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# Micro Assay of Carbon Tetrachloride in Beverages and in Pharmaceutical Preparations

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Carbon tetrachloride is slightly water soluble (0.08 g/100 g at 20 C) and boils at 77 C. AGARWAL (1977) showed that $CCl_4$ alters cells of the reticuloendothelial system of the liver and spleen. CAGEN and GIBSON (1977) showed that $CCl_4$ depressed hepatic excetory function in adult and developing rats. URYVAEVA and FAKTOR (1976) stated that administration of $CCl_4$ to mice causes massive necrosis of the central regions of the hepatic lobule. SZLAMKA et al (1975) reported that the stability of rat liver and the lysosomal membrane was affected by the $CCl_4$. BENEDETTI et al (1977) isolated highly abnormal products formed by the *in vivo* interactions of $CCl_4$ with membrane fatty acids. Published reports (internal communication, 1977) revealed that a chemical manufacturer allegedly spilled massive amounts (estimated to be 70 tons) of $CCl_4$ into the Kanawha River which empties into the Ohio River. The EPA determined that raw Ohio River water contained levels of $CCl_4$ as high as 300 ppb, and up to 100 ppb was found in the drinking water supply of several municipalities (internal communication, 1977). Since the river is a source of water used to manufacture many types of food and drug products, an expedient and quantitative method was needed to detect the extent of $CCl_4$ contamination. This report describes a rapid micro assay procedure for $CCl_4$ in beverages and in pharmaceutical preparations. An attempt was made to use a mass fragmentography system for the identification of the $CCl_4$ utilizing the combination of both gas chromatography and the ratio of 3 or more mass spectral fragment ions. This technique was first introduced by HAMMAR et al (1968).

## MATERIALS and METHODS

*Samples*. Various beverage samples, representing carbonated and non-carbonated soft drinks, reconstituted orange juice, beer, bourbon whiskey, gin and municipal drinking water were used.

*Extraction and trapping of $CCl_4$*. Figure 1 depicts the various components of the system used. The *n*-pentane (3 ml) was added to a 15-ml centrifuge tube, placed in the dry ice-acetone bath (-78 C), and allowed to stand for 1 min. The liquid sample (50 g) was then added to the 100-ml r.b. flask attached to the system. The magnetic stirrer was adjusted to medium speed and the sample swept with $N_2$ at the rate of 50 ml/min. After 20 min, the $N_2$ flow was stopped, the centrifuge tube containing $CCl_4$ removed, tightly capped, and allowed to reach room temperature before injection on the gas chromatographic column.

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Gas chromatography. Two independent gas chromatographic systems were used. The first consisted of a Tracor MT-222 with a $^{63}$Ni detector (linearized pulse width of 1.0), a glass column 6' x 1/4" containing 80/100 mesh Chromosorb 101 for gas solid chromatography (GSC) operation. The parameters used were: 5% methane in argon, flow, 35 ml/min; the temperatures of the column, inlet and detector were 170, 235, and 250 C, respectively. The second system consisted of a Packard 800 with a $^{3}$H detector, a glass column 6' x 1/4" containing 20% Carbowax 400 on 80/100 mesh Chromosorb W for gas liquid chromatography (GLC) operation. The parameters used were: 40 ml/min $N_2$ flow; temperature of column, inlet and detector were 35, 90 and 155 C, respectively. In quantitation using the GLC system, the sharp peaks were usually suitable for peak height measurements whereas in the GSC system the peaks are best suited for area measurement.

Gas-chromatography-mass spectrometry. A Finnigan model 3300 F quadrupole unit equipped with an all-glass separator interface, was used. The combined system in the mass fragmentography mode was controlled by a computer to monitor fragment ions at 117, 119 and 121 m/e. Specific GC/MS conditions were: column 5' x 2 mm packed with 3% OV-101 and 6% OV-210 on 80/100 mesh Chromosorb WHP; helium flow, 20 ml/min; column and inlet temperatures were 32 and 100 C, respectively; temperatures of ion source, transfer line and jet separator were 60, 110 and 110 C, respectively. The electron impact ionization had the following specific conditions: electron energy 70 eV; electron multiplier 3.0 kV.

