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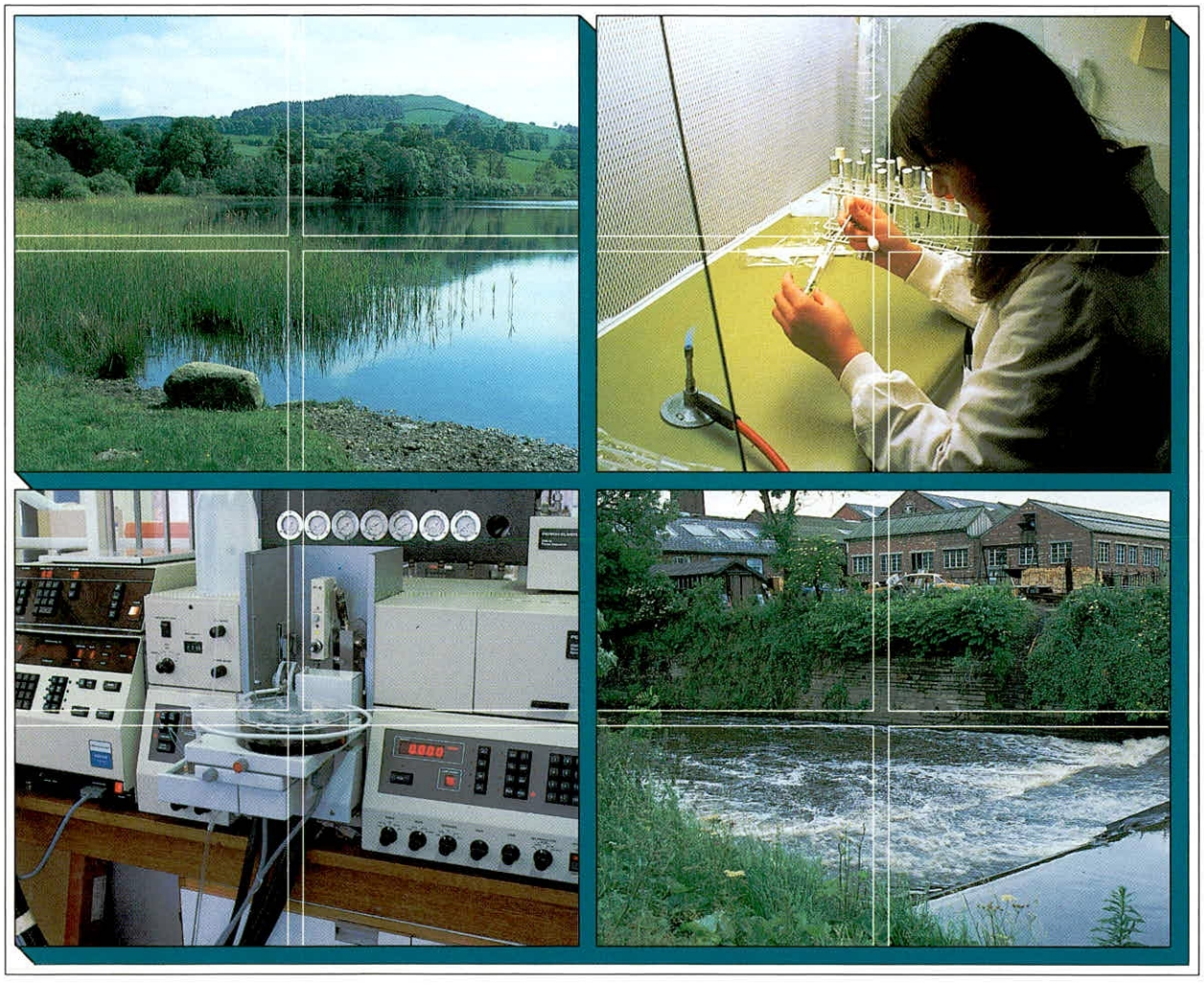

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CONTENTS

1. Introduction

This work follows the project on the preparation and long-term stability of permethrin and simazine in lyophilized water samples (contract no. 5174/1/9/332/89/06-BCR/UK(10)). The conclusions from the last study of a hard-water spiked with permethrin and simazine was that the reconstitution method is very reproducible and that the results of the long-term stability show that *cis*-permethrin is stable in the lyophilization and storage conditions employed. The long-term stability of simazine in the lyophilized samples has been clearly demonstrated. The results of the significance tests show that at the 5% level, no loss of simazine occurs during a storage of one year.

The purpose of the present project is to extend the homogeneity and stability trial to a lyophilized sample containing low concentrations of permethrin as well as other pesticides. The work reported here is specifically to examine the homogeneity and stability of two batches of lyophilized water prepared containing nominal concentrations of 1 and 2 μg dm^{-3} of *cis/trans* permethrin.

