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## Note

# Headspace gas chromatographic analysis for determining low levels of chloroform in human plasma

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The presence of trihalomethanes (THM), particularly chloroform, in the water and air in indoor swimming pools has been confirmed in many countries [1–6]. Swimming pool waters are usually disinfected with sodium hypochlorite or dichloroisocyanurate; THM are produced during chlorination of natural waters containing organic substances [7, 8] and are released into the air above the water surface. In this way THM are inhaled by swimming pool visitors, mainly by agonistic swimmers, who breathe under stress for a long time directly at the water surface. As chloroform is classified as having sufficient animal evidence as a carcinogenic substance, but inadequate epidemiological evidence [9], it is advisable to perform direct measurement of this substance not only in water and air, but also in fluids, such as plasma, from exposed subjects.

There are few reports of the levels of chloroform in plasma; so far it has been measured in human blood by a modified purge–trap–desorb method [10, 11]. However, this procedure has shown some difficulties in quality control, so that more work needs to be done before it can be accepted for field use in monitoring exposure to low levels of chloroform vapour [12]. Perhaps this difficulty is due to the temperature of the purging device (115°C), which may convert chloroform precursors into chloroform. For instance, trichloroacetic acid (TCA), if present in plasma, is decomposed to chloroform at 90°C [13, 14]. In previous experimental trials we found that TCA and chloral hydrate, when added to a human plasma pool at increasing concentrations in the range 10–200 μg/l, were decomposed and converted into proportional amounts of chloroform at 60°C.

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In order to differentiate between circulating chloroform (present prior to analysis) and circulating TCA, the blood plasma may be decomposed in the presence of deuterated water ($^2H_2O$), as proposed by Pfaffenberger et al. [15]. The reactive intermediate, the trichloromethyl carbanion, abstracts deuterium from $^2H_2O$ and forms $C^2HCl_3$, which is easily distinguished from $CHCl_3$ by mass spectrometry. However, mass spectrometry is not suitable for a routine screening survey; for this reason we present here a headspace gas chromatographic technique, suitable for determining chloroform in human plasma, in order to perform a direct measurement in subjects exposed to low levels of chloroform in air. This procedure can be performed using the apparatus that is commonly used, in Italy at least, for the measurement of all volatile halogenated organics in waters.

## EXPERIMENTAL

### *Sample collection*

Blood was drawn by a physician at the swimming pool, a few minutes after the end of a training session, from 107 agonistic swimmers. Eighty blood samples were collected from adult volunteers, carefully screened among non-exposed subjects. A 5-$cm^3$ volume of blood was drawn from an arm vein with a 10-$cm^3$ disposable syringe and immediately placed in capped disposable tubes, containing $K_3$EDTA as anticoagulant. No difference appeared in preliminary experiments performed with glass syringes and glass tubes pre-treated at 400 °C for 2 h. The tubes were gently shaken, chilled in ice and kept cold until taken for analysis. As soon as possible the samples were centrifuged (10 min at 1000 *g*), then the plasma was drawn using an Eppendorf micropipette and transferred into 3.7-$cm^3$ borosilicate glass vials with hole caps sealed with Teflon-faced silicone-rubber septa (Supelco, U.S.A.).

The same kind of vials were used for the headspace analysis. The glassware was previously placed in a muffle furnace at 400 ± 5 °C for 2 h in order to purge it of volatile contaminants.

As chloroform is volatile, it is advisable to perform the analysis as soon as possible; however, in previous experimental trials we found that a plasma sample can be stored for fourteen days at −20 °C after collection without a decrease in the chloroform content. This was confirmed by analyses performed on the same sample within fourteen days; after this period we found a slow decrease in chloroform content.

### *Preparation of samples, standards and blanks*

Analytical-reagent grade materials were used.

Plasma samples, when kept in a freezer, must reach room temperature without being warmed in order to avoid loss of chloroform. To a 3.7-$cm^3$ vial were added 1.5 $cm^3$ of chloroform-free water, 2 g of magnesium sulphate heptahydrate ($MgSO_4 \cdot 7H_2O$) to obtain a saturated solution and 5 $\mu$l of *n*-octyl alcohol (diluted 1:4 in methanol) as an antifoam agent. Then 0.5 $cm^3$ of plasma was rapidly added and the vial was tightly closed.

