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

RESULTS OF THE PILOT STUDY - RECONSTITUTION OF WATER SAMPLES

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The Institute of Freshwater Ecology is part of the Terrestrial and Freshwater Sciences Directorate of the Natural Environment Research Council.

ABSTRACT

1. Two pesticides of contrasting lypophilic nature, simazine and permethrin, were added to river water (R. Frome, Dorset, U.K.) immediately prior to freeze-drying.

2. Two methods of reconstitution of the river water were tested. The recovery of the inorganic solutes was good ie generally greater than 80%.

3. The reconstituted waters were analysed in triplicate for permethrin and simazine for 3 sub-samples of each water. The results indicate reasonable recoveries of both simazine and permethrin.

4. Good agreement between the results of the triplicate analysis of each of the sub-samples was obtained for simazine. The concentration of simazine in the 2 reconstituted waters was found to be 0.025 and 0.030 mg dm^{-3} with satisfactory homogeneity within each batch.

5. The results for permethrin were more variable than for simazine. The concentration in the 2 reconstituted waters was found to be 3.21 and 2.08 μg dm^{-3}. Reconstitution with CO_2 produced a homogeneous sample whereas the second method, using HCl, indicated the possibility of some heterogeneity.

CONTENTS

1. Preparation of river water samples

Three litres of R. Frome water were collected from East Stoke weir (National Grid Reference SY 868868) at 10.00 hours on 9 October 1989. The samples were returned to the laboratory where the pH, temperature and electrical conductance were measured. All three samples were then filtered through a 0.45 μm cellulose nitrate membrane filters (Sartorius 11306 No. 780 2110604209). One of the samples was analysed for major-ions and the results are shown in Table 1. The remaining 2 samples, hereafter referred to as Samples 1 and 2, were stored at 5°C in the dark and with a CO_2 head-space.

2. Preparation of freeze-dried samples

The river water was allowed to return to room temperature before being spiked with cis-permethrin and simazine. This was done by the addition of 1 ml of 5 mg dm^{-3} simazine and 1 ml of 5 mg dm^{-3} cis-permethrin dissolved in acetone. The bottles were shaken for 15 minutes and then frozen overnight. The concentration of simazine and permethrin in the river water was 0.05 mg dm^{-3} and 5 μg dm^{-3} respectively. These values were selected to allow for potential losses on freeze-drying and to obtain concentrations in the reconstituted water of at least 10 μg dm^{-3} for simazine and 1 μg dm^{-3} for permethrin. The frozen solids were then freeze-dried using an Edwards Modulo 4K machine. The drying took approximately 48 hours. The freeze-dried solids were transferred from the freeze-drier containers to 1 litre pyrex bottles with PTFE screw caps and stored at 5°C in the dark until they were reconstituted.

A small amount of solids remained in the freeze-drier containers after transfer to the 1 litre bottles. It was decided to recover this material separately to permit an assessment of the loss of inorganic solids and if necessary the loss of pesticides. Two procedures were used to do this:

(a) **Sample 1.** The freeze-drier containers were rinsed with MeOH and the sample rotary-evaporated to dryness. The distillate was stored in the dark at 6°C. The solid material was dissolved in 100 ml of 0.01 M HCl and the calcium concentration was determined.

(b) **Sample 2.** The freeze-drier containers were washed with 490 mls of 0.01 M HCl to dissolve the residual solids and the calcium concentration was determined.

3. Reconstitution of Sample 1.

Sample 1 was reconstituted on 1.11.89 using single distilled water with CO_2 gassing. The details of the method are as follows:

3.1 1 litre of single distilled water was added to the freeze-dried solids in the 1 litre pyrex vessel used for storage. A PTFE magnetic bar was added.

