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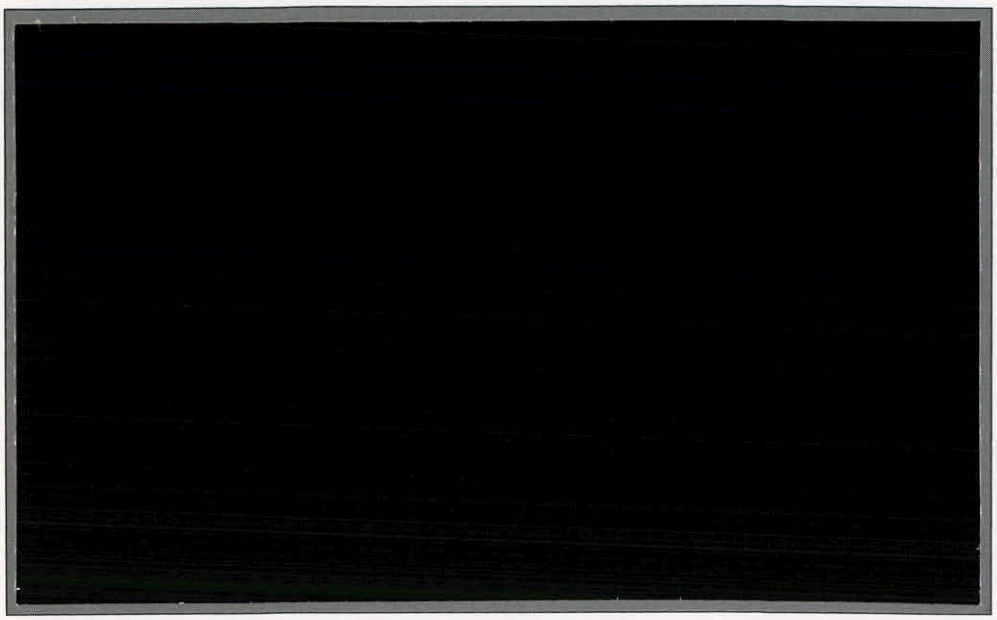

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INSTITUTE OF FRESHWATER ECOLOGY
River Laboratory, East Stoke, Wareham, Dorset BH20 6BB

Tel: 0929 462314
Fax: 0929 462180

RL 413

Preparation, reconstitution and homogeneity studies of lyophilized permethrin and simazine containing water samples

Long-Term Stability Study

W.A. House, PhD, FRSC, CChem
D.R. Orr

Project leader: W.A. House
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CONTENTS

ABSTRACT

The stability of a lyophilized river water containing *cis*-permethrin and a triazine, simazine, has been evaluated over a period of one year. The lyophilized powders were reconstituted at the reference time and subsequently after 1, 3, 6 and 12 months storage in the dark at room temperature. The reconstitution procedure has been found to be very successful and reproducible. This has been determined by the measurement of the major ion concentrations at the start of the trial and at the end, i.e. after one year's storage of the lyophilized samples. In addition, detailed conductivity measurements have been made throughout the trial to evaluate the performance and reproducibility of the reconstitution method. The results can be used to design a standard operating procedure for the reconstitution of lyophilized samples.

The analysis of the extract isolated from the reconstituted samples indicates that both pesticides are stable under the storage conditions employed. The results have been analysed using statistical F and t-tests with a 5% probability level. The mean concentration of simazine was determined as 0.041 ± 0.006 (SD) $mg\ dm^{-3}$ (CV = 15%) and *cis*-permethrin as 2.93 ± 0.64 (SD) $\mu g\ dm^{-3}$ (CV = 22%).

1. PREPARATION OF LYOPHILIZED SOLID

A fifty litre quantity of river water was collected from the R. Frome at the East Stoke weir (National Grid Reference SY868868) at 14.00 h on 5 January 1990. This was stored in a 60 litre polypropylene container prior to sampling one litre quantities for freeze-drying. The pH, temperature and conductivity of the river water was measured immediately after collection.

River water from the 50 litre sample was filtered through a 0.45 μm cellulose nitrate membrane filter (Sartorius 11306 No. 7802119604209) into a one litre pyrex bottle with PTFE screw cap and stored at 5 $^{\circ}$C in the dark. The first litre of filtered water was analysed for major ions and nutrients.

Freeze-dried samples of the river water spiked with pesticides were then prepared as follows (full details in report to BCR IFE/RL/T04053o1/2).

1. On 10.1.90 the samples were returned to room temperature and spiked with *cis*-permethrin and simazine to give final concentrations of 4.99 μg dm^{-3} and 0.052 mg dm^{-3} respectively. Five of the one litre samples were selected as blanks.

2. Five bottles of river water, previously spiked with pesticide, were selected at random and extracted to produce a bulk extract designated as the raw extract. This was stored in the dark at -20 $^{\circ}$C.

3. The remaining spiked samples and blank samples were then freeze-dried, the process being completed on 23.1.90.

4. The lyophilized material containing pesticides was bulked, homogenized and subsampled to give individual quantities of 0.3393 ± 0.0003 g (SD, n = 24). The individual weights have been recorded (Table 2, report to BCR IFE/RL/T04053o1/2).

