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Analysis of trichloroacetic acid in the urine of workers occupationally exposed to trichloroethylene by capillary gas chromatography

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Abstract

A gas chromatographic procedure is described for the determination of trichloroacetic acid in urine, the major metabolite of trichloroethylene exposure. Trichloroacetic acid was derivatised to its methyl ester with BF_3/methanol reagent and then extracted into toluene and analysed by capillary gas chromatography using electron-capture detection. The response was linear in the range 0.4–100 mg/l of trichloroacetic acid in urine and showed a relative recovery of 99.6%. The procedure is suitable for monitoring occupational exposure to trichloroethylene.

1. Introduction

Trichloroethylene is widely used in industry as a degreaser for metal parts, dry cleaning agent, thinner for paints and lacquers, and for extracting oils, fats and waxes from vegetable and animal products. In occupational exposure, inhalation is the main route of absorption, however, significant skin absorption can occur upon contact with liquid trichloroethylene [1]. The absorbed trichloroethylene is mainly metabolised into trichloroacetic acid (TCA) (18%) and trichloroethanol (TCOH) (33%) which are eliminated in urine. The fate of 20–30% of the absorbed amount of trichloroethylene remains unknown [2]. Minor metabolites, such as chloroform, chloral hydrate and monochloroacetic acid have been suggested [3]. The relationship between the degree of exposure and excretion of TCA remains linear if the exposure concentration does not exceed 50 ppm (268 mg/m^3) in air [2,4–6]. When measuring both major metabolites, TCA and TCOH, the sampling time is quite critical because of differences in elimination half-lives of the metabolites (TCA 50–100 h; TCOH 12–26 h [7]). Hence, TCA concentration in urine reflects exposure over the working week while TCOH reflects exposure over the previous day.

Our laboratory previously used a spectrophotometric method based on the Fujiwara reaction [8]. However, this method involved the use of toxic solvents, such as pyridine, and it has shown to lack selectivity, due to interferences from similar halogenated organic compounds [9], and to lack stability of the Fujiwara reaction product. Other gas chromatographic methods which require derivatisation with reagents such as diazomethane [10–12] have the disadvantages of needing the use of concentrated acid and the

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performance of multiple solvent extractions to achieve a reasonable extraction recovery. Furthermore, the use of a dangerous, toxic, carcinogenic derivatisation reagent is most undesirable. Other methods which have been reported include high-performance liquid chromatography [13], ion chromatography [14] and headspace gas chromatography [15–17]. Headspace analysis is attractive if one has an appropriate autosampler, but little time is saved due to the long incubation period. Analysis using dual columns and electron-capture detection (ECD) offers good specificity comparable to that of gas chromatographic–mass spectrometric determinations, e.g. the difluoroanilide derivatisation method [18]. The difluoroanilide derivatisation method looses specificity by monitoring an ion fragment which originates from the difluoroanilide reagent. Furthermore, difluoroanilide derivatives have shown to be unstable due to sensitivity to light [18]. Hence, the current method was developed to be simple, accurate and not requiring the use of toxic reagents or solvents such as pyridine. It involves the derivatisation of TCA to its methyl ester using methanol/BF_3 reagent. The ester is then partitioned into an organic solvent and analysed by capillary gas chromatography with electron-capture detection. The results obtained using this procedure were compared with those obtained using the Fujiwara spectrophotometric method.

The present method was developed to monitor occupational exposure to trichloroethylene. The occupational exposure limit is equivalent to 100 mg/l [19]. Therefore, the method was developed to monitor concentrations between 0.4 and 100 mg/l. The limit of quantitation of the method was hence designed to be 0.4 mg/l as concentrations below this level are not considered relevant to occupational exposure.

2. Experimental

2.1. Reagents and chemicals

The borontrifluoride–methanol complex, 14% BF_3 content was obtained from BDH Laboratory Supplies (Poole, UK); the toluene was nanograde obtained from Mallinckrodt (Germany); sodium trichloroacetic acid (97%) was obtained from Aldrich (Milwaukee, OR, USA); trichloroacetic acid methyl ester (>96%) from Tokyo Kasei Kogyo (Tokyo, Japan); Biorad Lyphochek Urine Metals Quality Control Level 1 from Bio-Rad Laboratories (Anaheim, CA, USA); anhydrous sodium sulphate (99.0%) from Ajax Chemicals (Sydney, Australia).

2.2. Apparatus

A Hewlett-Packard Model 5890 series II gas chromatograph with an electron-capture detection system, Model 7673A automatic liquid sampler and HP 3396 ChemStation data analysis system was used. The gas chromatographic capillary columns used were DB-17 (0.25 μm film thickness; 30 m × 0.32 mm I.D.) and DB-5.625 (0.25 μm film thickness; 30 m × 0.32 mm I.D.) from J&W Scientific (Folsom, CA, USA).

