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LARGE ON-COLUMN INJECTIONS FOR THE GAS CHROMATOGRAPHIC DETERMINATION OF LOW-MOLECULAR-WEIGHT HALOCARBONS

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SUMMARY

The potential of on-column injections of up to 250 μl of pentane solution for the determination of low-molecular-weight halocarbons at extremely low concentrations has been investigated by using a wide-bore capillary column with an immobilized stationary phase. The relationship between pressure in the column and boiling point of the solvent shows that the actual boiling point is never exceeded during the chromatographic run. The dependence of resolution and peak height on the volume injected is shown. Linearity, precision and detection limits, determined at an injection volume of 100 μl, show that the technique is applicable to quantitative work.

INTRODUCTION

For the determination of low-molecular-weight halocarbons in different natural and waste waters, a simple and sensitive method was presented by Eklund *et al.*[1]. It utilized a pentane extraction, followed by a glass capillary gas chromatographic (GC) separation and electron-capture detection. This method has been used successfully in studies of distribution patterns of industrial effluents in coastal waters. Chloroform was used as a tracer of waste water from a paper and pulp mill and bromoform as a tracer of sea water used for cooling in a power plant[2]. The distribution of bromoform in the Arctic Ocean indicated the possibility of using this compound for deep-water formation studies[3]. However, there was still a lack of sensitivity in offshore studies of some of the halocarbons. As the extraction step had already been optimized, a change in the chromatographic part of the analysis was considered.

Different sampling techniques for applications for which a high sensitivity is crucial have been described. They are suitable for the analysis of very dilute samples, where increased amounts of sample components have to be introduced into the GC column. Problems connected with the solvent matrix have been circumvented by various approaches. For the determination of medium- to high-boiling compounds, a pre-vaporization of the solvent has been shown to be useful, as the losses of sample components are then not too serious. A slow injection of up to 250-μl samples into a cold injector with the split-valve open for venting of the solvent, followed by rapid heating of the injector, was used by Vogt and co-workers[4,5]. A similar approach was

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demonstrated by Poy *et al.*[6], who described a temperature-programmed vaporizer. Elimination of solvent can also be accomplished with the "moving needle" technique, introduced by Van den Berg and Cox[7]. However, Brötell *et al.*[8] showed that pesticides could be separated without previous venting of the solvent by slow, splitless injections of volumes of up to 50 μl of pentadecane solutions isothermally at 120°C. They also made use of a double injection technique, where up to 120 μl of pentadecane was injected prior to a hexane sample.

Dual-column systems for selective sampling were utilized by Schomburg *et al.*[9], who improved the techniques introduced by Deans[10] and German and Horning[11]. The stationary phase in the pre-column may cause retardation of unwanted components, *e.g.*, water when aqueous samples are to be injected directly. The analytical column can thereby be selected more independently of the sample matrix. Another mode of operation, heart cutting, makes it possible to select the parts of the chromatogram of interest for a separation in the second column.

For the analysis of pentane extracts, containing volatile halocarbons at very low concentrations, all the methods mentioned are unsuitable. A direct approach to attain high sensitivity would be on-column injection of the sample volume necessary for the detection of the trace components. However, it is generally assumed that the maximum amount that can be injected successfully into a column is a few microlitres. Nevertheless, some applications have been reported where the injection of 10 μl has been found to be useful. For quantitative determinations of halocarbons in sea water, 10 μl of a pentane extract were injected into an SE-52, barium carbonate-treated column[2,3]. Wang *et al.*[12] reported the injection of 10 μl of a hexane solution of a hydrocarbon mixture into a column with a bonded phase at an oven temperature of 120°C (isothermal). The same group[13] used 100-μl on-column injections of hexane solutions where the solutes were concentrated in a cold trap at the end of the column after the elution of the solvent. Separation was thereafter obtained by reversing the flow of carrier gas.

The aim of this work was to study the potential of even larger injection volumes of extract in order to increase the sensitivity and thereby the precision of the halocarbon determinations. Injections of up to 250 μl of sample, isothermally separated on a wide-bore column with an immobilized stationary phase, are shown. Linearity, precision and detection limits with 100-μl injections are reported. An application to a sea water sample is described.

EXPERIMENTAL

Equipment

A Carlo Erba Fractovap 4160 gas chromatograph, equipped with an air-cooled on-column injector and a ^{63}Ni electron-capture detector was used. Hydrogen at a linear velocity of 70 cm/sec was used as the carrier gas. The detector temperature was 275°C and the detector scavenger gas was argon–5% methane at a flow-rate of 60 ml/min. All chromatograms were recorded on a Perkin-Elmer 56 recorder with a chart speed of 6 cm/min.