5. All the samples were stored in the dark at room temperature in a nitrogen gas atmosphere.

2. PROCEDURE FOR THE STABILITY STUDY

Three spiked samples and one blank sample were selected at random at the beginning of the stability trial on 31.1.90. This date will be subsequently referred to as the reference time. On 5.3.90, 32 days from the reference time, a further four samples, including a blank, were selected at random. Both series of samples were reconstituted and extracted as quickly as possible after selection using a procedure determined in the pilot study (report to BCR IFE/RL/T04053o1/1). The codes for the samples selected at the reference time and after 32 days storage, together with the final pH of solutions after reconstitution and conductivities corrected to 25 $^{\circ}$C of the solutions immediately after reconstitution with CO_2 but before the adjustment of pH with nitrogen gas, are shown in Table 1. Two of the samples, BCR9 and 23, were analysed for major ions and nutrients (see Table 2). The percentage recoveries calculated from the conductivity of the original R. Frome water and measurements on the reconstituted samples are shown in Table 1.

The final stage of the stability trial involved the further storage at room temperature and the reconstitution and analysis of triplicate samples chosen at random, a blank and the analysis of the raw extract stored at -20 $^{\circ}$C after 3, 6 and 12 months. The results of the measurement of the pH of the reconstituted solution and conductivity at 25 $^{\circ}$C are shown in Table 1. The

percentage recoveries were calculated from the relationship:-

$$\text{Percentage recovery} = \frac{\text{conductivity of reconstituted solution corrected to } 25^{\circ}C}{\text{conductivity of R. Frome water corrected to } 25^{\circ}C}$$

The recoveries were in good agreement with the mean recovery calculated from the major ion analysis (see Table 2).

The conductivity of the reconstituted river water, measured immediately after CO_2 treatment, was found to be remarkably consistent with the maximum coefficient of variation of <1%. The mean value of the conductivity, measured at the temperature of reconstitution and corrected to a standard temperature of 25°C using the procedure recommended by Talbot, House & Pethybridge (*Water Research*, 1990, 24, 1295-1304), was calculated as 436.3 $\mu S\ cm^{-1}$ (at 25°C) with a standard deviation of $\pm$ 2.84 $\mu S\ cm^{-1}$. The deviations of the sample conductivities from the mean value are shown in Figure 1, together with the limits for the standard deviation or confidence bands. The results show that only three of the samples (BCR3, 13 and 8) had conductivities outside the band width indicated in Figure 1.

The mean pH was 8.01 $\pm$ 0.14 (SD) for 19 values. The value for BCR3 of 6.7 was excluded from the calculation of the mean value.

The river water samples reconstituted after one year's storage were also analysed for the major inorganic ions. The results are collected in Table 3. Excellent agreement in the results obtained for the four samples is evident with differences of the order of the experimental errors in the analysis.

3. EXTRACTION AND ANALYSIS OF PESTICIDES IN RECONSTITUTED WATERS

The details of the method for the extraction and analysis of *cis*-permethrin and simazine have been given in a previous report to BCR (report IFE/RL/T04053o1/2). These methods have been used for the extraction and analysis of all the reconstituted samples. The method of extraction avoids the use of large quantities of solvent and is preferred to the more usual solvent extraction methods. The analysis of permethrin was performed using glc with detection by ecd (glc/ecd) and simazine was analysed using glc with NPD detection (glc/NPD).

In addition to this analysis, the samples reconstituted after one year's storage were also analysed using glc with a mass-spectrometer detector, MSD.

Simazine was determined using the m/z = 201 ion with confirmation using the m/z = 186 and 173 ions. *Cis*-permethrin was determined using the m/z = 183 ion with confirmation using the m/z = 163 ion.

4. RESULTS OF THE ANALYSIS

The results of the glc analysis of the extracts from the reconstituted samples are shown in Tables 4 and 5.

4.1 *Cis*-Permethrin

The results for *cis*-permethrin are plotted in Figure 2, together with the associated error bars ($\pm$ SD) and mean value (2.93 $\pm$ 0.64 $\mu g\ dm^{-3}$, CV = 22%) calculated from the results from glc/ecd over the one year trial, i.e. n = 15.

The results of the significance tests are shown in Table 6. The comparison of the variability, F-test, shows no significant difference (at the 5% probability level) between the results obtained at the reference time and any of the subsequent data. However, the t-test does indicate significant differences, $t_{5\%}$, between the mean values at 90, 181 and 368 days and the mean at the reference time. As shown in Figure 2, the results obtained at the reference time are considerably greater than the subsequent determinations. If the 32 d reconstitution is used as the reference in the t-test, then the results indicate no significant, $t_{5\%}$, differences in the concentration in the extracts from the 90 and 181 d reconstitutions (see Table 6), i.e. the lyophilized material is stable over 149 d. The results obtained after one year do indicate significant, $t_{5\%}$, loss of *cis*-permethrin with a t-value of 3.4. The loss is not significant at the 2% probability level, $t_{2\%}$ ($\emptyset = 4$) = 3.37.

The statistical significance of differences in the variation and mean concentration for the reconstituted samples and raw extracts was also tested. The results at the 5% probability level indicate no significant difference between the results of the analysis of the reconstituted samples and raw extract at each storage time with the noted exception of the results obtained after 181 d storage. This is caused by the exceedingly low value of the standard deviation in the analysis of triplicates of the raw extract at this time (see Table 4). Hence it is concluded that at the 5% significance level, no difference could be determined between the concentration of *cis*-permethrin in the reconstituted samples and in the raw extract analysed at the specified storage times.