2.3. Instrumental conditions

The gas chromatograph was used with dual capillary columns of different polarities, as above, to give added certainty to positive identification. A split injection mode was used as electron-capture detection was highly sensitive to the TCA-methyl ester. The inlet injection temperature was 240°C and a detector temperature of 300°C was used. The temperature program of the chromatograph was initial temperature 55°C for 0.5 min, then at a rate of 3°C/min to 80°C, then at a second rate of 50°C/min to 280°C and held for 4.17 min. This gives a total run time of 17.00 min. Hydrogen was used as carrier gas at a flow-rate of 2 ml/min. The split vent was equal to 40 ml/min and detector make up gas (5% methane in argon) was equal to 80 ml/min. The injection volume used was 1 μl. A 100 mg/l spiked urine sample chromatogram can be seen in Fig. 1.

2.4. Preparation of standards

A stock standard was prepared by weighing 0.5 g of sodium trichloroacetate and making up to volume in a 1-l volumetric flask with distilled

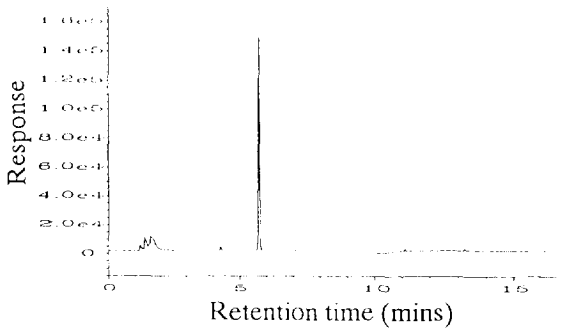

Fig. 1. Gas chromatogram of 100 mg TCA/l urine using a DB-17 (0.25 μm film thickness; 30 m × 0.32 mm I.D.) capillary column from J&W Scientific.

water. A range of working standards were prepared from this stock by appropriate dilutions to give a range of 0.5–100 mg/l. The sodium salt of TCA was preferred to the acid form because it was less hygroscopic. A Bio-Rad commercial quality control sample was used to determine the accuracy and precision of the method. The absolute recovery of the procedure was determined using standards prepared from the TCA-methyl ester made up directly in toluene.

2.5. Procedure

A 200-μl aliquot of urine sample was added to a 12-ml stoppered test tube. A 0.5-ml volume of BF_3–methanol complex was added to the sample in the test tube. The mixture was heated at 60°C for 30 min. After cooling, the sample was extracted with 8.0 ml of toluene for one hour on a rotator. A 2-ml volume of the toluene extract was then taken and shaken with anhydrous sodium sulphate in a glass vial. A portion of this dried toluene extract was then transferred to a vial for gas chromatographic analysis.

The standards and the quality control sample were prepared according to the same procedure as used for the samples.

3. Results and discussion

Gas chromatographic analysis of chlorinated hydrocarbons using electron-capture detection is a very sensitive detection technique. Hence, efforts were made in this procedure to optimise the linear range of the detector to the concentration range encountered in occupational exposures. A large volume of extracting solvent was used to give the effect of (1) dilution and (2) a satisfactory extraction efficiency of greater than 90%. It was found that at least a 1:2 ratio of sample to derivatising reagent was necessary for the maximum level of derivatisation to occur.

3.1. Recoveries

Absolute recoveries were quantified against a TCA-methyl ester standard, which was diluted to the appropriate concentration in nanograde toluene. The absolute recovery of TCA-methyl ester in water was 48.8% with a standard deviation of 1.9 ($n = 30$) over the range 0.1–100 mg/l. In urine the absolute recovery was 48.5% with a standard deviation of 2.5 ($n = 30$) over the range 0.1–100 mg/l. The fact that both water and urine have the same recoveries allows the use of water as the standard matrix.

The relative recoveries were obtained by comparing the spiked urine results to the water standards, which had both been subjected to the same procedure. This gave a relative recovery in urine of 99.6% ($n = 30$).

3.2. Accuracy and precision

The precision of the procedure was determined by using a commercially available quality control standard known as Lyphochek Urine Metals Control Level 1 obtained from Bio-Rad Laboratories (Anaheim, CA, USA). The mean value of this control was specified by the manufacturers as 24.4 mg/l with an acceptable range of 19.5–29.3 mg/l. This control standard was analysed, and gave a mean value of 28.45 mg/l with a standard deviation of 1.14 mg/l ($n = 11$). This gave a 95% confidence interval of the mean as 27.7–29.2 mg/l based on the two-sided t-test of $\times \pm ts/n^{1/2}$. A slightly higher bias can be seen in the mean of the procedure when compared to mean value given by the manufacturer. However, the confidence limits of the mean lie within the acceptable range given by the manufacturer.