The lyophilized water samples were prepared by CID-CSIC. The results reported here for permethrin refer to the homogeneity and stability study over one month.

2. Homogeneity study

Thirty-three bottles of batch A and batch B were received on 20 December 1991. Each bottle had a number allocated by CID-CSIC; this was retained and referred to as the BCR number. Each bottle had the weight of sample clearly marked. The BCR number and sample weight were recorded and are shown in Tables 1 and 2. Each sample bottle was allocated to a storage condition using a random number generator, viz. room temperature, RT; -20^{0}C and 40^{0}C with five samples from each batch used for the homogeneity study at the start of the stability trial, hereafter referred to as the 'time-zero' samples. One sample from each batch was reconstituted (see Appendix 1) and then analysed, with five-fold replication, to give the results shown in Table 3.

The composition of the water is very different from the samples analysed in the previous study. The concentration of calcium of approximately 0.1 mmol dm^{-3} indicates a soft-water compared with that used in the previous stability trial with chalk-stream water, a relatively hard-water. There are significant differences between batch A and batch B: for example the concentration of sodium is lower in batch B and the concentration of calcium is slightly higher in batch B. However, both waters are soft-waters and the conductivity is expected to be low (<100 μS cm^{-1} at 25^{0}C).

Five samples of batch A and five from batch B were reconstituted using the procedure in Appendix 1. No blank control samples were available until 21 January 1992. The pH and conductivities of the reconstituted samples were measured as described in Appendix 1 and the results are shown in Table 4.

Table 1. **Information on the lyophilized water samples of batch A. RT refers to room temperature and 'time zero' to the samples used in the homogeneity trial.**

BCR number	mass /g	Storage temp/^{0}C
1	2.474	-20
2	2.469	time zero
3	2.483	40
9	2.491	RT
10	2.470	40
11	2.492	time zero
12	2.481	-20
13	2.473	time zero
14	2.470	time zero
15	2.469	RT
16	2.468	RT
17	2.483	40
18	2.494	RT
19	2.481	40
20	2.487	-20
21	2.471	-20
22	2.464	40
23	2.488	-20
24	2.490	40
25	2.473	-20
26	2.467	RT
27	2.489	-20
28	2.484	-20
29	2.480	RT
30	2.465	-20
31	2.471	40
32	2.469	RT
33	2.464	time zero
34	2.490	RT
35	2.467	40
36	2.490	RT
37	2.472	40
40	2.474	time zero

Table 2. **Information on the lyophilized water samples of batch B. RT refers to room temperature and 'time zero' to the samples used in the homogeneity trial.**

BCR number	mass /g	Storage temp/^{0}C
2	2.670	40
3	2.696	time zero
4	2.677	RT
9	2.681	time zero
10	2.674	RT
11	2.674	RT
12	2.698	-20
13	2.683	40
14	2.679	-20
15	2.698	-20
16	2.655	RT
17	2.698	-20
18	2.668	-20
19	2.663	time zero
20	2.696	RT
21	2.668	time zero
22	2.663	time zero
23	2.664	40
24	2.665	-20
25	2.697	-20
26	2.672	40
27	2.664	RT
31	2.691	40
32	2.676	-20
33	2.678	RT
34	2.693	40
35	2.669	-20
39	2.680	RT
40	2.674	RT
41	2.675	40
47	2.668	time zero
49	2.675	40
55	2.690	40

Table 3. **Results of the major ion analysis of one sample from batch A and batch B. The analysis was done at the beginning of the stability trial.**

ion	BCR 40 batch A		BCR 3 batch B	
	c/mmol dm^{-3}	SD	c/mmol dm^{-3}	SD
Ca	0.085	0.0076	0.113	0.0010
Mg	0.019	0.0004	0.025	0
PO_4	0.267*	0.020	0.264*	0.055
NO_3	0.013	0.0003	0.014	0
SiO_2	0.0017	0.0003	0.0005	0
Na	0.171	0.001	0.099	0.001
K	0.002	0.0001	0.003	0.0001

* units μmol dm^{-3}. All results are five-fold replicate analysis.

Table 4. **Results of the homogeneity study at the start of the stability trial. The conductivity and *trans*-permethrin concentrations are normalized to a mass of 2.4 g of batch A and 2.6 g of batch B.**

BCR code	batch code	conductivity /μS cm^{-1} at 25°C	pH	concentration permethrin /μg dm^{-3}	SD
11	A	104.6	4.75	0.869	0.006
13	A	48.9	5.20	0.438	0.004
14	A	69.0	5.42	0.033	0.004
33	A	55.0	5.39	7.322	0.026
2	A	70.7	5.41	1.088	0.005
Mean		69.6(21.6)	5.23(0.29)	0.607(0.468)*	
9	B	49.1	5.28	0.315	0.013
19	B	73.4	5.28	0.186	0.014
21	B	73.4	5.17	0.599	0.094
22	B	69.2	4.74	0.239	0.013
47	B	73.9	5.07	0.250	0.008
Mean		67.8(10.6)	5.11(0.22)	0.318(0.164)	

Note: The values in brackets are the standard deviations of the means.
*, the mean excludes the result for BCR 33.