## RESULTS and DISCUSSION

$CCl_4$ in water and other samples. Products manufactured using water which passed the city on the date of highest risk were collected when available. In most cases, both in-process water samples and finished beverage products were obtained simultaneously. Table 1 depicts a compilation of inspectional (internal communication, 1977) and analytical results of samples obtained from various firms, using municipal water subject to contamination from the $CCl_4$ spill. A positive correlation was noted between $CCl_4$ levels in the water and finished product. The concentration of $CCl_4$ in all samples examined ranged from trace (non-detected) to 6.4 ppb. This high level was observed in a sample of bourbon whiskey, obtained from a firm in Louisville, KY during the week of February 9, 1977. The level of $CCl_4$ in water processed by different manufacturers in various cities and collected in March ranged from none detected (ND) to 3.0 ppb, indicating the persistant nature of this contaminant. The stripping of volatile organic substances from water by contact with an inert gas was examined by several investigators (GROB 1973, NOVAK et al. 1973, and GROB and ZURCHER 1976). NOVAK et al (1973) concluded that volatile substances dissolved in water were most effectively removed by stripping them from water with an inert gas. GROB and ZURCHER (1976) also stated that methods for stripping organic substances from water were readily accepted in water research laboratories for a variety of reasons.

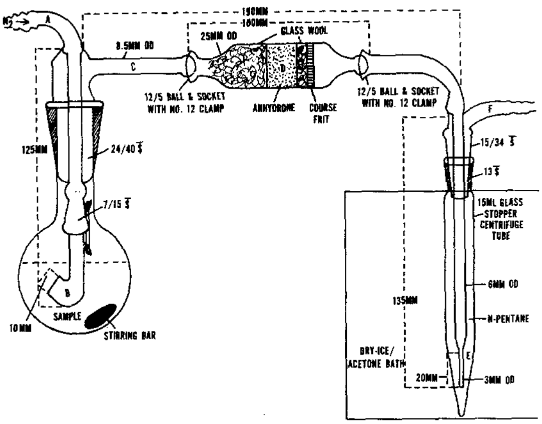

Figure 1. A schematic diagram of the $CCl_4$ trap and extraction apparatus. Purified $N_2$ enters joint A from a calibrated flow meter (50 ml/min) and passes through the liquid sample (25 C). It continues upward to line C and dryer D, then downward through cold n-pentane contained in a condenser (centrifuge) tube at E (kept in a dry ice-acetone mixture) and out at F. The dryer contains 4 g anhydrone (magnesium perchlorate) between glass wool pledgets. The magnetic stirrer is positioned to one side of the flask away from the glass frit at B to avoid $N_2$ entrapment in the stirring vortex.

The use of either GLC or GSC chromatographic systems was found suitable for the quantitation of the $CCl_4$. Linear response was also established between 0.016 and 0.15 ng $CCl_4$ (standard curve). Some degree of specificity was obtained by using both GSC and GLC systems. Using the parameters established for the GLC (system B), the retention time of $CCl_4$ and $CHCl_3$ was 1.5 and 3.0 min, respectively. On the other hand, using the parameters established for the GSC (system A) $CCl_4$ and $CHCl_3$ exhibited a retention time of 4.6 and 3.6 min, respectively. Thus, the order of elution could be reversed ($CHCl_3$, $CCl_4$). It is of interest to report that standard $CH_2Cl_2$ was eluted prior to either $CHCl_3$ or $CCl_4$ for both systems. The validity of this procedure for determining $CCl_4$ at various fortified levels was established by recovery experiments using a variety of carbonated sodas, alcoholic beverages, reconstituted orange juice and pharmaceutical liquid preparations. Summary of recovery data are recorded in Table 2 for both GLC and GSC systems. The recovery data for $CCl_4$ using the GLC system was also verified by three analysts referred to as A, B and C, who independently performed the assay procedure as recommended. The data revealed that the recoveries obtained ranged from 91 to 108% and was not correlated to either the type of beverage or the level of the $CCl_4$. Figure 2 depicts

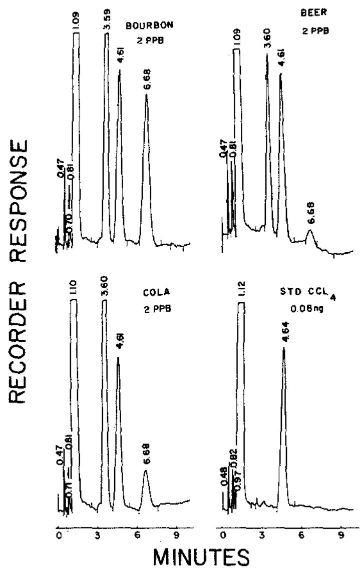

Figure 2. GSC chromatograms of bourbon, beer and cola fortified at 2 ppb as compared to 0.08 ng standard $CCl_4$.