The chromatograms for the glc/ecd analysis of the samples reconstituted after one year's storage, i.e. BCR5, 8, 13 and raw extract did show some unexpected details. The chromatograms in Figure 4 illustrate the good separation of permethrin for the standard and raw extracts but some interference by a co-eluting compound with the permethrin peak for all the reconstituted samples. The substantial negative peak in the region of permethrin does lead to uncertainty in the integration and a probable underestimation in the concentration of permethrin. As an additional check, the extracts obtained after one year's storage of the lyophilized material were analysed using glc/MSD. The m/z = 183 ion-chromatograms, Figure 5, were used for the quantification and gave the results listed in Table 7. The mean concentration of 3.06 ± 0.13 (CV = 4%) $\mu g\ dm^{-3}$ is plotted in Figure 2 and is close to the mean value calculated for all the glc/ecd data.

4.2 Simazine

The results of the analysis of simazine are shown in Figure 3 with the associated error bars (± SD) and the mean value (0.041 ± 0.006 mg dm^{-3}, CV = 15%) calculated from the results from the glc/NPD over the one year trial, i.e. n = 15. The results of the significance tests are shown in Table 6. All the results were compared with the analysis at the reference time. No significant difference ($F_{5\%}$) could be detected in the variability of the analytical results. At the 5% probability level there is no significant difference between the mean value calculated at the reference time and any of the subsequent results obtained after storage of the lyophilized samples (see Table 6). It is therefore concluded on this basis that the lyophilized samples containing simazine are stable for a period of at least one year.

A comparison of the data from the raw extract and reconstituted samples also indicates no significant ($t_{5\%}$) differences for all the samples except the

final ones (Table 5). In this case the analysis of the raw extract produced a higher value than expected and led to the failure of the t-test at this level of significance. An analysis by glc/MSD produced similarly higher results for the raw extract.

5. CONCLUSION

The lyophilization and reconstitution procedures have proved to be successful. The reconstitution method is reproducible and various methods for the assessment of the performance of the techniques are available.

The reconstituted samples are close to the composition of the original freshwater. This has been demonstrated by the analysis of the original batch of river water prior to lyophilization and of reconstituted waters at the start and finish of the stability trial.

The measurement of the conductivity of the reconstituted water immediately following reconstitution, is a valuable method for the assessment of the success of the procedure.

The results of the long-term stability study show that *cis*-permethrin is stable in the lyophilization and storage conditions employed in this study. The final reconstitution and extraction of the material stored for one year, led to glc/ecd results which deviate significantly from the results obtained after 32 days storage. It is suggested that the apparent loss of permethrin arises from problems associated with the glc/ecd chromatography. The glc/MSD results for the analysis of the final extracts demonstrate that no significant loss of permethrin occurs over the storage interval.

The long-term stability of simazine in the lyophilized samples is clearly demonstrated. The results of the signifance tests show that at the 5% level no loss of simazine occurs during storage.

A comparison of the results for the analysis of the raw extract and reconstituted samples at each time interval also lead to the conclusion that the lyophilized samples stored at room temperature are as stable as the raw extract stored at -20°C. This is demonstrated by the application of the F and t-tests. Some problems associated with the analysis of the raw extract after one year's storage may be linked with the small volume of material remaining and possible losses of solvent during handling.

The results suggest various possibilities for improving the test procedures. These include: (i) using glc/MSD for the pesticide analysis if the concentrations of the analytes are high enough to permit quantification, (ii) preparation of a larger volume of raw extract.

Table 1. Conductivity of the reconstituted water samples measured at pH ≈5.1 and corrected to 25°C

Sample Number BCR #	Final pH	Conductivity /μS cm^{-1}	Percentage Recovery
	Results at the reference time		
3	6.7	466.8	84.5
17	7.9	460.9	83.5
22	7.8	465.8	84.4
Blank 9	8.1	462.5	83.8
			Mean 84.1 ± 0.5 (SD)
	Results after 32 days		
6	8.0	464.4	84.1
14	7.8	464.9	84.2
24	8.0	464.8	84.2
Blank 23	8.0	464.9	84.2
			Mean 84.2 ± 0.05 (SD)
	Results after 90 days		
7	8.26	465.3	84.3
15	8.20	463.2	83.9
29	8.23	462.8	83.8
Blank 16	8.28	461.7	83.6
			Mean 83.9 ± 0.3 (SD)
	Results after 181 days		
10	7.93	464.3	84.1
11	7.97	464.5	84.1
21	7.94	465.4	84.3
Blank 2	7.99	463.1	83.9
			Mean 84.1 ± 0.2 (SD)
	Results after 368 days		
5	7.98	462.0	83.7
8	8.07	459.3	83.2
13	7.92	454.0	82.2
Blank 30[+]	7.91	464.6	84.2
			Mean 83.3 ± 0.8 (SD)

Footnote[+] correction applied to compensate for lower mass of freeze-dried powder, viz 0.3148 g as compared to mean of 0.3393 g in other subsamples (ref. Table 2 of report RL/T04053o1/2).