The waters were analysed using the method described in Appendix 2, i.e. IFE, River Laboratory Standard Operating Procedure, SOP:6/070192 "Extraction of a one litre water sample through 500 mg C8 SPE column for permethrin analysis". The final volume of the extract was 2 ml of 5% acetone in hexane solvent spiked with an internal standard of 300 ng of phosalone. The reproducibility of this method for *cis* and *trans* permethrin is better than 6% (coefficient of variation for triplicate analysis of spiked: 0.1 μg dm^{-3}, one litre samples).

The extracts were analysed by GLC with ECD using the same conditions employed in the pilot study (Appendix 3). The presence of *trans*-permethrin was confirmed by GLC/MS in selective-ion mode.

The results shown in Table 4 have been calculated from a 3-point calibration (0.05, 0.5 and 1.0 μg ml^{-1} of *cis/trans*-permethrin with 0.15 μg ml^{-1} of internal standard, phosalone) with triplicate analysis of each extract. The GLC runs involved a sequence of standards, solvent (to check for injection "carry-over") and individual injections from each bottle extract followed by a further series of multi-level standards. This was repeated for each replicate of the samples in the batch. The standard deviations of the replicate analysis of samples from batch A were good with CV's generally <1% when the concentration of permethrin was >0.1 μg dm^{-3}. The CV's for the samples in batch B were higher with values between 3 and 15%. The between sample variation in concentration within a batch was very high with a CV for batch A and B of 77% and 52% respectively which is a much greater variation than the within sample variability. The between sample variability of the conductivity (corrected to a temperature of 25^{0}C and mass of 2.4 and 2.6 g for batch A and B respectively) is also much higher than expected, viz. CV of 31% and 15.6% for batch A and B respectively. Previous results with a hard-water sample produced a CV <1% for the between sample variability.

The concentration of *trans*-permethrin was much lower than expected with only a trace of *cis*-isomer detected in some of the samples. When *cis*-permethrin was detected, the concentration was too low for quantification: see for example the results of the analysis of BCR 13 and 49 from batch B illustrated in Figures 1 and 2.

The results of the homogeneity study (Table 4) show that the lyophilized samples from batch A and B are too heterogeneous with respect to the permethrin distribution. The poor reproducibility of the conductivity of the reconstituted samples indicates that differences exist in the ionic composition of the reconstituted samples. The reconstitution procedure itself is very reliable as demonstrated in the previous study.

3. Results after one month's storage

It was decided to continue the study and reconstitute the stored samples after one month at 20^{0}C, room temperature and 40^{0}C. The same methods were used except that approximately 500 ml of each sample was passed through the adsorbent C8 column. This avoided problems caused by the progressive blocking of the column when large samples were used.

The results of the analysis are shown in Tables 5 and 6 for batch A and Tables 7 and 8 for batch B samples. The triplicate analysis of individual extracts were good and in all but one case produced CV's <10%. The inter-sample variability was high, i.e. 40-120%, again indicating sample inhomogeneity. *Trans*-permethrin was also detected in the blank sample. (Note: permethrin is used to treat water mains and can occur in tap water.) Traces of *cis*-permethrin were detected in some of the samples but at concentrations too low for reliable quantification. Because of the high inter-sample variability of the samples stored at each temperature, no systematic differences between the samples stored at different temperatures was discerned. However, a significant difference (t-test, 5%) between the initial analysis (time zero) and the results after one month's storage was determined ($t = 2.46$ and 2.94 for batch A and B respectively) indicating a significant loss of permethrin during storage.

Raw extracts were prepared and analysed but the results have not been used because of the inhomogeneity of the samples and ensuing problems in evaluating the stability of the sample.

The results do not show a systematic relationship between the final conductivity of the samples and the concentration of permethrin therein.

4. Conclusion

In view of the inhomogeneity of samples from batch A and B in terms of the distribution of permethrin and also the very low concentration of permethrin detected in the samples, the stability trial is not continued. *Cis*-permethrin was only detected at trace levels in some of the samples. *Trans*-permethrin was present at a concentration <1 μg dm^{-3} in both preparations.

The replicate analysis of the sample extracts was satisfactory with coefficients of variation <10%. The inter-sample variability was high (40-120%) indicating the heterogeneous nature of the lyophilized material. It is probable that lipophilic pesticides behave differently from more polar compounds during the lyophilization process, particularly in the presence of stabilization agents. The previous study, using a hard-water without a stabilization agent, probably worked because of the incorporation of permethrin with organic matter in occlusions within the calcium carbonate matrix. This mode of protection is not possible with soft-waters.

