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Note

Gas–liquid chromatographic determination of 2-mercaptoethanol

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2-Hydroxy-1-ethanethiol, $HSCH_2CH_2OH$, commonly known as 2-mercaptoethanol, and also known under various synonyms[1,2] is primarily used in clinical laboratories as a reducing agent. One popular example of such use is the cleavage of disulfide cross-links of cystine to form the simpler amino acid cysteine. Recently, the clinical and medical use of this compound has increased considerably whereby 2-mercaptoethanol is also used for living-cell transformation investigations, studying the enzyme activity in cellular systems and cell mediated cytotoxicity studies[3–14]. Limited mention of the toxicity of 2-mercaptoethanol has been reported[15]. A major toxicological investigation of 2-mercaptoethanol was undertaken by White *et al.*[16] who demonstrated its toxicity toward skin. No threshold limit value (TLV) for this compound has been established. However, LD_{50} and LD_{Lo} exposure values have been reported[17]. The increased use of this compound in laboratories, coupled with the fact that it is toxic and has an obnoxious odor, led the National Institute for Occupational Safety and Health (NIOSH) to determine the extent of worker exposure to this compound. Hence, a rapid and sensitive analytical method was required pursuant to a request[3].

In the past, analysis of 2-mercaptoethanol has been done by redox titration[18,19]. This procedure is lengthy and is not suitable for use at low levels. Gas–liquid chromatography (GLC) was chosen for the present study because of its simplicity, rapidity and sensitivity. This paper describes the development of a GLC procedure for the determination of 2-mercaptoethanol.

EXPERIMENTAL*

Chromatographic apparatus and conditions

Analyses were performed using a Varian 3700 gas chromatograph equipped with a flame photometric detector (FPD) operated in the sulfur mode (GC–FPD) and an autosampler. The gas chromatograph was interfaced with a Hewlett-Packard 3350 laboratory data system which was used to process the experimental data. The chromatographic column was a 200 cm × 6.35 mm (O.D.) × 2 mm (I.D.) coiled glass packed

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with 10% Carbowax 20 M on 80–100 mesh Supelcoport. The column temperature was 230°C isothermal and the injection and detector ports both were at 240°C. Nitrogen was used as the carrier gas with a flow-rate of 30 ml/min.

All injections were made by autosampler.

Reagents and standards

2-Mercaptoethanol, 99.9% pure as supplied by Chem. Service (West Chester, PA, U.S.A.) was used throughout the investigation. Spectrograde methanol was obtained from Burdick & Jackson Labs., Muskegon, MI, U.S.A.).

Standards were prepared in the range of 1–150 ng/μl. A stock solution of 2.2 μg/μl was first prepared by dissolving a known amount of 2-mercaptoethanol in methanol, then the working standards were prepared by appropriate dilutions of the stock solution. The stock solution was found to be stable up to 3 months when stored in the refrigerator. The standards in the low nanogram concentration range were stable up to 4 weeks if stored in the refrigerator after each use.

Analytical procedure

Stock solutions were prepared in 25-ml volumetric flasks having ground glass stoppers. Dilutions for the standards were made in aluminum foil-capped 20-ml scintillation vials using Supelco micropipets.

The refrigerator-stored standards were brought to room temperature prior to their injection into the gas chromatograph. A 1-μl injection of the samples was used for the GC analysis. As expected, the FPD response for 2-mercaptoethanol was found non-linear with concentration[20]. The linear amplifier of the Varian 3700 chromatograph was not used because of the lack of sensitivity of this amplifier. A linear standard curve was made by plotting the logarithm of the concentration *versus* logarithm of the peak area.

Preparation of silica gel samples

For spiked and generated samples, standard 2-section, 130 mg/65 mg silica gel tubes were used. Desired spikings were done by directly injecting the calculated amount of concentrated solution onto the front section of each silica gel tube. Calculations were made prior to the injections, so that the injected volume never exceeded 5 μl for any concentration. After spiking, each silica gel tube was sealed using Parafilm and was allowed to stand overnight at room temperature.

A dynamic flow-vapor generator equipped with a syringe injection system was used to generate known concentrations of 2-mercaptoethanol vapor which was then collected on silica gel tubes. Some tubes were analyzed immediately and others were stored at room temperature for seven days before desorption and analysis.

Desorption of samples

Each silica gel tube sample containing spiked or generated sample was desorbed with 1 ml methanol, agitated in an ultrasonic bath for 15 min and was allowed to stand for 30 min at room temperature before analysis by the GC–FPD. No breakthrough was found over the concentration range of this investigation. Hence, after a few initial trials, only the front sections of the silica gel tubes were desorbed.

RESULTS AND DISCUSSION

Several stationary phase of varying polarity were tried to chromatograph this compound. A Carbowax 20M was selected for this investigation because the analyte was well separated from the solvent peak and well formed. This may be due to relatively high polarity of 2-mercaptoethanol. Precision (R.S.D.) for the analytical method using standard solutions of 2-mercaptoethanol in methanol were 16.4%, 6.7% and 5.5% at 5.45 ng, 10.9 ng and 21.8 ng per 1-μl injection, respectively. Fig. 1 shows the chromatographic separations of two low nanogram concentrations of 2-mercaptoethanol in methanol on this stationary phase. The minimum quantifiable level could be lowered to 2 ng but with less precision. Thus, a reasonable minimum quantifiable limit was judged to be 5 ng. A log–log calibration curve of the average computer-acquired peak area *versus* concentration of 2-mercaptoethanol is shown in Fig. 2. For unknown reasons, the slope of this linear curve is not exactly 0.5 as expected.