A stock standard solution of chloroform was prepared by dissolving 10 $\mu$l of

chloroform (stabilized with 0.75% of absolute ethanol) in 14.81 $cm^3$ of methanol. A working standard solution was prepared by diluting a convenient aliquot of the stock standard solution in order to obtain 0.2 mg/l chloroform in methanol.

Calibration standards were prepared by adding a convenient aliquot of the working standard solution to a chloroform-free plasma solution diluted 1:4.

Blanks were prepared by filling vials with 2 $cm^3$ of chloroform-free water, 2 g of $MgSO_4 \cdot 7H_2O$ and 5 $\mu$l of antifoam solution.

*Gas chromatography*

A Varian 3700 gas chromatograph with a $^{63}Ni$ electron-capture detector was used. The stainless-steel chromatographic column (3 m×3.18 mm I.D.) was packed with 10% OV-1 on Chromosorb W AW (80–100 mesh). The carrier gas (nitrogen) flow-rate was 30 $cm^3$/min, oven temperature 70°C, inlet temperature 150°C, detector temperature 280°C and sensitivity $8 \cdot 10^{-11}$ a.u.f.s.

*Procedure*

After vigorous shaking for 1–2 min, all the vials were placed in a 30°C thermostated water-bath for at least 1 h. The vials were occasionally shaken to aid dissolution of the salt and to avoid stratification of the medium. Standards, samples and blanks were handled similarly.

A 200-$\mu$l aliquot of the gas phase was injected into the gas chromatograph using an air-tight syringe (Hamilton, U.S.A.). Quantitative analysis was performed by using a Perkin-Elmer $\Sigma$15 Chromatography Data System.

The external standard technique, with injection of the headspace volumes from vials with known amounts of chloroform in plasma, was used for calibration. This method has been preferred by most workers when studying the presence of THM in waters by headspace gas chromatography [16–18]. The confirmation of the identity of chloroform was performed by gas chromatography–mass spectrometry (GC–MS).

*Gas chromatography–mass spectrometry*

The GC–MS system consisted of a Carlo Erba Fractovap 2900 gas chromatograph equipped with a 25-m fused-silica capillary column (Hewlett-Packard, U.S.A.) with cross-linked 5% phenylmethylsilicone interfaced with a Finnigan-MAT Model 700 ion trap detector. We examined standard samples of chloroform in *n*-pentane and standard samples of chloroform-fortified human blood plasma at increasing concentrations (0.1–1000 mg/l) after extraction with *n*-pentane.

The identification of chloroform is based on both retention times measured on a total ion current chromatogram and on mass chromatograms of the molecular ion and the most significant fragments of chloroform (*m/z* 47–50, 82–87 and 117–124). The analyses of the previous standards were only qualitative because of the relatively low sensitivity of the ion trap (1 mg/l).

## RESULTS AND DISCUSSION

Fig. 1 shows chromatograms of laboratory air, of a blank (chloroform-free water), of a plasma sample and of the same plasma sample fortified with a known