3.2 A stream of CO_2 gas was passed through the solution at a rate of ≈60 ml min^{-1} whilst the solution was stirred.

TABLE 1. Comparison of R. Frome water and reconstituted waters

Sample	Conductivity† at 25°C /μS cm^{-1}	pH	Alkalinity meq dm^{-3}	Na^+	K^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}	Cl^-	NO_3^-	SiO_2	Fe μmol dm^{-3}	PO_4
				mmol dm^{-3}									
R. Frome 9.10.89	546.0	8.14	4.36	0.60	0.06	2.30	0.08	0.43	0.56	0.27	0.06	0.54	8.1
Reconstitution 1	437.5	8.40	3.45	0.47	0.05	1.75	0.07	0.33	0.28	0.26	0.05	ND	5.4
Reconstitution* 1	470.0	8.40	3.71	0.50	0.05	1.88	0.08	0.35	0.30	0.28	0.05	ND	5.8
% Recovery	86	-	85	83	83	82	100	81	54	104	83	0	72
Reconstitution 2	1810	8.2	1.25	7.22	0.04	1.57	0.06	0.30	-	0.21	0.02	ND	4.6
Reconstitution* 2	-	8.2	1.25	7.22	0.05	1.94	0.07	0.37	-	0.26	0.02	ND	5.7
% Recovery	-	-	-	-	83	84	88	86	-	96	33	0	70

*: Corrected for losses on container walls obtained from the calcium analysis of separate washings.
ND: Not detected.

3.3 Measurements of the temperature and conductance were made from the time of addition of the water and then at 15 minute intervals. The conductance readings were corrected to 25°C (Wagner 1971). The results were as follows:

Time /min	Conductivity at 25°C /μS cm^{-1}
0	2.42
15	370.2
30	411.96
45	433.2
60	442.2
75	444.3
90	445.6
105	446.4

3.4 After 105 minutes, the pH was adjusted to 8.4 by replacing the CO_2 gas with nitrogen. The solution was then left stirring a further 15 minutes. The increase in pH was made to facilitate the dissolution of soluble silicon minerals and humic substances.

3.5 The visible absorbance of the water at 340 nm was measured to estimate the reconstitution of the humic substance. The absorptivity of the humic acid material in the R. Frome sediment had previously been determined as 6.4 $dm^3\ g^{-1}\ cm^{-1}$ at 340 nm. The absorbance of the original R. Frome water sampled on 9.10.89 was 0.0154 ± 0.0001 (SD) at 340 nm (SD: = Standard Deviation). The absorbance of the reconstituted water was 0.0134 ± 0.0015 (SD) indicating a recovery of absorbing species of ≈ 87% for Sample 1.

3.6 250 ml of the reconstituted water was analysed for major ions. The results are reported in Table 1.

3.7 Two of the three remaining 250 ml quantities were immediately extracted to isolate the pesticides. The remaining 250 ml was stored overnight at 5°C and in the dark with a CO_2 filled headspace and extracted during the following morning.

4. Reconstitution of Sample 2

Sample 2 was reconstituted 6.11.89 using 0.01 M HCl. The details of the method are as follows:

4.1 1 litre of 0.01 M HCl (prepared using BDH, ConVol standard) was added to the freeze-dried solid in the 1 litre Pyrex vessel used for storage. A PTFE magnetic bar was added.

4.2 The bottle was sealed with a PTFE screw cap and stirred for 1 hour. The solution cleared in this period.

4.3 The pH was adjusted from ≈ 1.9 to 8.2 by the addition of 1 M NaOH (AR grade). The conductance of the reconstituted sample was recorded (see Table 1).

4.4 The visible absorbance of the water at 340 nm was measured as described in §3.5. The absorbance was 0.0137 ± 0.0010 giving a recovery of absorbing species at 340 nm of ≃ 89% for Sample 2.

4.5 250 ml of the reconstituted water was analysed for major-ions. The results are reported in Table 1.

4.6 Two of the three remaining 250 ml quantities were immediately extracted for pesticides. The remaining 250 ml was stored overnight at 5°C and was extracted during the following morning.

5. Extraction procedure

Simazine and permethrin were extracted together using solid-phase extraction techniques (SPE). A Bond-Elut adsorption column containing octyl (C8) bonded phase silica as the adsorbent (Analytichem International Code P606303) was used. The method was as follows.

5.1 The C8 column was fitted to a Vac-Elut (Analytichem International, model AI6000) assembled and washed with 2-3 ml of HPLC grade MeOH. The column was left for 5 minutes in contact with MeOH. The column was not allowed to become dry.

5.2 Approximately 15 ml of HPLC grade water was passed through the column.

5.3 The column was then transferred to the entrance of a 250 ml capacity polycarbonate reservoir which could be evacuated as required. The top of the column was connected to the sample to be analysed using small bore PTFE tubing. The sample in the 1 litre bottle was stirred continuously using a PTFE magnetic bar and motor.

