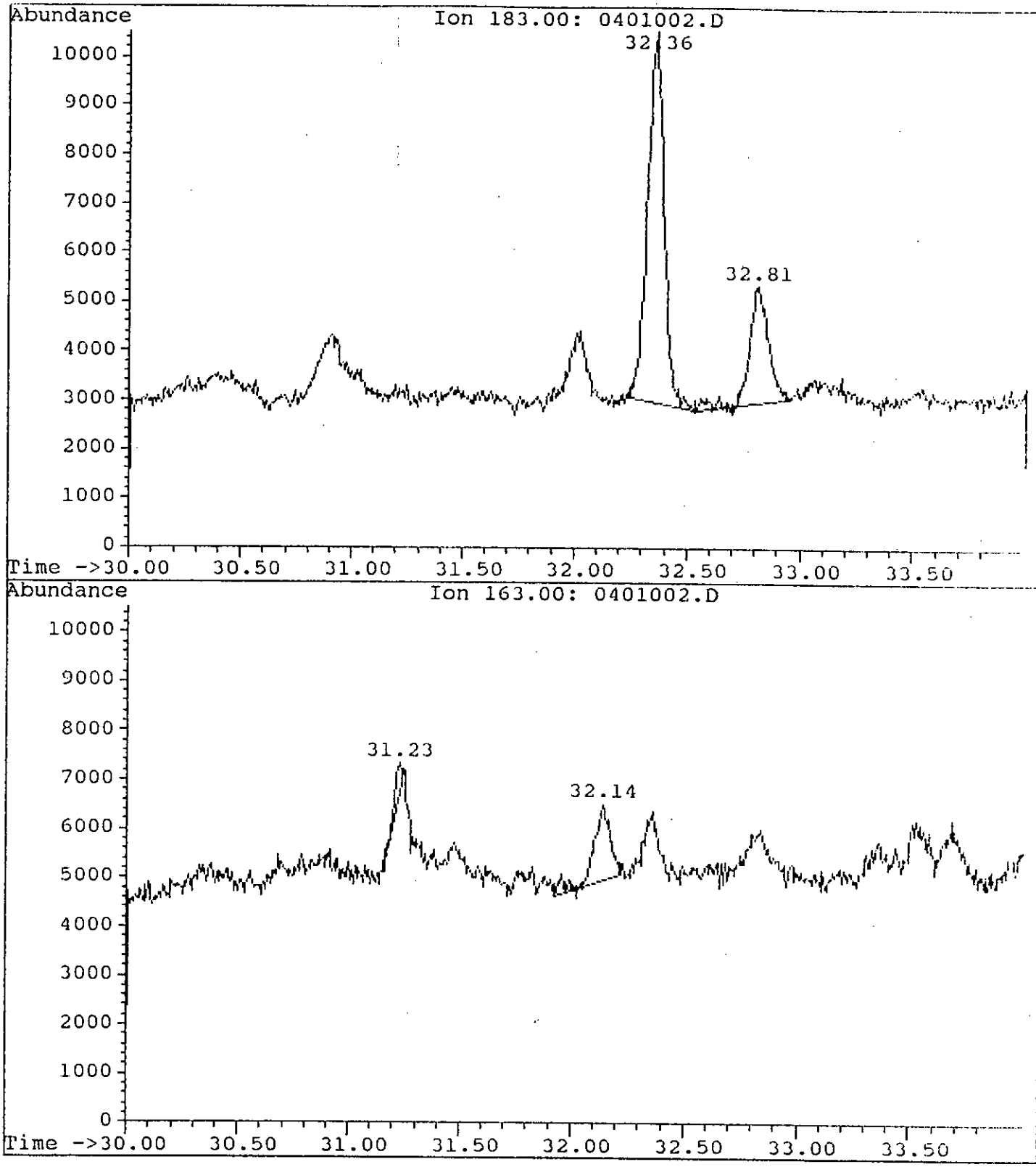


FIGURE 14 Identification of permethrin in the DCM extract.