Table 5. **Results obtained for batch A from the analysis of the reconstituted samples after one month's storage at -20°C, RT and 40°C. The conductivities and concentrations are normalized to a mass of 2.4 g of sample A and 2.2 g of the blank.**

BCR code	storage temp. /°C	conductivity /μS cm^{-1} at 25°C	concentration permethrin /μg dm^{-3}	SD /μg dm^{-3}
1	-20	69.8	0.217	0.019
20	-20	71.6	0.457	0.043
23	-20	48.1	0.266	0.022
mean	-	63.2(13.1)	0.313	0.113
18	RT	49.9	0.136	0.008
29	RT	68.8	0.146	0.004
32	RT	73.0	0.339	0.030
mean	-	63.9(12.3)	0.207	0.100
24	40	68.7	0.148	0.002
31	40	61.9	0.152	0.001
37	40	59.0	0.082	0.004
mean	-	63.2(5.0)	0.128	0.152
8	blank	66.7	0.192	0.006
		All data: 0.216 ± 0.119 μg dm^{-3}		

Table 6. **Summary of the results of the t-test for batch A.**

Storage temp. /°C	mean concentration of permethrin /μg dm^{-3}	SD /μg dm^{-3}	t-test	P
-20	0.313	0.113	-	-
RT	0.207	0.100	NS, 2.11	0.051
40	0.128	0.152	S, 4.72	0.0002

Key: NS no significant difference with -20°C result
S significant difference with -20°C result

Table 7. **Results obtained for batch B from the analysis of the reconstituted samples after one month's storage at -20°C, RT and 40°C. The conductivities and concentrations are normalized to a mass of 2.6 g of sample B and 2.2 g of the blank.**

BCR code	storage temp. /°C	conductivity /μS cm^{-1} at 25°C	concentration permethrin /μg dm^{-3}	SD /μg dm^{-3}
15	-20	48.3	0.190	0.017
17	-20	69.1	0.073	0.002
35	-20	70.1	0.168	0.005
mean	-	62.5(12.3)	0.143	0.054
16	RT	66.2	0.021	0.001
20	RT	59.0	0.047	0.002
40	RT	50.4	0.196	0.008
mean	-	58.5(7.9)	0.088	0.082
13	40	74.3	0.082	0.018
23	40	69.2	0.210	0.005
49	40	48.4	0.205	0.004
mean	-	64.0(13.7)	0.166	0.063
8	blank	66.7	0.192	0.006
		All data: 0.132 ± 0.076 μg dm^{-3}		

Table 8. **Summary of the results of the t-test for batch B.**

Storage temp. /°C	mean concentration of permethrin /μg dm^{-3}	SD /μg dm^{-3}	t-test	P
-20	0.143	0.054	-	-
RT	0.088	0.082	NS, 1.69	0.051
40	0.106	0.063	NS,-0.80	0.043

Key: NS no significant difference with -20°C result
S significant difference with -20°C result