5.4 Approximately 250 ml of sample was then passed through the column at a rate of ≃ 5 min^{-1}. The volume which had passed through the column was calculated from the weight of water in the reservoir with appropriate buoyancy correction.

5.5 The column was washed with 20 ml of HPLC grade water and dried for ≃ 20 minutes at maximum air flow.

5.6 The column was then transferred to the Vac-Elut assembly and eluted with ≃ 2 ml of MeOH. The volume of eluate was calculated from the change in mass of the collecting vial and assuming a density of MeOH of 0.7910 g ml^{-1}. Precautions were taken to avoid evaporation of MeOH during and after weighing.

5.7 Extracts were stored in 4 ml PTFE screw-capped glass vials at 6°C before analysis by glc.

6. GLC analysis

The extracts were analysed using a Perkin-Elmer glc model 8700 fitted with a split/splitless injector ECD detector and PTV injector - NPD detector.

6.1 Analysis of simazine

Configuration

Injector	-	PTV	(Programmable Temperature Vaporizer)
Column	-	DB1301	Jones Chromatography
Detector	-	NPD	(Nitrogen Specific Detector)

Oven conditions:	Initial temperature	140°C for 1 minute
	First ramp	20°C per minute
	Isothermal	240°C for 7 minutes

Injector conditions:	Initial temperature	100°C for 1 minute in split mode
	Vaporization temperature	280°C for 5 minutes in splitless mode for 1 minute
	Final temperature	150°C

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

Analysis by external standard mixture of 5 mg dm^{-3} Simazine and 0.5 mg dm^{-3} cis-permethrin.

6.2 Analysis of Permethrin

Configuration:

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

Oven conditions:

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

Injector conditions:

Temperature 310°C
Splitless for 30 seconds

Detector condition:

Temperature 350°C

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

Analysis: Sample 1 by external standard. Sample 2 with external standard and internal reference.

7. Results of the glc analysis

The three extracts from Sample 1 were designated No. 1 BCR 1-3. Similarly, the extracts for Sample 2 were designated No. 2 BCR 1-3. The analysis of each extract was done in triplicate. The mean concentrations of each pesticide in each extract are reported.

7.1 Performance of the extraction and analysis procedures

Three 1 litre samples of filtered R. Frome water were spiked with simazine and cis-permethrin as described in §2. Each sample was then extracted using the method described in §5. Average recoveries of both pesticides were considered satisfactory.

Test number	Percentage Recovery of Simazine	Percentage Recovery of Permethrin
1	98.3	49.5
2	100.0	62.4
3	73.9	66.2
Mean percentage recoveries:	91%	59%

7.2 Results of the analysis of Sample 1

All extracts were analysed by glc in triplicate with an external standard. The calibration lines for both Permethrin and Simazine were linear over the relevant range of concentration. The concentration of pesticides in the MeOH extract are reported below together with the concentration in the reconstituted water. The values for the reconstituted water were corrected for losses associated with the extraction procedure (see average recoveries in §7.1) and loss of material when transferring freeze dried powder from the freeze-drier containers to the bottles used for the reconstitution (see Table 1). The overall recoveries are reported below. Typical chromatograms are shown in Figures 1 and 2.

7.2.1 *Results for Simazine*

Extract Number	Concentration in extract (mg dm^{-3})	Concentration in water (mg dm^{-3}) SD in brackets	Concentration in water after corrections for losses (mg dm^{-3})	Overall recovery %
NO1 BCR 1	2.95	0.0182 (0.0002)	0.023	46
NO1 BCR 2	3.01	0.0195 (0.0007)	0.024	48
NO1 BCR 3	3.30	0.0209 (0.0003)	0.027	54
Mean	N/A	0.0195	0.025	49
Standard Deviation	N/A	0.0011	0.002	3

N/A: not applicable because the volumes of water from which the pesticides were extracted varied.

7.2.2 *Results for permethrin*

Extract Number	Concentration in extract (mg dm^{-3})	Concentration in water ($\mu g\ dm^{-3}$) SD in brackets	Concentration in water after corrections for losses ($\mu g\ dm^{-3}$)	Overall recovery %
NO1 BCR 1	0.241	1.49 (0.09)	2.97	59
NO1 BCR 2	0.236	1.53 (0.12)	3.05	61
NO1 BCR 3	0.287	1.81 (0.19)	3.61	72
Mean	N/A	1.61	3.21	64
Standard Deviation	N/A	0.14	0.28	5.7

N/A: not applicable because the volumes of water from which the pesticides were extracted varied.