The wide-bore column (Duran glass, 33 m × 0.50 mm I.D.) was prepared according to Grob *et al.*[14]. It was silylated with divinyltetramethyldisilazane, and the statistically coated stationary phase (SE-54, 0.4 μm) was immobilized by introducing

diisopropylbenzene peroxide (dicumyl peroxide). After about 100 injections, the column was washed with dichloromethane (10 ml) and pentane (10 ml). After washing, a column test[15] showed no significant reduction in the film thickness.

For injections, the removable needles of Hamilton gas-tight syringes (10, 100 and 250 μl) were exchanged for fused-silica capillaries (I.D. 0.10 mm, O.D. 0.20 mm).

Chemicals

A mixture of eight halocarbons dissolved in *n*-pentane, together with bromotrichloromethane as internal standard, was used in all the experiments. This mixture, the composition of which is shown in Table I, is referred to as H in the following discussion. The mixture contained $CHCl_3$, CCl_4 (both p.a. grade; Merck), CH_3CCl_3 (Fisher Scientific), $CHClCCl_2$ (analytical-reagent grade; Mallinckrodt), $CHBrCl_2$ (purum grade; Fluka), $CBrCl_3$ (practical grade; Eastman), $CHBr_2Cl$ (practical grade; Fluka), CCl_2CCl_2 (Uvasol grade; Merck) and $CHBr_3$ (reinst grade; Merck), all dissolved in *n*-pentane (p.a. grade; Merck).

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

In optimizing the gas flow-rate and the oven temperature, a compromise was made in order to obtain a chromatogram from which all the halocarbons in Table I could be quantitatively determined within an acceptable analysis time. The GC parameters were chosen as follows: carrier gas pressure, 0.35 kg/cm² above atmospheric pressure, which gave a flow-rate of 70 cm/sec; oven temperature, 40°C (isothermal); purge gas flow-rate, 60 ml/min; and speed of injection, 10 μl/sec.

Gas chromatograms of three separations, representing different injection volumes, are shown in Fig. 1a–c. All of them were prepared under the same chromatographic conditions. Injection of small volumes (2 μl, Fig. 1a) showed slight broadening of the leading edges of the peaks in the earliest part of the chromatogram. On the other hand, when large amounts (250 μl, Fig. 1c) were injected, the same kind of

TABLE I

COMPOSITION OF THE HALOCARBON MIXTURE IN *n*-PENTANE SOLUTION, REFERRED TO IN THE TEXT AS H

Peak No.	*Compound*	*Concentration (μg/l)*
1	$CHCl_3$	3.6
2	CH_3CCl_3	1.0
3	CCl_4	0.4
4	$CHCl=CCl_2$	1.4
5	$CHBrCl_2$	1.2
i.s.*	$CBrCl_3$	1.0
6	$CHBr_2Cl$	0.8
7	$CCl_2=CCl_2$	0.8
8	$CHBr_3$	1.7

* Internal standard.

distortion appeared only in the later peaks. Otherwise, no deterioration (*e.g.*, double peaks) was observed. As a comparison a 2-μl split-injection of an eight times more concentrated sample was made at the same oven temperature (Fig. 1d).