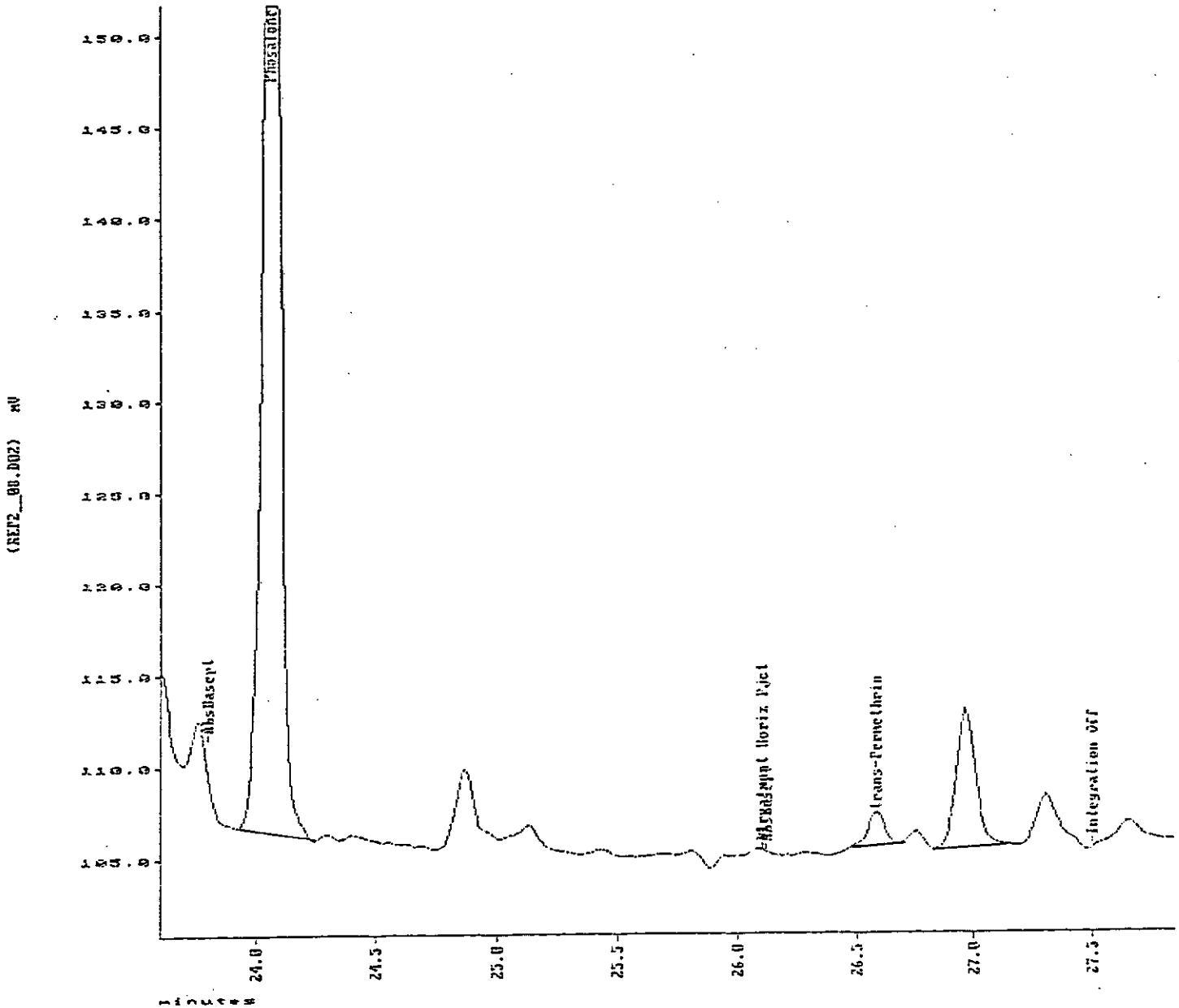

Figure 1. Chromatogram for sample BCR13 from batch B showing the internal standard peak (phosalone at RT ~24 min.) and *trans*-permethrin. Note the absence of *cis*-permethrin.