Table 2. Comparison of R. Frome water and reconstituted waters

	Conductivity† at 25°C /μS cm^{-1}	pH	Alkalinity meq dm^{-3}	Na^+	K^+	Ca^2	Mg^{2+} /mmol	SO_4^{2-} dm^{-3}	Cl^-	NO_3^-	SiO_2	PO_4 /μmol dm^{-3}
R. Frome 5.1.90	552.1	7.95	4.09	0.51	0.03	2.50	0.10	0.57	0.69	0.44	0.07	4.9
BCR 9 5.2.90	462.5	8.14	3.50	0.38	0.04	2.07	0.09	0.48	0.48	0.39	0.06	2.6
% Recovery	84		86	75		83	90	84	70	89	86	53
			Mean recovery of major-ions listed is 83 ± 7% (SD)*									
BCR 23 5.3.90	463.6	7.96	3.70	0.40	0.05	2.04	0.08	0.49	0.51	0.39	0.06	4.1
% Recovery	84		91	78		82	80	86	74	89	86	84
			Mean recovery of major-ions listed is 83 ± 5% (SD)									

†corrected to 25°C according to the method of Talbot, House and Pethybridge (1990)

*excludes PO_4

Table 3. Results of the major-ion analysis of samples BCR5, 8, 13 and 30 reconstituted after one year's storage.

	Concentration /mmol dm^{-3}						
Ion	BCR5	BCR8	BCR13	BCR30[+]	Mean[x]	SD	CV/%
Ca^{2+}	2.00	1.99	1.98	2.03	1.99	0.009	0.5
Mg^{2+}	0.090	0.079	0.086	0.074	0.085	0.006	6.6
Na^{+}	0.42	0.48	0.41	0.39	0.44	0.04	9.1
K^{+}	0.051	0.051	0.049	0.049	0.05	0.001	2
HCO_3^{-}	3.02	3.18	3.12	3.05	3.11	0.08	2.6
Cl^{-}	0.43	0.56	0.54	0.43	0.51	0.07	1.4
SO_4^{2-}	0.55	0.56	0.51	0.57	0.54	0.03	5.6
NO_3^{-}	0.39	0.38	0.38	0.38	0.38	0.006	1.6
PO_4^{*}	3.49	3.49	3.39	3.48	3.46	0.06	1.7
SiO_2	0.029	0.026	0.026	0.019	0.027	0.002	7.4

Footnote: *Units μmol dm^{-3}

[x]Cation balance 4.56 mequiv dm^{-3}
Anion balance 4.54 mequiv dm^{-3}

Table 4. Results of Permethrin analysis

Sample designation	Storage interval/d	Concentration in water/$\mu g\ dm^{-3}$	Standard deviation	Number replicates	Normalized concentration
BCR 3	0	3.67	0.25	3	-
BCR 17	0	4.16	0.13	2	-
BCR 22	0	3.89	-	1	-
Mean		*3.87*	*0.29*	*6*	-
BCR 6	32	2.92	0.30	3	1.01
BCR 14	32	3.19	0.39	3	1.11
BCR 24	32	3.15	0.55	3	1.09
Mean		*3.09*	*0.39*	*9*	*1.07*
Raw	32	2.88	0.12	3	1.00
BCR 7	90	2.22	0.85	3	1.03
BCR 15	90	2.35	0.70	3	1.09
BCR 29	90	2.44	0.52	3	1.13
Mean		*2.34*	*0.62*	*9*	*1.08*
Raw	90	2.16	0.48	3	1.00
BCR 10	181	3.35	0.46	3	1.43
BCR 11	181	2.96	0.07	3	1.26
BCR 21	181	2.92	0.17	3	1.25
Mean		*3.08*	*0.32*	*9*	*1.32*
Raw	181	2.34	0.01	3	1.00
BCR 5	368	2.36	0.18	2	1.17
BCR 8	368	2.12	0.21	3	1.05
BCR 13	368	2.25	0.21	3	1.11
Mean		*2.23*	*0.20*	*8*	*1.10*
Raw	368	2.02	0.25	2	1.00

No Permethrin in the blank samples.

Note: Normalized concentration = $\frac{\text{concentration of pesticide in the sample}}{\text{concentration of pesticide in the raw extract}}$

Table 5. Results of Simazine analysis

Sample designation	Storage interval/d	Concentration in water/mg dm^{-3}	Standard deviation	Number replicates	Normalized concentration
BCR 3	0	0.041	0.001	2	-
BCR 17	0	0.042	0.0004	3	-
BCR 22	0	0.037	0.001	3	-
Mean		*0.040*	*0.002*	*8*	-
Blank	0	0.002	0.002	3	-
BCR 6	32	0.047	0.005	3	1.12
BCR 14	32	0.046	0.005	3	1.10
BCR 24	32	0.048	0.001		1.14
Mean		*0.047*	*0.004*	*9*	*1.12*
Blank	32	Not detected			
Raw	32	0.042	0.005	3	1.00
BCR 7	90	0.032	0.003	3	0.94
BCR 15	90	0.038	0.004	3	1.12
BCR 29	90	0.041	0.004	3	1.21
Mean		*0.037*	*0.005*	*9*	*1.09*
Raw	90	0.034	0.003	3	1.00
Blank	90	Not detected			
BCR 10	181	0.029	0.002	3	1.07
BCR 11	181	0.035	0.002	3	1.30
BCR 21	181	0.045	<0.001	3	1.67
Mean		*0.036*	*0.007*	*9*	*1.33*
Raw	181	0.027	0.002	3	1.00
Blank	181	<0.001	<0.001	2	
BCR 5	368	0.041	0.006	3	0.67
BCR 8	368	0.049	0.002	3	0.80
BCR 13	368	0.051	0.001	3	0.84
Mean		*0.047*	*0.005*	*9*	*0.77*
Raw	368	0.061	0.006	3	1.00
Blank	368	Not detected			

Table 6. Result of the significance tests applied to the stability trial results.