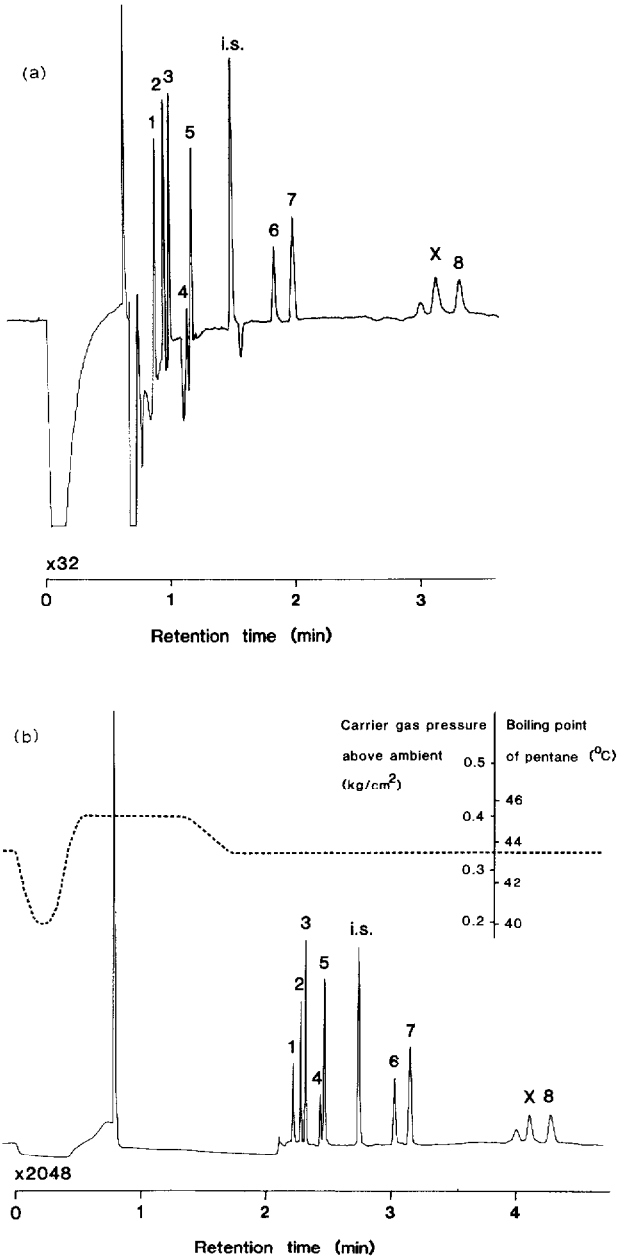

Fig. 1.

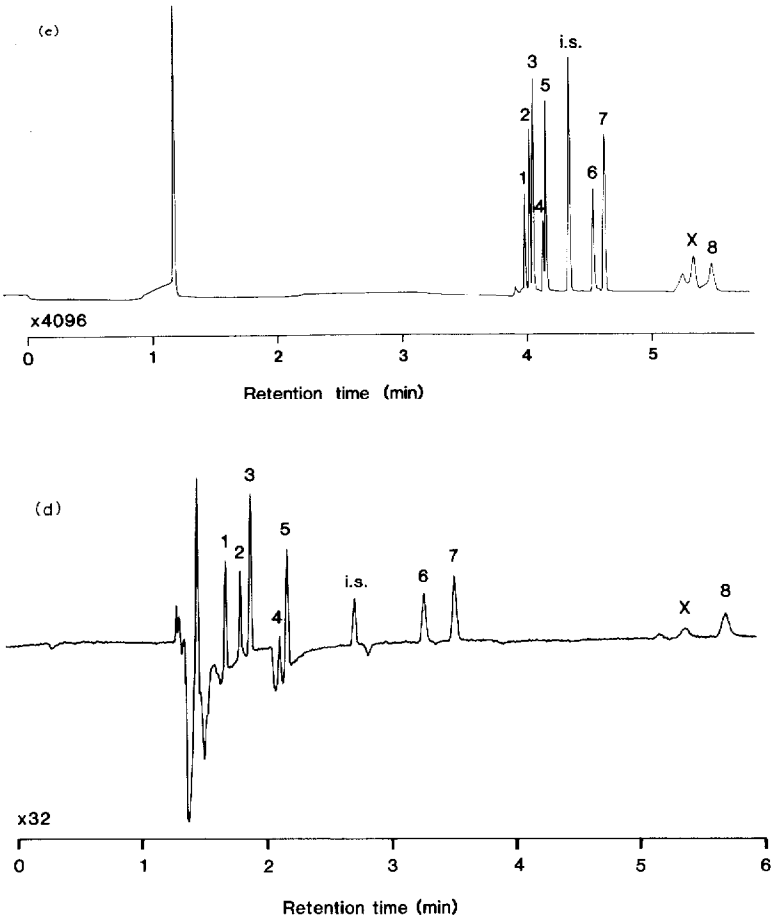

Fig. 1. Gas chromatograms of a halocarbon mixture in *n*-pentane solution (H); peak numbers as in Table I. GC conditions: column, 33 m × 0.5 mm I.D., SE-54, immobilized; column temperature 40°C (isothermal); carrier gas, hydrogen at 70 cm/sec; electron-capture detector temperature, 275°C; purge gas, argon–5% methane at a flow-rate of 60 ml/min. (a) Cold on-column injection of 2 μl of solution. (b) Cold on-column injection of 100 μl. The relationship between the excess pressure above 1 atm., read from the carrier gas manometer, and the boiling point of *n*-pentane is plotted. In this range, the relationship is almost linear. (c) Cold on-column injection of 250 μl. (d) Split-injection of 2 μl of a solution with eight times the concentrations used in (a)–(c) (8 × H). Splitting ratio, 1:30; carrier gas flow-rate, 50 cm/sec; injector temperature, 250°C.

