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Recovery and Reproducibility in Determination of Volatile Halocarbons in Water and Blood

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Electron capture gas chromatography and mass spectrometry have been used earlier to detect volatile halocarbons in serum and tap water (Dowty *et al.* 1975, Eklund *et al.* 1978, Vogt *et al.* 1980) and especially chloroform in serum samples through purging (Pfaffenberger *et al.* 1980, 1980a, Pfaffenberger and Peoples 1982). We have developed our own modification of the methods to analyse volatile halocarbons through eluation with n-pentane in water, solutions made with water and blood samples. The aim with the present study has been to study the recovery and reproducibility of the earlier presented method (Kroneld and Reunanen 1983).

MATERIALS AND METHODS

The linear dynamic range and detection limit during the analysis are shown in Table 1. The methods used for the analysis were both rapid and specific for the different compounds studied. The chromatographic system is based on the resolving power of the glass capillary column and the high sensitivity of electron capture detection. Low column bleed and decreased band spreading with capillary columns enhance the sensitivity at very low levels. The relative standard deviation for calibration of standard mixtures within the linearity ranges was itself determined to be less than 1 %, as measured from 25 extractions on each of seven concentration levels.

Special experiments to determine recovery or extraction efficiency of volatile halocarbons were performed on both water and blood samples. The quantification of the methods was also tested with spiked samples. Bottles of 500 ml were filled with well-water samples shown to be free of interfering compounds. They were sealed with septums, ensuring no air bubbling inside. A 5 µl portion of an ethanol solution, consisting of a known concentration of the reference halocarbons, was then injected. The bottles were left to stand for one hour until analysis. For serum, plasma and blood cells, the test samples were prepared in a similar way to the calibration specimens. Such series were made to determine within-day and from day-to-day variation.

The results given in the text and tables are presented as arithmetic means and standard deviation values. The Student's t-test was used to analyse the significance of the differences between samples. A probability level of $p < 0.05$ was considered as statistically almost significant, $p < 0.01$ as significant and $p < 0.001$ as highly significant.

RESULTS AND DISCUSSION

The recovery or extraction efficiency of volatile halocarbons from water and both plasma and blood cells was analysed. In Table 2 the recovery in water is described as a function of amount of n-pentane used as an extraction solvent. There was a highly significant increase in the eluation process to 5 and 10 ml, but the increase was not significant from 5 to 10 ml. Evidently a water pentane ratio lower than 20 means a dilution of the extract. The relative standard deviation for the extraction procedure was less than 5 %, as determined from 12 extractions. This includes the within-day and day-to-day variation and the relative standard deviation for the split injection, which was measured to 2 % from 18 injections. A higher sensitivity is thus received using a higher water-to-pentane ratio. Despite the lower extraction efficiency, the concentration of the extractable compounds in the extract is higher.

The recovery as a function of extraction time in water (Table 3) was also analysed. There was a significant increase in the eluation process to 5 and 10 minutes. Continued extraction or shaking of the test tubes for more than 5 minutes did not evidently increase the recovery in a significant way. The relative standard deviation for the extraction procedure was less than 5 %, as determined from 14 extractions.

In Table 4 the recovery from plasma and cells is described as a function of extraction solvent. There was a significant increase in the eluation process to 100 and 200 µl. The relative standard deviation for the extraction process was less than 5 %, as determined from 21 extractions. This includes the within-day and day-to-day variation and the relative standard deviation for the split injection, which was measured

Table 1. Linear dynamic range, relative response, sensitivity and approximative detection limit during the analysis for the volatile halocarbons studied

Substance	linear range peak height ratio (mm)	dynamic conc. (µg/l)	relative response peak height ratio (mm)	Sensi-tivity (µg/l)	Approximative detection limit water (µg/l)	blood (nmol/l)
$CHCl_3$	0.07 -4.6	0.5 -100.0	1.1	0.05	2.5	20 (2 µg/l)
$CHBrCl_2$	0.1 -5.8	0.1 - 20.0	7.8	0.01	0.5	1 (0.1 µg/l
$CHBr_2Cl$	0.07 -4.2	0.1 - 20.0	5.5	0.01	0.5	1 (0.1 µg/l
$CHBr_3$	0.01 -1.2	0.045- 9.0	1.3	0.004	0.2	
$CHCl=CCl_2$	0.003-0.14	0.01-- 2.0	2.1	0.001	0.05	
CCl_4	0.06 -2.8	0.01 - 2.0	29.4	0.001	0.05	
$CBrCl_3$	0.01 -0.58	0.12 - 4.0	3.2	0.002	0.1	
$BrCH_2CH_2Br$	0.005-0.32	0.08-- 6.6	1.1	0.004	0.2	
$Cl_2C=CCl_2$	0.03 -1.2	0.02 - 4.0	6.8	0.002	0.1	
CH_3CCl_3	0.01 -0.68	0.013- 2.6	7.5	0.001	0.05	
C_4H_9I = S			1.0	0.02	1.	5.5