7.3 Results of the analysis of Sample 2

Same conditions of analysis as Sample 1 except that an internal standard was used in the permethrin analysis to improve the precision of the result.

7.3.1 *Results for Simazine*

Extract Number	Concentration in extract (mg dm^{-3})	Concentration in water (mg dm^{-3}) SD in brackets	Concentration in water after corrections for losses (mg dm^{-3})	Overall recovery %
NO2 BCR 1	3.68	0.0213 (0.0005)	0.027	54
NO2 BCR 2	3.72	0.0240 (0.0001)	0.030	60
NO2 BCR 3	4.09	0.0247 (0.0004)	0.032	64
Mean	N/A	0.0233	0.030	59
Standard Deviation	N/A	0.0015	0.002	4

N/A: not applicable because the volumes of water from which the pesticides were extracted varied.

7.3.2 *Results for Permethrin*

Extract Number	Concentration in extract (mg dm^{-3})	Concentration in water ($\mu g\ dm^{-3}$) SD in brackets	Concentration in water after corrections for losses ($\mu g\ dm^{-3}$)	Overall recovery %
NO2 BCR 1	0.250	1.45 (0.11)	2.28	46
NO2 BCR 2	0.239	1.53 (0.24)	2.40	48
NO2 BCR 3	0.163	0.99 (0.04)	1.55	31
Mean	N/A	1.32	2.08	42
Standard Deviation	N/A	0.24	0.38	7.6

N/A: not applicable because the volumes of water from which the pesticides were extracted varied.

8. Comparison of the composition of the reconstituted waters

8.1 Major-ion components

The method of reconstitution of the second sample precluded the comparison of recovery of Na^+ and Cl^- ions.

8.1.1 Both methods of reconstitution produced similar overall recoveries (see Table 1) for alkalinity as HCO_3^-, K^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, NO_3^- and phosphate (Soluble Reactive Phosphate, SRP). The recovery of calcium may be used to evaluate the efficiency of recovery of the other ions. The inorganic phosphorus concentration (SRP) is slightly lower than expected and the nitrate concentration is higher. If the first method of reconstitution is used the recovery of these ions may be estimated from the conductivity of the water.

8.1.2 The R. Frome water contains very low concentrations of iron. No Fe was detected in the reconstituted waters.

8.1.3 The first method of reconstitution gives a good recovery of Na^+ (83%) and relatively poor recovery of Cl^- (54%).

8.1.4 The recovery of dissolved Si was good for method 1 ie 83%, whereas for method 2 the recovery was poor (33%). This may reflect differences in the kinetics of dissolution of the silicon fractions in the different conditions of reconstitution.

8.1.5 The first method of reconstitution produces a freshwater that is similar in composition to the original river water. In contrast, the addition of HCl and NaOH in the second method produce a water of much higher conductivity ie 1810 $\mu S\ cm^{-1}$ compared with 546 $\mu S\ cm^{-1}$ for the original water.

8.1.6 Both methods produce good recoveries of organic solutes absorbing at 340 nm.

8.2 *Simazine*

The concentration of Simazine in both reconstituted freshwaters was very similar with good agreement between the results of the triplicate analysis of each extract. The mean concentrations and recoveries are as follows:

Reconstitution method	Mean concentration in water ($mg\ dm^{-3}$)	Overall recovery %
1	0.025 ± 0.002 (SD)	49
2	0.030 ± 0.002 (SD)	59

8.2.1 About 50% of the simazine is lost, either by adsorption to the glass container prior to extraction, or degradation during the processing. The recovery is considered large enough to permit the preparation of a matrix reference material.

8.2.2 There is evidence for a systematic increase in the simazine concentration for consecutive extractions within each sample. Even though the solution in the 1 litre bottle was well-stirred during the sampling for extraction, it appears that there is some inhomogeneity in the sample. This may be caused by particulate material containing adsorbed simazine remaining near the bottom of the flask and determined in the last extract ie NO1 BCR 3 and NO2 BCR 3. This effect is however small and may in the future be reduced by avoiding the possibility of the introduction of extraneous material into the sample eg via sensors such as pH and conductivity used in this pilot study.