Pressure–boiling point relationship for the solvent

The pressure variations read from the carrier gas manometer during the analysis are plotted in Fig. 1b. During the injection, the pressure drops because the injector valve is opened. Then, the pressure increases owing to the evaporation of the solvent, and finally, it slowly decreases to the original pressure as the solvent leaves the column.

The pressure plot in Fig. 1b also represents the variation in the boiling point of the solvent, *n*-pentane, during the analysis. The boiling point is higher than the

oven temperature (40°C) throughout the analysis. The relationship between the boiling point and the pressure was calculated from a modified Clausius–Clapeyron equation[16]:

$$\log p = 2.8808 - \frac{4.6\,(36.07 - t)}{273.1 + t - 0.15\,(36.07 - t)}$$

where p is the observed pressure (mmHg), t is the actual boiling point (°C), 4.6 is the entropy of vaporization (cal/mol · K) for *n*-pentane at 760 mmHg and 36.07 is the boiling point (°C) of *n*-pentane at 760 mmHg.

During the optimization of the temperature and pressure conditions it was observed that, whenever the oven temperature was about the same as or higher than the actual boiling point, problems arose, such as chromatograms with irreproducible peak shapes and retention times as well as backflushing in the injector.

Resolution versus volume injected

For three pairs of peaks in different parts of the chromatogram, CH_3CCl_3–CCl_4, $CHBr_2Cl$–CCl_2CCl_2 and X (unknown)–$CHBr_3$, the resolution was plotted *versus* the volume injected (Fig. 2). In general, no drastic differences were observed up to 100 µl. Even when 250 µl were injected, the decrease in resolution was moderate. However, the severe distortion of the last pair of peaks made the calculation of the resolution questionable for 250-µl injections. The resolution (R) was calculated according to the equation $R = \Delta t_R/(2\sigma_I + 2\sigma_{II})$, where Δt_R is the difference in retention times for peaks I and II, and σ is the standard deviation of each peak. For a Gaussian peak 2σ is the peak width at 0.67 × peak height.

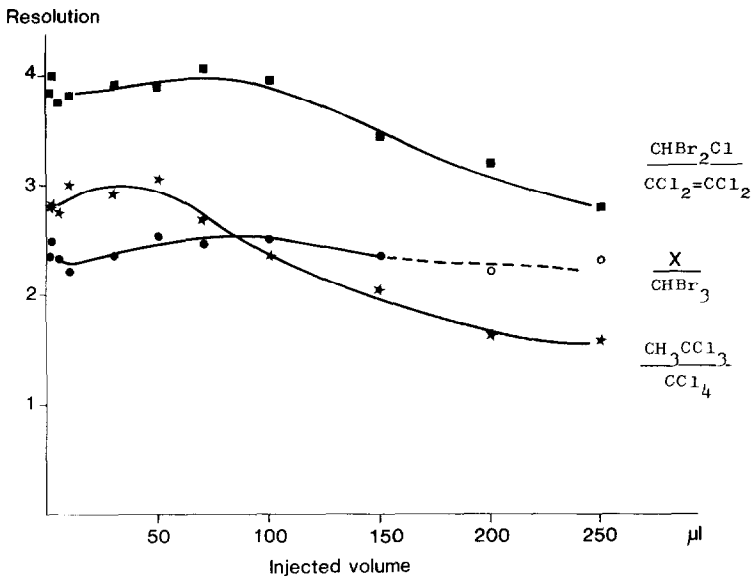

Fig. 2. Resolution *versus* volume injected for three pairs of peaks. GC conditions as in Fig. 1.