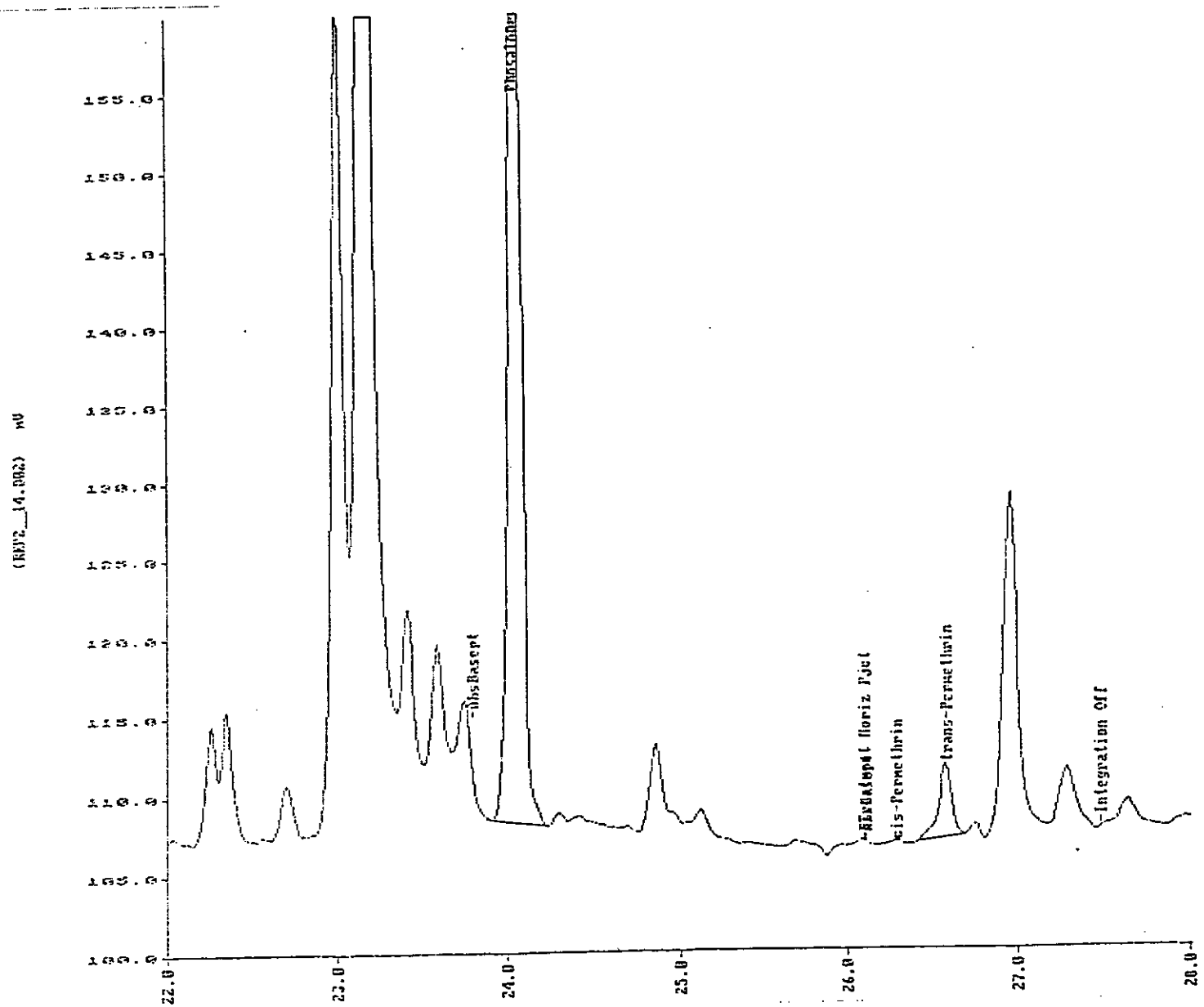

Figure 2. Chromatogram for sample BCR49 from batch B showing the internal standard peak (phosalone at RT ~24 min.) and *trans*-permethrin. Note the trace of *cis*-permethrin.