The precision of the procedure can be seen by observing the relative standard deviations (R.S.D.) over the range of concentrations shown in Table 1. This concentration range of 0.1–100 mg/l TCA gave a range of R.S.D. of 2.2–4.9% with an overall mean of the R.S.D. of 3.3%.

3.3. Linearity

The procedure shows good linearity (Fig. 2) over the range 0.4–100 mg/l with a correlation coefficient of 1.000. However, to extend the range of analysis to 0.05–100 mg/l it was found that a power function was necessary to achieve the same correlation coefficient.

Figure 2. Linearity of TCA Analysis in Urine

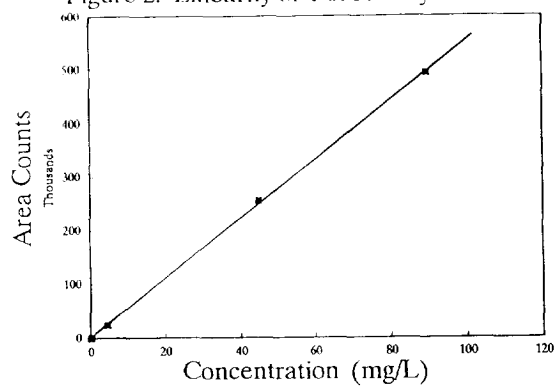

Fig. 2. Linearity of TCA analysis in urine over the range 0.4–100 mg/l with a correlation coefficient of 1.000.

3.4. Detection limits

The detection limit of this procedure was found to be 0.05 mg/l based on a signal-to-noise ratio of 5 and a power regression curve. This detection limit can easily be lowered by using a splitless chromatographic injection mode and less toluene to extract the TCA-methyl ester.

3.5. Comparison to the Fujiwara method

Urine samples were taken in the field from 10 workers employed in a degreasing operation who had been exposed to trichloroethylene. These urine samples were analysed by both the above procedure and the Fujiwara method [8]. The two procedures are compared in Fig. 3 which shows the values obtained from the gas chromatographic procedure plotted against the values obtained for the same samples analysed by the Fujiwara method. For the procedures to give identical values the points should lie on the 45° angle line. There appears to be a positive bias towards the Fujiwara reaction method. This is not unreasonable as the Fujiwara reaction is not absolutely specific for TCA [9]. This bias may be attributable to chloroform and chloral hydrate which have been suggested as a minor metabolites of trichloroethylene [3].

Table 1
The absolute and relative recoveries of TCA in water and urine

Range studied (mg/l)	Absolute recovery in water[a]		Absolute recovery in urine[a]		Relative recovery of urine to water[a]	
	Mean	R.S.D.	Mean	R.S.D.	Mean	R.S.D.
0.1 ($n = 6$)	50.9	2.1	46.4	3.1	91.2	3.7
0.4 ($n = 6$)	45.7	1.2	46.6	4.8	102.0	4.9
5.0 ($n = 6$)	49.2	1.7	49.5	1.7	100.6	2.4
50.0 ($n = 6$)	50.8	1.8	50.9	1.3	100.2	2.2
100.0 ($n = 6$)	47.2	2.4	49.1	1.4	104.0	3.2
Overall mean ($n = 30$)	48.8	1.9	48.5	2.5	99.6	3.3

[a] Values are in %.

Figure 3. Comparision of GC to Fujiwara Method

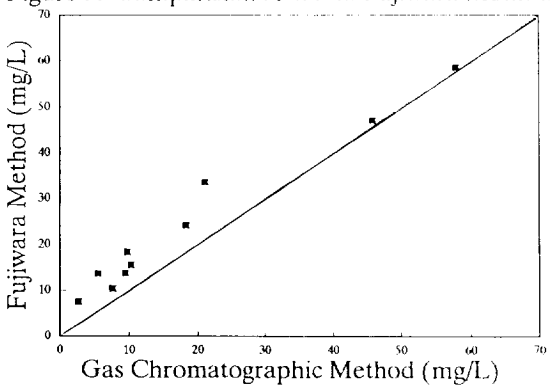

Fig. 3. Comparison of the gas chromatographic procedure to the Fujiwara reaction method with 10 urine specimens taken from trichloroethylene exposed workers.

4. Conclusion

The present method was developed for the purpose of monitoring exposure of workers to trichloroethylene in the workplace. Hence, it was optimised to determine TCA in the range 0.4–100 mg TCA/l urine. However, this method could easily be made more sensitive by using a splitless injection mode and a lower volume of toluene for the extraction step and still achieve satisfactory recoveries. It is a simple method which gives an accurate and sensitive analysis and does not suffer from the interferences of the Fujiwara method and avoids the use of toxic solvents such as pyridine.

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