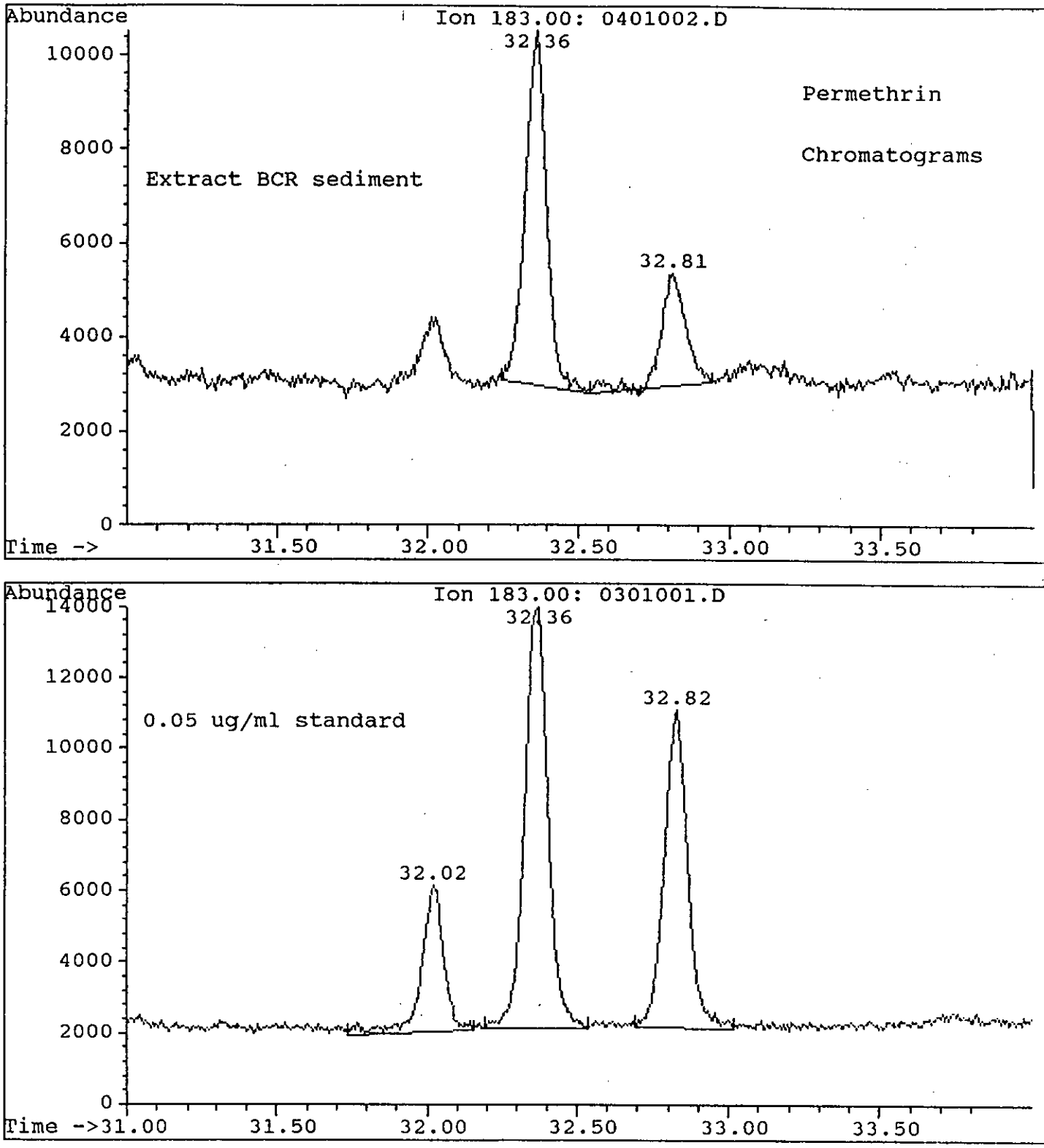


FIGURE 15 Identification of permethrin in the DCM extract.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