	Simazine		*cis*-Permethrin			
Time /d	F-test	t-test	F-test	t-test	F-test	t-test
0	R	R	R	R	-	-
32	4	2.7	1.8	2.8	R	R
90	6.2	0.96	4.6	*3.9	2.5	1.8
181	12.3	0.95	1.2	*3.2	1.5	0.03
368	6.2	2.3	0.48	*8.1	3.8	*3.4

Key: *Values outside ±t limits (5% probability level)
R = Reference analysis
$F_{5\%}(2,2) = 19$
$t_{5\%}(Ø=4) = 2.78$

Table 7. Results of the glc/MSD analysis of the extracts isolated from the reconstituted waters after one year's storage of the lyophilized samples. The ion m/z = 183 was used for the quantification.

Sample code	Concentration in the aqueous phase /$\mu g\ dm^{-3}$	Standard deviation /$\mu g\ dm^{-3}$
Blank BCR 30	ND	-
BCR 5	3.16	0.11
BCR 8	3.12	0.04
BCR 13	2.91	0.05
Mean	*3.06*	*0.13*
Raw	1.26	0.10

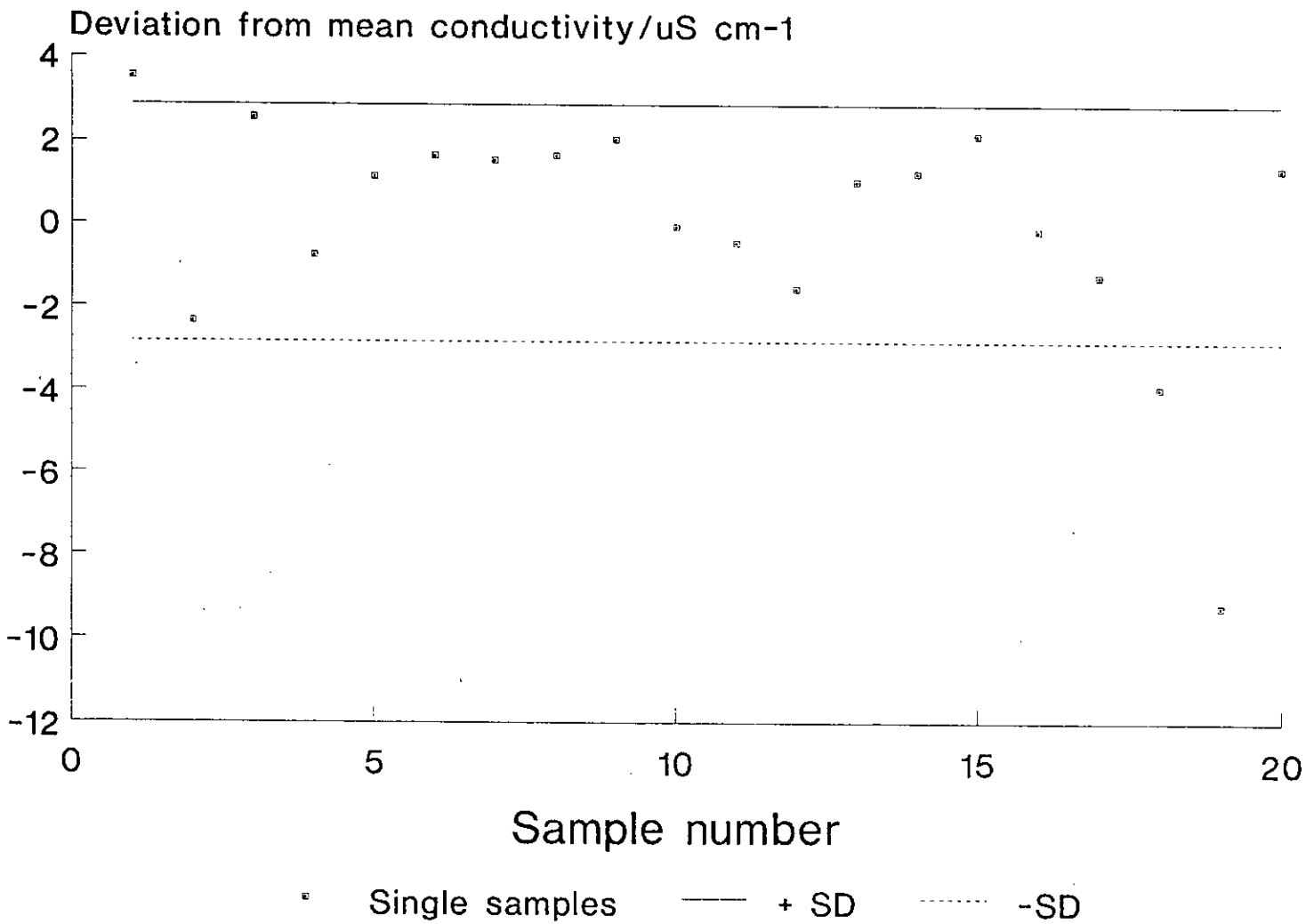


Figure 1. Comparison of the deviations from the mean conductivity (at 25 C) for all the reconstituted samples.