Table 2. Recover (%) in 100 ml water sample as a function of the amount of extracted solvent

Substance n = 12	2.5 ml	5.0	10.0
$CHCl_3$	64.2±1.1	82.3±1.6	82.5±1.8
$CHBrCl_2$	63.3±0.8	88.6±2.0	88.9±0.7
$CHBr_2Cl$	59.3±2.1	84.2±1.8	84.7±0.9
$CHBr_3$	57.1±1.3	86.3±1.0	86.4±1.1
$CHCl=CCl_2$	54.3±1.6	86.9±0.7	87.4±0.6
CCl_4	64.2±0.4	86.4±0.7	88.1±0.5
$CBrCl_3$	63.1±0.7	85.3±0.8	87.1±1.1
$BrCH_2CH_2Br$	61.3±1.2	86.2±0.8	86.8±0.6
$Cl_2C=CCl_2$	53.1±1.4	87.0±1.3	87.3±1.1
CH_3CCl_3	49.6±1.3	82.8±1.6	83.0±1.4
		$p < 0.001$	N.S. (5-10.0) $p<0.001$ (2.5-10.0)

N.S. = not significant

Table 3. Recovery (%) in 100 ml water sample as a function of extraction time.

Substance n = 14	2 min	5 min	10 min
$CHCl_3$	70.1±0.7	82.4±1.2	83.5±1.1
$CHBrCl_2$	72.2±0.5	86.6±1.8	87.5±1.3
$CHBr_2Cl$	71.1±1.2	84.1±1.0	85.1±0.8
$CHBr_3$	66.3±0.7	86.2±0.9	86.6±1.1
$CHCl=CCl_2$	64.1±1.0	87.2±0.4	88.1±1.1
CCl_4	60.7±1.1	85.3±0.8	85.9±0.9
$CBrCl_3$	71.2±0.8	85.2±0.7	86.7±0.9
$BrCH_2CH_2Br$	59.3±1.0	86.1±1.1	87.0±1.2
$Cl_2C=CCl_2$	73.2±1.0	84.9±1.1	85.6±0.9
CH_3CCl_3	65.6±0.8	83.8±0.9	84.5±1.0
		9<0.001	N.S. (5-10) p<0.001 (2-10)

N.S. = not significant

Table 4. Recovery (%) from 1.1 ml blood sample as a function of the amount of extraction solvent.

Substance n = 21		50 µl	100 µl	200 µl
$CHCl_3$	plasma	50.0±1.1	60.1±0.8	60.8±2.3
$CHBrCl_2$		51.2±2.4	61.3±2.6	61.7±1.6
$CHBr_2Cl$		43.6±2.3	62.8±1.7	63.1±1.6
			$p \leq 0.05$	N.S.
$CHCl_3$	cells	52.2±2.1	60.2±2.2	62.1±2.6
$CHBrCl_2$		47.1±0.7	61.8±3.1	61.2±3.3
$CHBr_2Cl$		45.2±1.4	63.1±1.2	64.1±0.9
			$p<0.001$	N.S. (100-200), $p<0.001$ (50-200)

N.S. = not significant

Table 5. Recovery (%) from 1.1 ml blood sample as a function of extractions.

Substance n = 22		5	10	30	80	100	150
$CHCl_3$	plasma	28.1±1.6	38.2±1.2	50.0±2.3	58.3±2.4	61.2±1.2	62.3±3.2
$CHBrCl_2$		28.7±1.8	38.3±2.3	45.4±1.5	61.2±2.1	63.2±1.3	65.3±1.8
$CHBr_2Cl$		10.3±0.8	26.2±0.7	43.6±1.3	59.2±2.1 $p<0.05$	61.7±3.2 N.S.	63.6±2.4 N.S.
$CHCl_3$	cells	30.0±1.6		50.0±0.9	60.2±1.1	60.4±0.8	61.7±2.2
$CHBrCl_2$		26.1±1.3		50.0±0.8	62.3±0.9	63.3±1.4	65.7±1.7
$CHBr_2Cl$		21.2±1.2		45.1±1.7	60.2±0.9 $p<0.001$	61.7±1.6 N.S. (80-100) $p<0.001$ (30-100)	63.1±1.9 N.S. (80-150) $p<0.001$ (30-150)

N.S. = not significant

Table 6. Reproducibility of volatile halocarbon determination in test analysis from water and blood samples. The individual GC variations and the over-all precision are given.