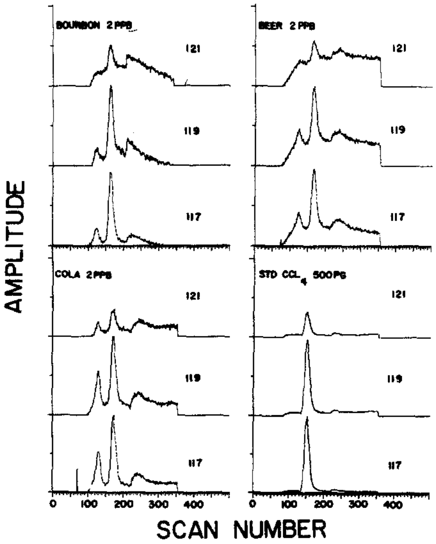

Figure 3. Mass fragmentograms of bourbon, beer and cola fortified at 2 ppb as compared to 500 pg standard $CCl_4$.

TABLE 1

Level of Carbon Tetrachloride Found in Various Samples Obtained from Different Manufacturers

| Firm Location and No.[a] | Samples Examined[b] | Date[c] 1977 | $CCl_4$ ppb |
|---|---|---|---|
| Louisville, KY | | | |
| 1 | water | 3/9 | 0.17 |
| | bourbon | 3/9 | 0.79 |
| | bourbon | 2/9 | 6.4 |
| | bourbon | 2/9 | 5.4 |
| 2 | water | 3/4 | 0.28 |
| | reconstituted orange juice | 3/4 | ND[d] |
| 3 | water | 3/10 | 0.58 |
| | bourbon | 3/10 | 0.30 |
| | bourbon | 2/24 | 1.4 |
| | bourbon | 1/20 | 0.67 |
| 4 | water | 3/10 | 1 |
| | whiskey | 2/21 & 3/10 | 1 |
| 5 | water | 3/3 | 1 |
| | soda | 2/5 & 3/3 | 1 |
| 6 | water | 3/9 | 2.8 |
| | bourbon | 3/9 | 1 |
| | bourbon | 2/24 | 5.2 |
| | bourbon | 12/28[e] | 0.54 |
| 7 | water | 3/3 | 1.1 |
| | fruit drink | 3/3 | 0.9 |
| 8 | water | 3/3 | 2.0 |
| | soda | 3/3 | 1.0 |
| 9 | water | 3/2 | 1 |
| | soda | 2/7, 2/23 & 3/2 | 1 |
| 10 | water | 3/2 | 1.3 |
| | soda | 3/2 | 1.1 |
| | soda | 2/5 | 0.21 |
| 11 | water | 3/3 | 3.0 |
| | bourbon | 3/3 | 0.8 |
| | bourbon | 12/9 | 0.3 |
| 12 | water | 3/3 | 1.2 |
| | wort | 3/3 | ND |
| | beer | 1/22 & 3/3 | ND |
| 13 | soda | 2/22 | 1.1 |
| | soda | 3/2 | 1.0 |
| Shirley, KY | | | |
| 1 | water | 3/10 | 0.78 |
| | bourbon | 3/10 | 0.50 |
| | gin | unknown | 1.8 |
| | bourbon | unknown | 1.3 |
| | gin | 2/10 | 0.3 |
| Newport, KY | | | |
| 1 | beer | 2/4 | 1 |
| | beer | 2/22 & 3/3 | 1 |
| | water | 3/3 | 1.2 |
| Paducah, KY | | | |
| 1 | water | 3/2 | 1 |
| | soda | unknown | 1 |
| 2 | soda | 2/25 & 3/2 | ND |
| 3 | water | 3/2 | ND |
| | soda | 3/2 | ND |
| | soda | 2/28 | 1 |

[a] water treatment used at these firms consisted of additional carbon filtration except at Paducah, KY (firm 1).

[b] water refers to plant process water.

[c] sample manufactured and/or water collected.

[d] none detected (ND).

[e] manufactured during 1976.