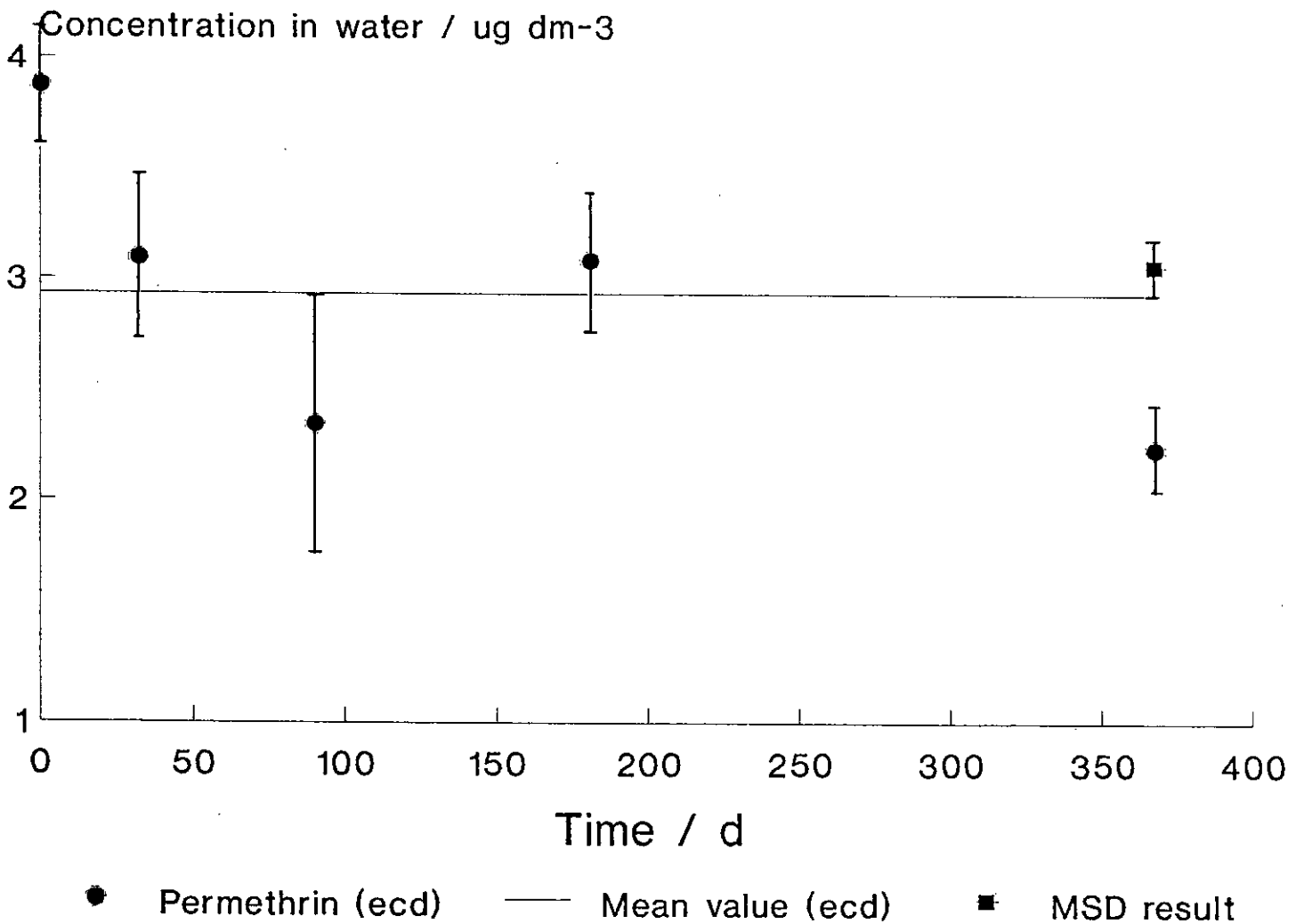


Figure 2. Results of the stability trial for cis-Permethrin.