8.3 Permethrin

The analytical determination of the pyrethroids is generally more difficult than for the organo-chlorine pesticides such as Dieldrin, Endrin and DDT. This is because of the lower sensitivity of the ECD detector to the compounds and hence lower response factors.

The concentration of cis-permethrin in Sample 1 was higher than in Sample 2. The results are summarized below:

Reconstitution method	Mean Concentration in water ($\mu g\ dm^{-3}$)	Overall recovery %
1	3.21	64
2	2.08	42

8.3.1 The standard deviation of the results of the triplicate analysis of Sample 1 extracts are similar to the standard deviation obtained for the entire sample. Hence no inhomogeneity in the sample could be quantified.

8.3.2 The third extract of Sample 2 produced a much lower concentration of permethrin that the other 2 extracts. The triplicate analysis of NO2 BCR 3 produced consistent results with a standard deviation of 0.04 $\mu g\ dm^{-3}$. The results suggest that some inhomogeneity in the sample existed.

8.3.3 The glc analysis of the extracts of Samples 1 and 2 using the ECD detector indicated the presence of some organics (not identified) which were not expected in the R. Frome water. These organics may have been present in the orginal R. Frome water or be contaminants introduced during the freeze-drying or reconstitution. These did not interfere with the analysis of permethrin or with the internal standard peak. They did not appear in the chromotograms obtained during the performance trials (§ 7.1) which suggests that they were contaminants. The source of the contaminants may be associated with the various sensors used to monitor the reconstitution ie pH and conductivity, which need not be used in the main stability study. The membrane filter and Bond-Elut column are not the source of the contaminants (see § 7.1).

9. Conclusions

9.1 The two pesticides studied were chosen because of their different physicochemical properties and concern for their effects on the ecology of rivers and lakes. Permethrin has a low solubility in water (20-70 $\mu g\ dm^{-3}$) and is lypophilic and readily associates with particulate material. In contrast, simazine is moderately water soluble (5 $mg\ dm^{-3}$) and also less likely to associate with particulate matter.

9.2 The first method of reconstitution ie with distilled water and CO_2, is considered to be superior to the second method because the reconstituted water is closer in composition to the original river water. The second method of reconstitution is however much easier to perform requiring only HCl and NaOH without the need for compressed gases.

9.3 The results for simazine, for both methods of reconstitution, are very optimistic with reasonable overall recoveries of simazine and good chromatography results.

9.4 The results for permethrin are less encouraging. The first method of reconstitution ie distilled water and CO_2, gave the best recovery of permethrin and satisfactory homogeneity of the sample. It is suggested that in future a preliminary analysis of the river water be undertaken before the water is freeze-dried to ascertain the presence of ECD active compounds.