# TABLE 2

Recovery of $CCl_4$ from Beverages, Water and Pharmaceutical Preparations Fortified at Various Levels Using Both GLC and GSC Systems

| Samples Examined | Analyst | Parts Per Billion | | | % Recovered |
|---|---|---|---|---|---|
| | | Found | Added | Recovered | |
| GLC System | | | | | |
| Water | C | ND[a] | 9.5 | 8.7 | 91 |
| Water | A | ND | 9.5 | 9.1 | 95 |
| Water | C | ND | 9.5 | 8.8 | 93 |
| Water | B | ND | 9.5 | 9.0 | 95 |
| Water | C | ND | 9.5 | 8.9 | 94 |
| Cola | A | ND | 9.1 | 9.7 | 107 |
| Cola | B | ND | 9.2 | 8.6 | 94 |
| Orange juice | C | ND | 9.2 | 8.4 | 92 |
| Dry gin (86 proof) | C | 1.8 | 10.2 | 11.1 | 91[b] |
| Bourbon (80 proof) | A | 0.8 | 9.5 | 10.2 | 99 |
| Bourbon (80 proof) | C | 0.7 | 10.1 | 10.5 | 98 |
| Beer | B | ND | 9.4 | 9.5 | 100 |
| GSC System | | | | | |
| Distilled water | | ND | 2.0 | 2.0 | 100 |
| | | 0.1 | 10.0 | 9.8 | 97[b] |
| Cola | | ND | 2.0 | 1.9 | 94 |
| | | ND | 10.0 | 9.9 | 99 |
| Orange soda | | ND | 2.0 | 2.2 | 108 |
| | | ND | 10.0 | 9.2 | 92 |
| Beer | | ND | 2.0 | 2.1 | 104 |
| | | ND | 10.0 | 10.5 | 105 |
| Bourbon | | 0.6 | 2.0 | 1.7 | 86[b] |
| | | 0.5 | 10.5 | 11.2 | 102[b] |
| Phenobarbitol elixir | | ND | 2.0 | 2.2 | 108 |
| | | ND | 9.1 | 9.1 | 100 |
| Mannitol (15%) in distilled water injection[c] | | ND | 2.0 | 1.8 | 92 |
| | | ND | 9.9 | 9.4 | 94 |
| Ringer's injection[c] | | ND | 2.0 | 1.8 | 88 |
| | | ND | 10.0 | 10.1 | 101 |

[a] None detected.

[b] Corrected for incurred residue.

[c] U.S.P.

the GSC chromatograms of different samples fortified at 2 ppb as compared to 0.08 ng standard $CCl_4$.

In the test samples for alcoholic beverages, CCl4 was quantitatively recovered as long as the alcohol content did not exceed 50% by volume. The addition of water to bring the sample below 50% alcohol was found suitable. Quantitative recoveries of CCl4 using viscous pharmaceutical preparations, such as elixir and/or syrup preparations, were obtained only after modifying the procedure by using less sample (25 g) and adding water for dilution purposes. Proper control samples were evaluated several times daily to ensure no contamination or losses occurred during sample handling or assay. No GLC peaks were introduced by the nitrogen, anhydrone, or the solvent n-pentane. This was determined by sweeping the control system with nitrogen in the absence of sample. It was also established that the N2 sweep could be varied between 15 and 35 min with no detectable effect on the recovery levels of $CCl_4$.

McLAFFERTY (1973) reported that mass spectrum of CCl4 exhibits the most intense peak at 117 m/e, representing the loss of Cl ion from the molecular species. The characteristic chlorine clusters two mass units apart are characteristic features of $CCl_4$. Although identification of CCl4 was not attempted by mass spectrometry on the samples examined (Table 1), it was felt that it would be of value to establish that the cleanup provided by this procedure is suitable for MS confirmation at 2 ppb levels in samples. The elution of CCl4 from the gas chromatographic column was monitored by using GC/MS system at selected ionic masses of 117, 119 and 121 m/e. Representative samples, as compiled in Table 2, were used to obtain individual mass fragmentation (Figure 3) on sample injections fortified at 2 ppb levels as compared to standard $CCl_4$ at 500 pg. The data obtained revealed that the cleanup provided by this procedure is adequate for GC/MS identification. Again, it was also established that the injection of untreated samples produced no response above the s/n ratio at the retention time established for $CCl_4$.

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