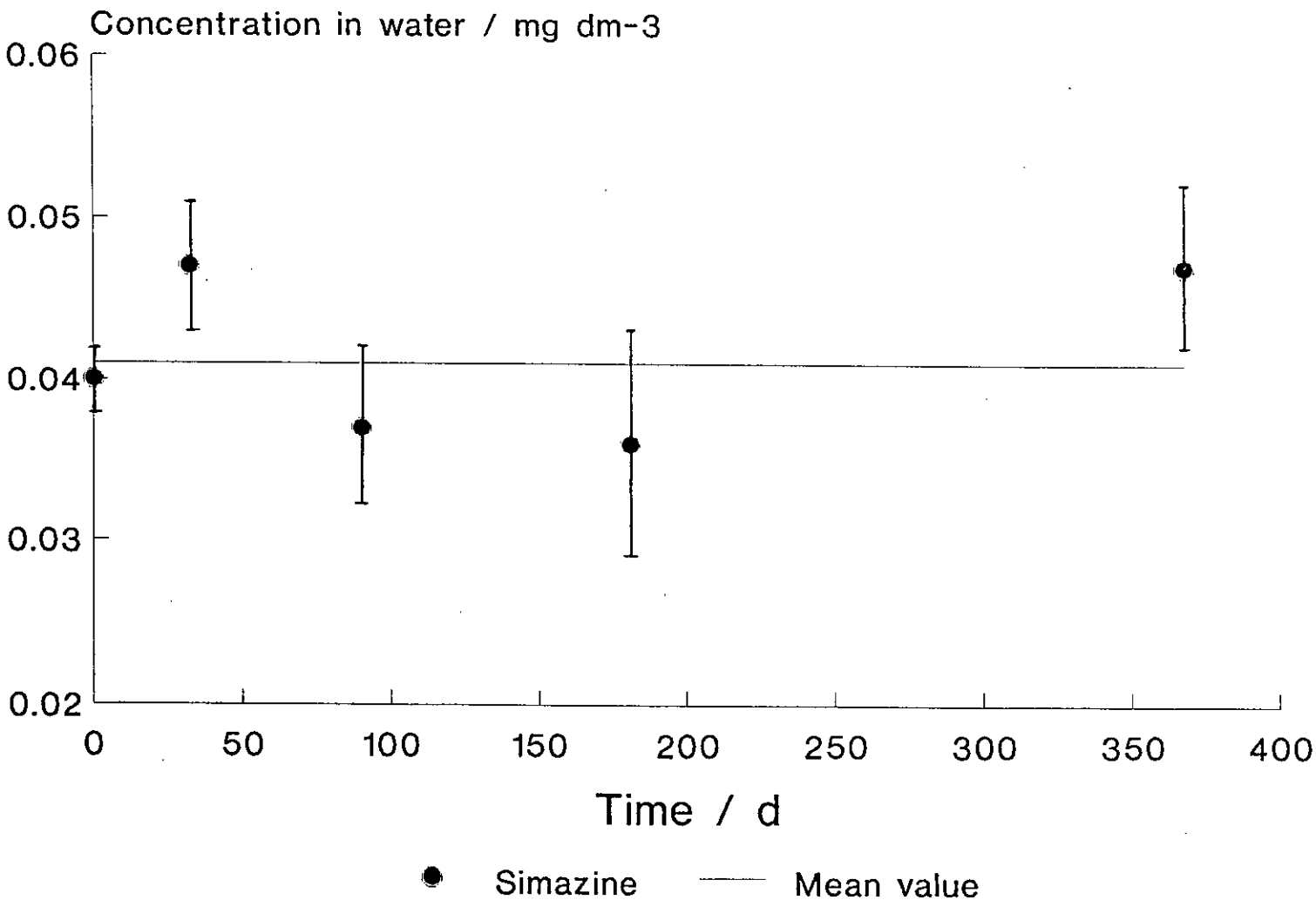
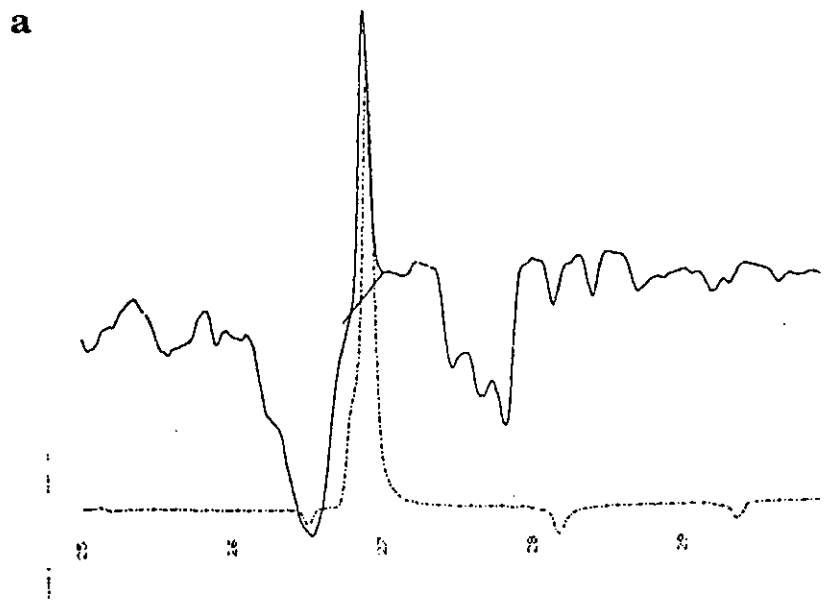


Figure 3. Results of the stability trial for Simazine.