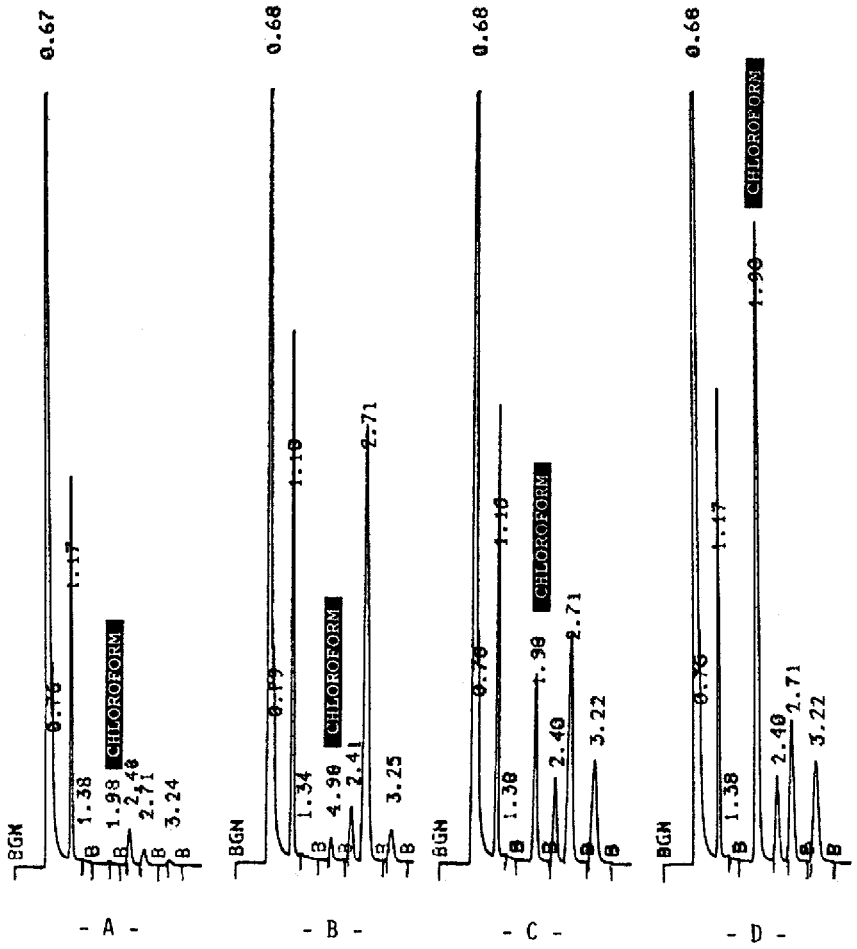


Fig. 1. Chromatograms of (A) environmental air, (B) a blank (chloroform-free water), (C) a plasma sample and (D) the same plasma sample fortified with 1 μg/l chloroform (working standard). The plasma sample (C) has a chloroform concentration of 0.39 μg/l. As the sample is diluted 1:4, the actual chloroform concentration in C is 1.56 μg/l.

amount of chloroform (working standard). The retention time of chloroform is 1.98 min; the peaks prior to the chloroform peak are due to impurities present in the laboratory air (as can be seen in chromatogram A), while the following peaks are due to the antifoam sulution and to unknown contaminants related to the handling of the sample.

The gas chromatographic analysis is completed in less than 5 min, and it does not need any pre-treatment step apart from placing the vials in a thermostated water-bath. The detection limit of the procedure depends on the blank value (chloroform-free water) and its variability; during 30 different series of chloroform determinations, the mean blank concentration was 0.0323 μg/l with a standard deviation of 0.009 μg/l. Based on 2.5 times the standard deviation, the limit of detection of the procedure was 0.0225 μg/l. As the dilution of samples and standards is 1:4, the limit of quantitation of chloroform in plasma was set at 0.09 μg/l.

None of the samples belonging to non-exposed subjects showed levels of chloroform above the limit of quantitation; the levels of chloroform measured in exposed subjects, such as swimmers, ranged from 0.13 to 3 μg/l, depending on the water and air concentration, the length of the training session and the time lapsed after leaving the pool. A future paper will present these results.

The precision of the procedure was determined by analysing two samples in duplicate in each of the 30 different series of chloroform determinations; one of these samples had a concentration of chloroform near the highest level ever found in our studies, whereas the other sample was near the lower end of the application range. The mean value for the first sample was 2.975 μg/l with a standard deviation of 0.305 μg/l and a coefficient of variation (C.V.) of 10.25%. The mean value of the second sample was 0.103 μg/l with a standard deviation of 0.018 μg/l and a C.V. of 17.8%.

The accuracy was evaluated by analysing three independent series of three plasma samples, fortified with 3 μg/l chloroform, and three independent series of three plasma samples, fortified with 0.1 μg/l chloroform. The relative error from the true value in the former three series (accuracy) ranged from −3.3% to +2.5% and in the latter from to+1% to +3%.

The sensitivity that can be achieved seems to be adequate for low-level environmental exposure to chloroform in air, and may be improved further. The selectivity is demonstrated by the selectivity of the column that was used; no peak appeared that overlapped the chloroform peak under our experimental conditions. The selectivity has also been confirmed in previous experiments performed using different columns in comparison.

The precision and accuracy are as required for this kind of analysis, as for control screening a C.V. ranging from 10 to 20% is adequate [16]; however, the precision and accuracy may be improved when the analyses are performed by an automatic sampling headspace system; such a study is in progress.

Finally, the procedure reported here does not require any expensive or complex apparatus, it is rapid and allows monitoring programmes for plasmatic circulating chloroform in subjects exposed to low environmental levels, together with the monitoring of chloroform in water and air of the same environment.

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