APPENDIX 1

Chemicals

1. HPLC Grade Methanol, Rathburns

2. Acetone, Pesticide Grade, BDH Chemicals, Poole

3. C8 Bond-Elute, Analytichem International

4. HPLC grade water HiPerSolv, BDH Chemicals, Poole

5. All other chemicals AR grade, BDH Chemicals, Poole

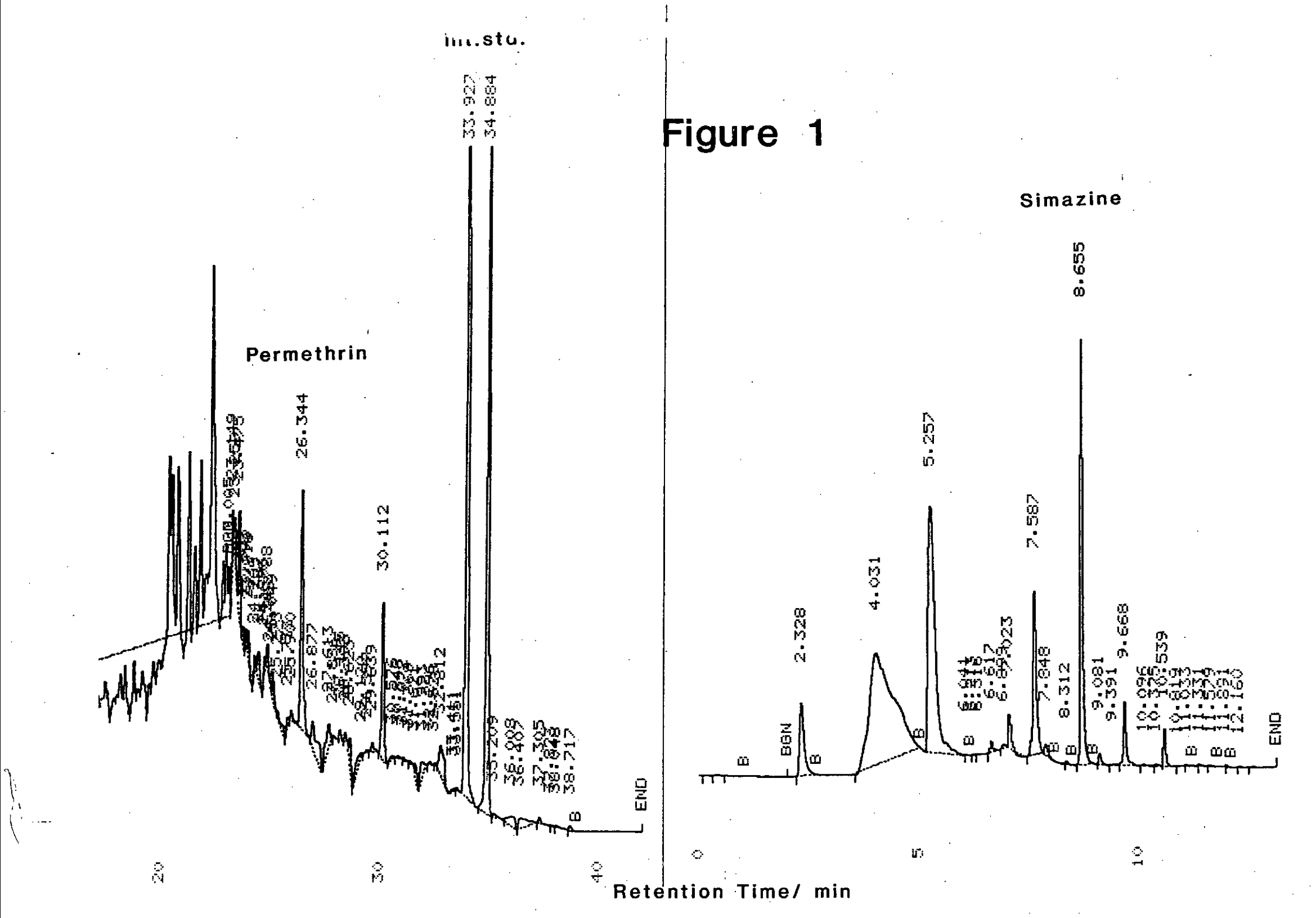
6. Simazine: 2-chloro-4,6-bis (ethylamino)-1,3,5- triazine, Standard Sample No. R2677 LOT P103, 99.9 ± 0.1 mole per cent. National Physical Laboratory, Middlesex.

7. CIS-Permethrin. 3-phenoxybenzyl-(IRS)cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate. Standard Sample NO. 149 R0341, 99.1 mass per cent. National Physical Laboratory, Middlesex.

REFERENCES

Wagner, I.W. (1971) Über die Temperaturabhängigkeit der elecktrischen Leitfähigkeit von Wässein. Vom Wass. 38, 27-48.

Figure 1



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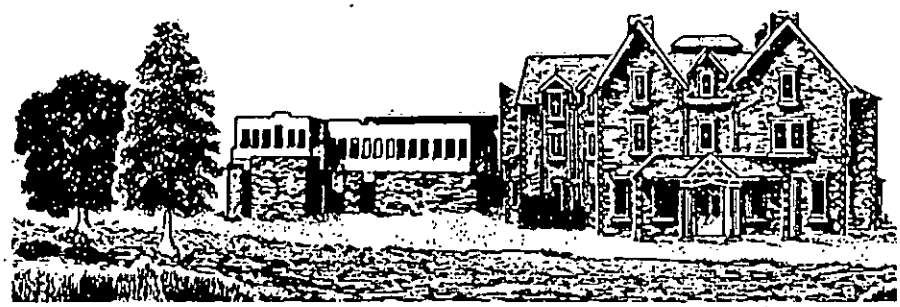

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