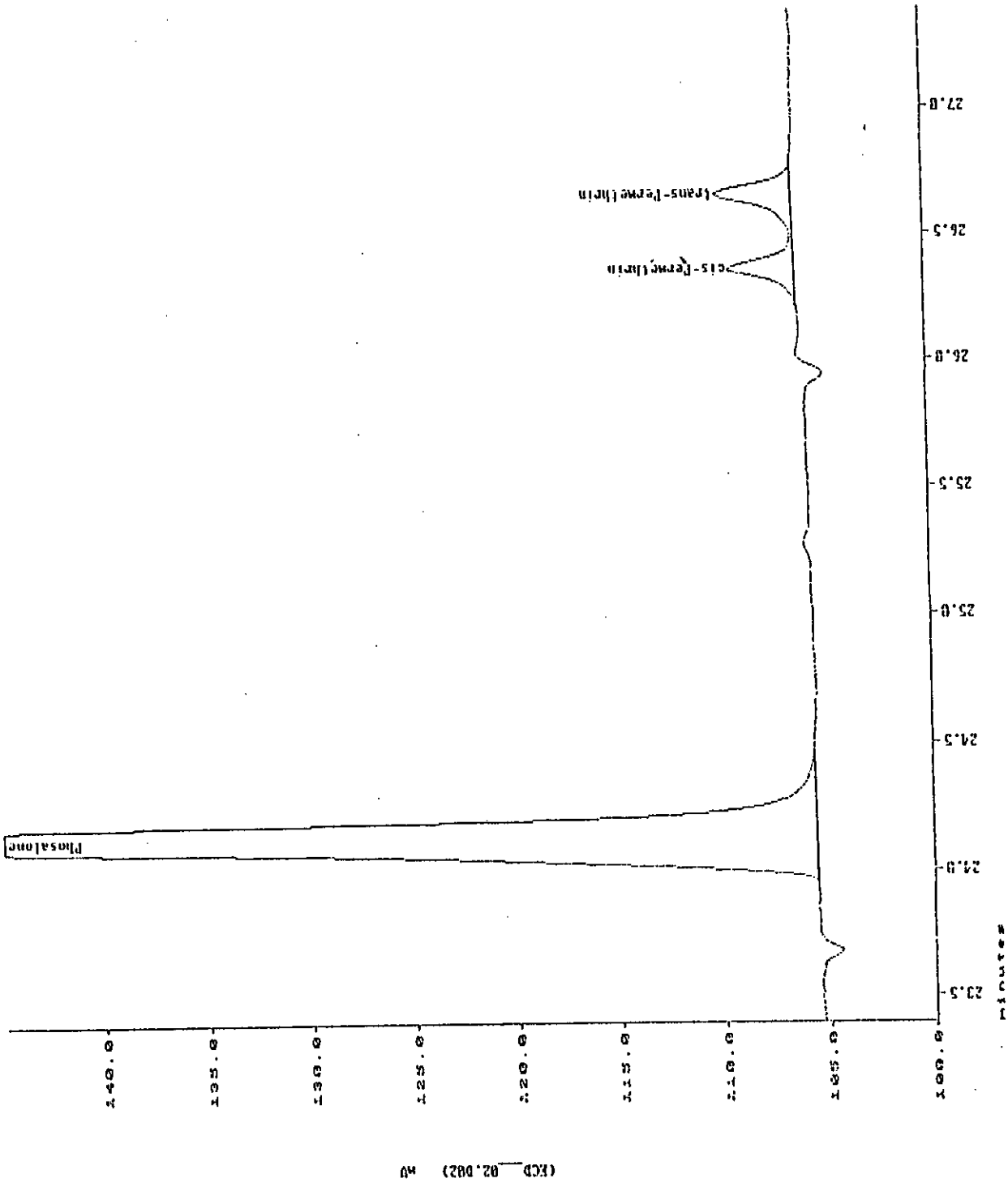

Figure 3. Chromatogram for a standard (0.05 μg ml^{-1}) of *cis* and *trans*-permethrin.

APPENDIX 1. Method for the reconstitution of aqueous pesticide references

Each sample of extract contains an amount of powder to enable the reconstitution of 1 litre of freshwater of ionic composition as near as possible to the original sample.

The performance of the reconstitution procedure may be evaluated by measurement of the electrical conductivity of the final solution measured at 25°C (or corrected to 25°C using a method described here) and if necessary from the measurement of the major ions in solution, viz Na^+, K^+, Ca^{2+}, Mg^{2+}.

1. Materials and apparatus

1 litre borosilicate glass bottle with PTFE sealed screw cap.

Gas distribution tube with grade P40 glass frit (BS grade 3).

High-purity water (single distilled and pesticide free; conductivity <4 μS cm^{-1} at 25°C).

PTFE stirrer bar 4-6 cm in length.

Carbon dioxide supply (purity ≥99.998%).

Conductance meter and measurement cell (glass-cell with platinised parallel-plate electrodes with a cell constant ~1 cm^{-1} is recommended). The cell should be calibrated in dilute potassium chloride solutions at 25°C. Reference: J. Barthel, F. Feuerlein, R. Neueder & R. Wachter, 1980. Calibration of condutance cells at various temperatures. *J. Solution Chem.* **9**, 209-

pH meter with glass electrode. Buffer calibration using KH_2PO_4-Na_2HPO_4(1+3.5) and $Na_2B_4O_7.10H_20$ is recommended.

Mercury in glass thermometer or platinum resistance thermometer (PRT) to measure temperature to ±0.1°C.

2. Reconstitution procedure

2.1 All glassware must be adequately cleaned to avoid contamination of the sample. Gas lines should be either copper or PTFE tubing. Contact with plasticisers must be avoided.

2.2 Prepare a 1 litre volume of distilled water in a volumetric flask (grade A) at room temperature.

2.3 Transfer the powder extract to a 1 litre borosilicate glass bottle and flush the sample bottle with approximately 50 ml of distilled water with care to avoid loss of the sample. Repeat the rinsing of the sample bottle a further two times.

2.4 Add the remaining distilled water to the glass bottle to obtain a final volume of 1 litre.

2.5 Insert a gas-distribution tube and PTFE stirrer bar into the solution. Bubble CO_2 gas through the solution at a rate of approximately 10 ml min^{-1} for a period of 120 minutes ensuring that the solution is well mixed during the process (PTFE stirrer bar).

2.6 Turn the CO_2 supply off and then remove sufficient solution (40 ml) for conductance and temperature measurement.

2.7 Pass nitrogen gas at a flow-rate of approximately 150 ml min^{-1} for a period of 10 minutes. Raise the gas-distribution tube into the head-space in the bottle and stop the gas flow. Remove sufficient solution (10 mls) for a measurement of the final pH. Seal bottle and store for analysis.

3. Correction of conductivity measured at t°C to the value at 25°C.

The electrical conductivity measured at t°C may be corrected to the value at 25°C using a form of the modified Walden product, equation (1) (Talbot *et al.*, 1990):

$$\kappa(25^0) = \kappa(t)[\eta(t)/\eta(25^0C)]^{K_1} \quad (1)$$

where κ is the electrical conductivity, μS cm^{-1}
$\eta(t)$ is the viscosity of water (cpoise) at temperature t(°C)
$\eta(25^0C)$ = 0.8905 cpoise
and K_1 is a constant with a value of 0.896.

The values of $\eta(t)$ are given in Table 1 as calculated from the equations produced by Weast (1986).

References

J.R. Talbot, W.A. House & A.D. Pethybridge, *Water Research*, 1990. "Prediction of the temperature dependence of electrical conductance for river waters".