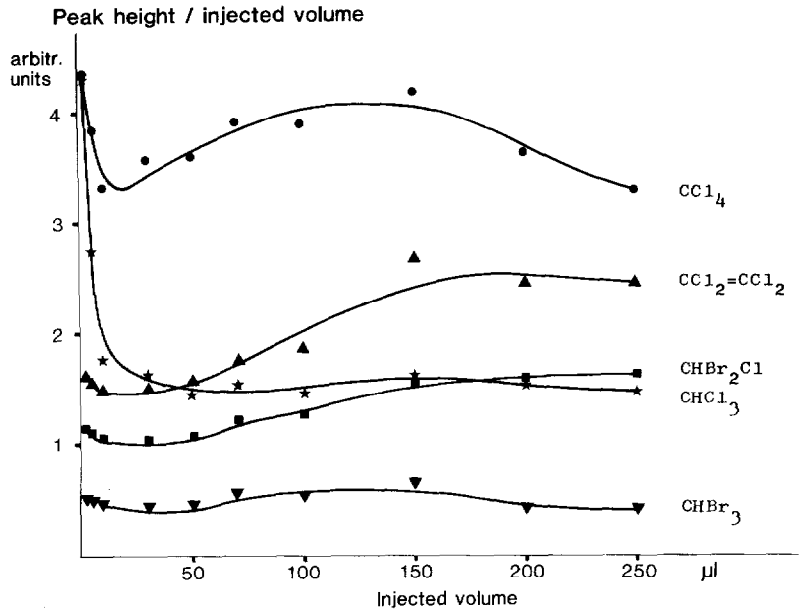

Fig. 3. Peak height per microlitre of sample injected *versus* volume injected for five of the halocarbons. GC conditions as in Fig. 1.

Peak height versus volume injected

The peak height per microlitre of sample injected was plotted against the injected volume for five of the halocarbons (Fig. 3). Injections of up to 30 µl resulted in a considerable decrease in the relative peak heights of the earliest peaks. This could be caused by a change in the detector response due to the large amount of *n*-pentane passing the detector just before these peaks. The same effect should then be less significant for the later peaks, and this corresponds to the results obtained. Measuring the peak areas gave the same results.

The greater the amount of solution injected, the more condensed is the chromatogram after the solvent. This is reflected in fairly constant peak heights, although the resolution is diminished. This could be a result of the decrease in effective column length as the flooded zone expands with increasing sample volumes.

Optimal volume for injection

From the results in Figs. 1–3, the injection of 100 µl was found to be a practical compromise for further investigations.

Linearity

In Fig. 4, the mean values of peak heights from three 100-µl injections are plotted against halocarbon concentration, all peak heights being normalized to the internal standard peak height. The halocarbon mixture H in Table I was used as a stock solution, and the concentrations in Fig. 4 are given relative to those in H. The intercepts reflect impurity levels in the solvent. A good linear relationship with a correlation coefficient higher than 0.999 was obtained for most of the compounds.

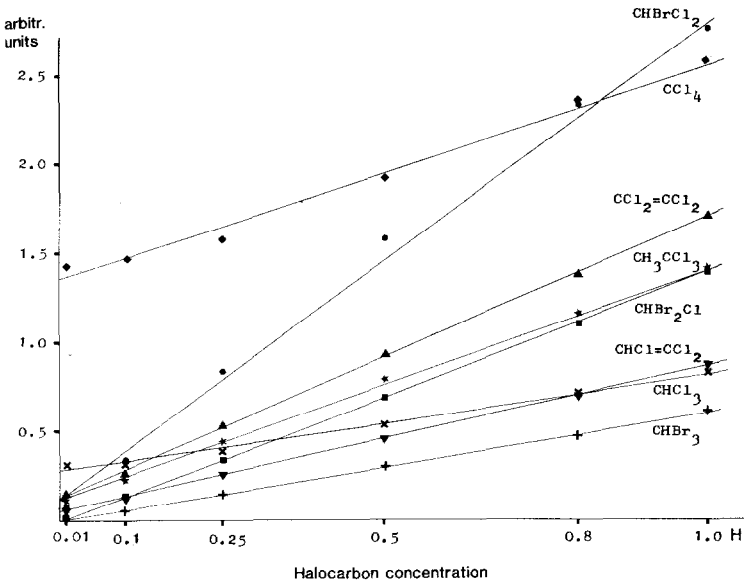

Fig. 4. Peak heights normalized to the internal standard, plotted against the concentrations in the solutions injected. The concentrations are given relative to the halocarbon mixture in Table I (H), which was used as a stock solution. Cold on-column injections of 100 μl under GC conditions as in Fig. 1. The intercepts reflect impurities in the *n*-pentane.

Reproducibility

In Table II, the relative standard deviations of absolute peak height measurements of five subsequent 100-μl injections are given. It was observed that the reproducibility was better for these large injection volumes than for volumes less than 10 μl.

Detection limits

The detection limits presented in Table III were calculated on the basis of a

TABLE II

REPRODUCIBILITY OF PEAK HEIGHTS

Five subsequent injections of 100 μl of pentane solution under optimized GC conditions.