Substance	water			serum, plasma			blood cells	
	added µg/l	determined		added nmol/l	determined		determined nmol/l	
		GC	over-all		GC	over-all	GC	over-all
$CHCl_3$	25.0	24.3±1.2	24.6±1.7	125.7	127.4±8.7	127.8±12.3	124.2±9.3	125.1±11.8
$CHBrCl_2$	5.0	4.9±0.3	5.2±1.2	18.3	18.8±1.0	19.1± 2.3	18.6±1.1	19.3± 1.9
$CHBr_2Cl$	5.0	5.1±0.2	5.2±0.8	14.4	14.6±1.1	14.8± 1.3	14.2±0.8	14.6± 1.4
$CHBr_3$	2.0	2.1±0.1	2.1±0.2					
$CHCl=CCl_2$	0.5	0.6±0.04	0.6±0.08					
CCl_4	0.5	0.4±0.06	0.6±0.07					
$CBrCl_3$	1.0	1.1±0.07	1.2±0.11					
$BrCH_2CH_2Br$	1.5	1.4±0.11	1.4±0.13					
$Cl_2C=CCl_2$	1.0	1.0±0.05	1.1±0.08					
CH_3CCl_3	0.5	0.5±0.03	0.5±0.07					

$p<0.001$ for all determinations

to be less than 2 %, as determined from 20 injections.

The recovery as a function of extractions from plasma and cells was also analysed (Table 5). There was a significant increase in eluation to an extraction number of 80, 100 and 150, both in plasma and cells. From 80 upwards, however, or turning the test tube until the phase had been passed through the tube, did not significantly increase the recovery. The relative standard deviation for the extraction procedure including within-day and day-to-day variation was less than 5 %, as determined from 22 extractions.

The relative standard deviation for extractions from water and blood samples was found to be less than 5 % for within-day and day-to-day variation. The losses of compounds owing to the extraction procedure were counteracted by calibrations. Quantification, on the other hand, had then to be based on comparison of peak height ratios between extracted compounds and the added internal standard with the same ratios in the standard mixture; account must also be taken of variations in GC and over-all. The variation in gas chromatography conditions and the over-all system regarding reproducibility was then tested with the results given in Table 6.

When the reproducibility counteracted by calibration was tested for GC variation and over-all precision for the whole system, it was found that the standard deviation was less than 10 %, measured from 18 extractions. This 10 % included not only daily and day-to-day variation, but also losses due to extraction and split injection. Statistical comparison with the hypothesis, that the measured values do not deviate from the added values, was found to be highly significant.

Repeated analyses of the spiked samples attested to the good reproducibility and accuracy of the tested method. The accuracy of the method was tested by means of spiking experiments.

The peaks analysed in this investigation with ECD-GC were also confirmed both with double columns giving equal retention times for the same peaks analysed and with mass spectrometry.

When analysing water and blood samples it was noted that the volatile halocarbons at the beginning of the extraction passed quite efficiently to the pentane phase. A balance between the two phases was achieved at a certain moment of extraction. To achieve good accuracy it was important to calibrate the system and determine the compounds at the equilibrium levels.

The extraction time needed for suitable recovery was found to be five minutes in water and ten to fifteen minutes for blood samples. 5 ml of pentane solvent for water to 100 ml water samples and 100 ul of pentane solvent to 1.1 ml blood samples was found to be satisfactory for the extraction procedure in this respect.

The standard deviation for recovery including daily and day-to-day variation from different samples tested was under five per cent. When calibrating the whole system, mean values of both several extractions and gas chromatographic determinations including daily and day-to-day variation have to be considered. The standard deviation for the over-all experimental errors was less than then percent in this respect.

However, for many halocarbons the detection limit is extremely low and therefore minor contaminations may interfere. Syringes, etc, which have been in contact with the reference halocarbons or with a concentrated solution of them should not be used in handling the samples, even if flushed with a solvent. At the beginning of the experiments the glassware (including the syringes) should be cleaned with soap and water, rinsed with distilled water, acetone, petroleum ether, pentane, and stored at 120°C for then to twenty hours prior to use. During the work each piece of apparatus should be used for a given purpose only.

Exposure through work is beside laboratory contamination also possible from dry-cleaning and degreasing agents such as perchloroethylene, a source of trichloroethylene with the major metabolite trichloroacetic acid, which in turn gives rise to serum chloroform (Pfaffenberger _et al_. 1980a). The individuals and patients in this study were, however, chosen so that possible exposure through work was avoided.

A different method for serum analysis, by purging the samples at 115°C for thirty minutes, has been developed and used in U.S.A. (Peoples _et al_. 1979, Pfaffenberger _et al_. 1980, 1980a). The method gave recovery values for chloroform between 90 and 110 %. Combs _et al_. (1982), on the other hand, have shown that no quantitative differences appeared whether heat was applied or not. Their analytical techniques had a relative standard deviation of less than 7.5 % for chloroform. The differences in recovery could then be counteracted quite well with calibration.

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