R.C. Weast, *C.R.C. Handbook of Chemistry and Physics*, 67th edition, 1986, CRC Press, Fla.

Table 1. Viscosity of water between 15 and 29°C.

Temperature /°C	Viscosity /cpoise	Temperature /°C	Viscosity /cpoise
15.0	1.139	22.0	0.9548
15.2	1.133	22.2	0.9503
15.4	1.127	22.4	0.9458
15.6	1.121	22.6	0.9414
15.8	1.115	22.8	0.9370
16.0	1.109	23.0	0.9326
16.2	1.103	23.2	0.9282
16.4	1.097	23.4	0.9239
16.6	1.092	23.6	0.9196
16.8	1.086	23.8	0.9154
17.0	1.081	24.0	0.9111
17.2	1.075	24.2	0.9069
17.4	1.069	24.4	0.9028
17.6	1.064	24.6	0.8986
17.8	1.059	24.8	0.8945
18.0	1.053	25.0	0.8905
18.2	1.048	25.2	0.8864
18.4	1.043	25.4	0.8824
18.6	1.037	25.6	0.8784
18.8	1.032	25.8	0.8745
19.0	1.027	26.0	0.8705
19.2	1.022	26.2	0.8666
19.4	1.017	26.4	0.8628
19.6	1.012	26.6	0.8589
19.8	1.007	26.8	0.8551
20.0	1.002	27.0	0.8513
20.2	0.9971	27.2	0.8476
20.4	0.9923	27.4	0.8438
20.6	0.9975	27.6	0.8401
20.8	0.9827	27.8	0.8364
21.0	0.9780	28.0	0.8328
21.2	0.9756	28.2	0.8291
21.4	0.9686	28.4	0.8255
21.6	0.9640	28.6	0.8220
21.8	0.9594	28.8	0.8184

APPENDIX 2. Extraction of a 1 litre water sample through a 500 mg C8 SPE column, for permethrin analysis

1. Preparation stage

Ensure that all glassware, tubing, connectors, reservoirs and taps are well cleaned with three washings of pesticide grade acetone, followed by three washings of pesticide grade hexane and finally rinsed with HPLC grade water.

Allow to dry.

Use the same washing techniques for the 'ports' of the SPS-24 unit (Vac Elut). (Lid of the SPS 24 unit set to 'WASTE'.)

Repeat for the ports on the Baker SPE-10 unit.

2. Extraction stage

Condition each of the extraction columns with 2 to 3 ml of HPLC grade methanol.

Draw the methanol through the column, under low vacuum, until the column is half full. Take about 1 minute for this stage. Then leave the column soaking in the methanol for 5 minutes.

Take 5 ml of HPLC grade water and place onto the column in three batches. Draw the water through the column slowly (1 to 2 ml min^{-1} for this stage).

Repeat with the next 2 ml of water etc. Do **not** allow the column to dry out at any time during this conditioning stage.

Finally, half fill the column with the HPLC grade water, to prevent any accidental drying out. Connect the reservoirs and tubing to the columns and start the extraction. Aim for a flow rate of 10 ml min^{-1} (about 20"Hg). When the water samples have been extracted, rinse out the sample bottles with 4 x 50 ml of HPLC grade water and take up the washings through the tubing, onto the columns. Reservoirs also rinsed out with 5 ml of water.

3. Drying stage

Place a C8 guard column on top of each of the extraction column. Dry the column by drawing air through for 20 min at maximum air flow.

4. Elution stage

Transfer the extraction columns to the Baker SPE-10 unit, and elute with about 2.5 ml of HPLC grade methanol. Use 4 ml screw capped vials, as the collection vessels. Apply low vacuum and adjust flow rate to approximately 1 ml min^{-1}.

Increase the vacuum when the column is empty, to remove any remaining methanol.

Store all samples in the dark in the fridge at 4^0C.

Evaporate the sample to dryness using a gentle stream of dry nitrogen gas over the solvent surface. Add internal standard (15 μl of 20 mg l^{-1} phosalone) and reconstitute with 2 ml of 5% acetone in hexane. Mix thoroughly before glc analysis.

APPENDIX 3. Chromatography conditions used in the analysis of permethrin.

Configuration

Injector	-	Split/splitless
Column	-	DB5 Jones Chromatography
Detector	-	ECD (Electron Capture Detector)

Oven conditions

Oven temperature (^{0}C)	50	170	240	280
Isothermal time (min)	2.0	0.0	7	2
Ramp rate (^{0}C min^{-1}	30.0	10.0	2.0	

Injector conditions

Temperature	310^{0}C
Splitless for 30 seconds	

Detector condition

Temperature	350^{0}C

Gases

Makeup:	N_2
Carrier:	He
Septum purge	~ 5 ml min^{-1}
Flow rate	~50 ml min^{-1}


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