Peak No.	*Compound*	*Relative standard deviation (%)*	*n*
1	$CHCl_3$	0.80	5
2	CH_3CCl_3	1.9	5
3	CCl_4	1.9	5
4	$CHCl=CCl_2$	2.4	5
5	$CHBrCl_2$	2.0	5
i.s.	$CBrCl_3$	1.2	5
6	$CHBr_2Cl$	1.4	5
7	$CCl_2=CCl_2$	2.3	5
8	$CHBr_3$	2.0	5

TABLE III

DETECTION LIMITS IN *n*-PENTANE SOLUTION CALCULATED FROM THE DETECTOR RESPONSE WITH A SIGNAL-TO-NOISE RATIO OF 5:1

Volume injected: 100 μl. Estimated limits for sea water samples and sensitivity improvements (*cf.*, refs. 2 and 3).

Peak No.	*Compound*	*Detection limit in n-pentane (ng/l)*	*Detection limit in sea water (pg/l)*	*Relative increase in sensitivity (×)*
1	$CHCl_3$	6	150	20
2	CH_3CCl_3	0.7	8	20
3	CCl_4	1	10	5
4	$CHCl=CCl_2$	2	30	15
5	$CHBrCl_2$	0.4	6	–
6	$CHBr_2Cl$	0.6	9	75
7	$CCl_2=CCl_2$	0.5	6	25
8	$CHBr_3$	3	50	50

signal-to-noise ratio of 5:1 for 100-μl injections. This is, however, an ideal situation for a pure solvent. Further, experience has shown that for some of the compounds contamination of the solvent from the laboratory atmosphere can be significant (*cf.*, Fig. 4). Nevertheless, the sensitivities for the different compounds obtained in earlier work[2,3] were improved by factors between 5 and 75. The detection of halocarbons in water samples is then possible at low picogram per litre levels.

Applications

Preliminary data show that direct on-column injection of large volumes (≥

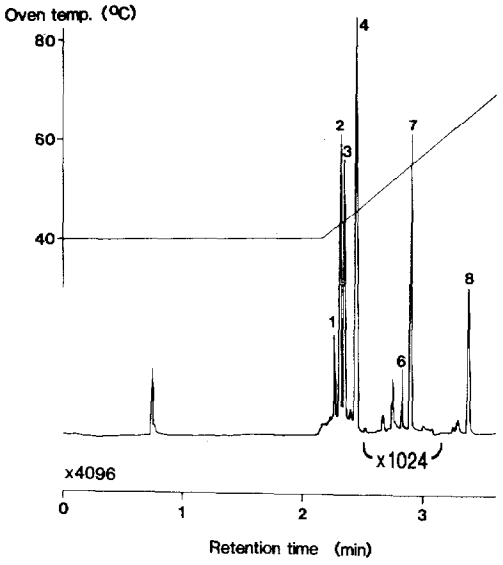

Fig. 5. Chromatogram of a sea water sample from the Swedish West Coast. Peaks (concentration in sea water, ng/l): 1 = $CHCl_3$ (65); 2 = CH_3CCl_3 (16); 3 = CCl_4 (1.8); 4 = $CHCl=CCl_2$ (30); 6 = $CHBr_2Cl$ (0.39); 7 = $CCl_2=CCl_2$ (2.8); 8 = $CHBr_3$ (27). The upper curve represents the temperature programme.

100 μl) is applicable to temperature programming without use of a cold trap. As an example, a temperature-programmed analysis of a sea water sample from the Swedish West Coast is shown in Fig. 5. A 100-ml water sample was extracted with 1 ml of *n*-pentane, and 100 μl of extract were injected. Application of this technique to other solvents and solutes will be presented elsewhere.

The use of large injection volumes is, of course, limited to some special applications. For dirty samples, the presence of high-molecular-weight compounds, which are deposited in the column inlet, may impair the chromatographic performance. On the other hand, columns with immobilized stationary phases can be restored by washing. This study has dealt with pure *n*-pentane solutions. Even after hundreds of large-volume injections, no deterioration of the column was observed. The main limit is set by the solvent purity, *i.e.*, the detection limits of some of the compounds are set by the background content in the solvent, rather than by the detector response.

CONCLUSION

The feasibility of injecting large amounts (up to 250 μl) of *n*-pentane solutions directly on-column has been demonstrated. As the sample volume was increased, the time of analysis was only slightly increased and the decrease in resolution was moderate. Determinations of halocarbons in *n*-pentane solution, after the injection of 100 μl, showed high precision and the sensitivity was improved by factors between 5 and 75.

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