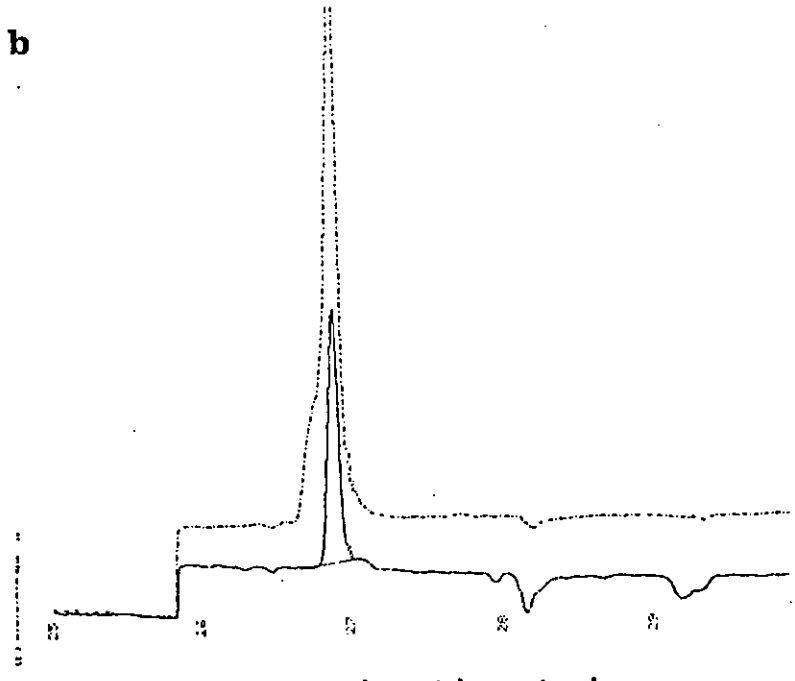


Figure 4. Comparison of glc/ecd chromatograms obtained for cis-permethrin.
(a) chromatogram for the BCR5 extract showing the permethrin peak (-). (b) chromatogram for the raw extract.
In both cases the dotted line is the 0.5 mg dm-3 standard peak.

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Sample Name: bcr 5
Misc Info:
Bottle Number: 3

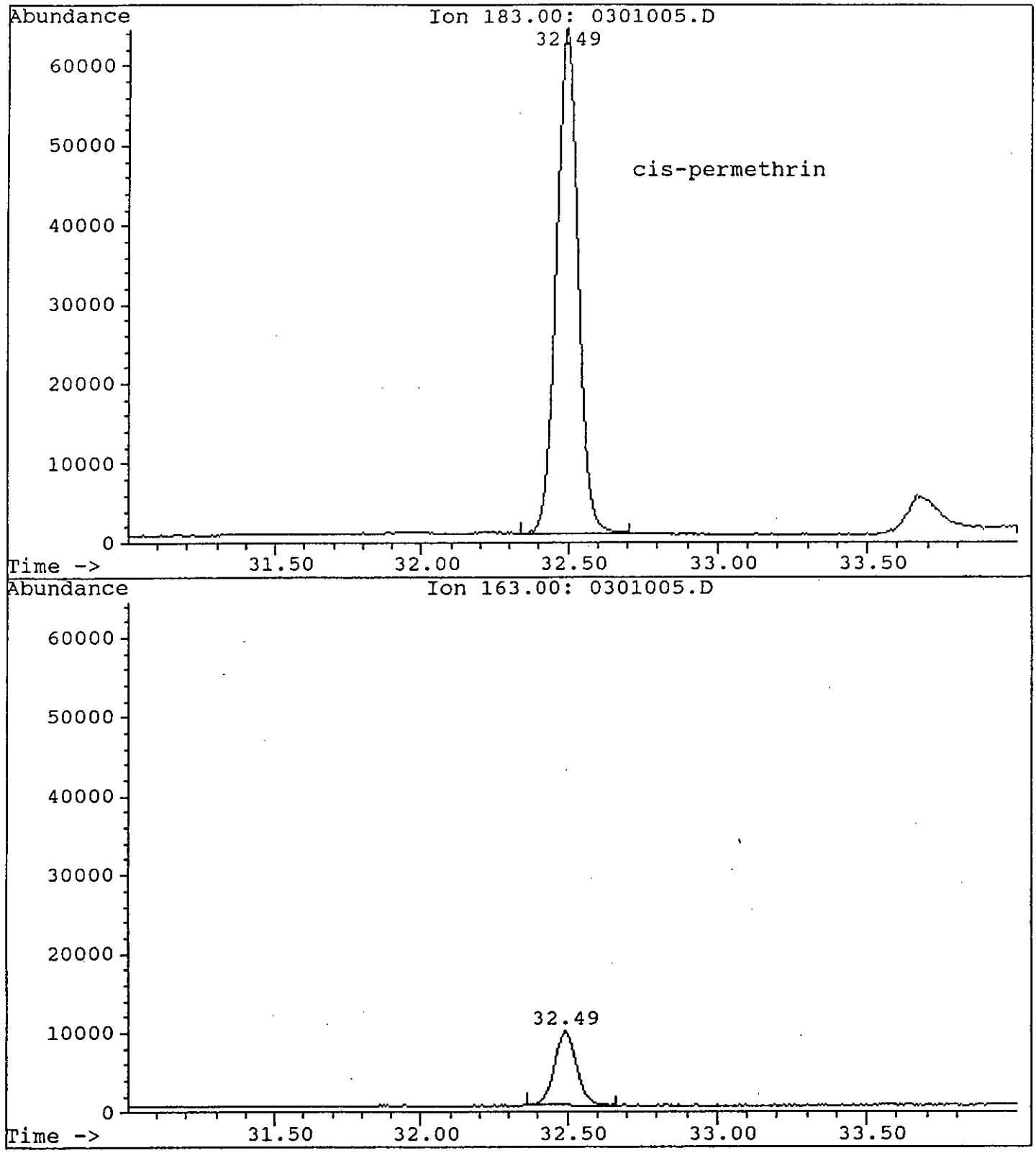

Figure 5.Chromatograms from glc/MSD for cis-permethrin. Sample BCR5 reconstituted after 1 years storage.

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