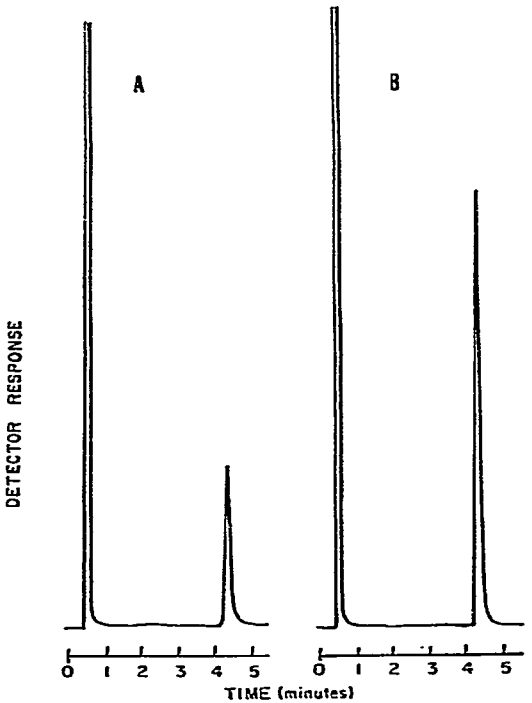

Fig. 1. Representative chromatograms for two low nanogram concentrations, 21.8 ng (A) and 32.0 ng (B), of 2-mercaptoethanol on 10% Carbowax 20M coiled glass column at 230°C. Column, 200 cm × 1/4 in. O.D. × 2 mm I.D.; nitrogen flow-rate, 30 ml/min.

For desorption efficiency purposes, 130-mg portions of silica gel were spiked with 1–5 μl of the standard solutions, capped and allowed to remain overnight. Analyses were performed after desorbing the spiked samples with methanol. Table I presents the desorption efficiency data.

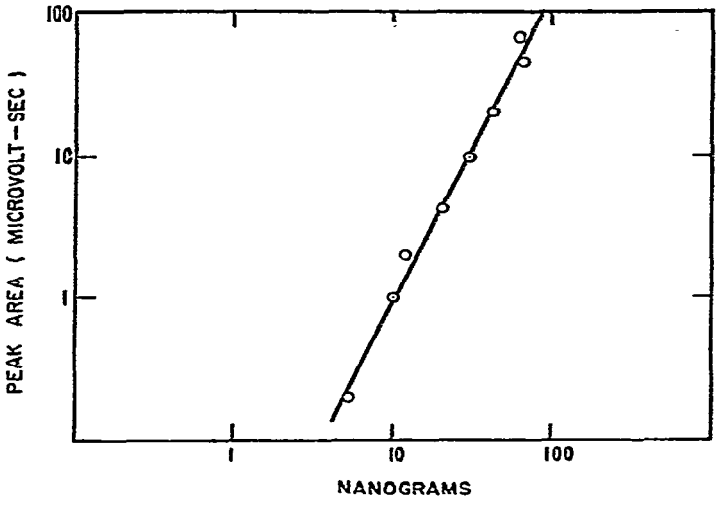

Fig. 2. Calibration curve (a plot of peak area *vs.* amount) for 2-mercaptoethanol. Chromatographic conditions are as stated in Fig. 1.

TABLE I

DESORPTION EFFICIENCIES FOR SPIKED SAMPLES OF 2-MERCAPTOETHANOL ON SILICA GEL OBTAINED BY GLC

2-Mercaptoethanol added (ng)	*2-Mercaptoethanol recovered (ng)*	*Desorption efficiency (%)*
4.8	4.3	89.6
4.8	4.4	92.0
10.5	9.8	93.3
10.5	9.6	91.4
15.4	14.6	94.0
15.4	14.6	91.0
21.9	20.3	92.6
21.9	20.8	94.9
28.5	27.3	95.9
28.5	27.4	96.2
46.0	45.0	96.2
46.0	44.9	98.0
88.5	86.7	98.0

In order to check the reliability of the developed analytical method, a series of laboratory generated samples in the concentration range of 15 to 88 μg per silica gel tube were analyzed. The recoveries varied from 86 to 93%. No significant effect of storage time (varying from 2 to 75 h between sampling and analysis) on the recoveries was found.

Duplicate chromatographic runs were made for all the generated tube samples and the precision was generally good (below 10% R.S.D.). Two silica gel tubes each containing approximately 25 μg of generated samples of 2-mercaptoethanol were stored at room temperature one for 5 and the other for 7 days prior to their desorption

and analysis. The recovery from these tubes was found to be about 80%. Although more stability tests are warranted, it appears that the developed method may be useful for collecting field samples and for laboratory analysis and evaluation.

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