ELSEVIER

Journal of Chromatography B, 665 (1995) 271–279

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Measurement of volatile organic compounds in exhaled breath as collected in evacuated electropolished canisters

Joachim D. Pleil, Andrew B. Lindstrom*

Atmospheric Research and Exposure Assessment Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, USA

First received 21 September 1994; revised manuscript received 15 November 1994; accepted 22 November 1994

Abstract

A set of three complementary analytical methods were developed specifically for exhaled breath as collected in evacuated stainless steel canisters using gas chromatographic–mass spectrometric detection. The first is a screening method to quantify the carbon dioxide component (generally at 4–5% concentration), the second method measures the very volatile high-level endogenous compounds [e.g. acetone and isoprene at 500–1000 parts per billion by volume (ppbv), methanol, ethanol, dimethylsulfide at 2–10 ppbv], and the third method is designed to measure trace-level environmental contaminants and other endogenous volatile organic compounds (VOCs) (sub-ppbv) in breath. The canister-based sample format allows all three methods to be applied to each individual sample for complete constituent characterization. Application of these methods is shown to be useful in the following ways: analysis of CO_2 levels indicates the approximate quantity of alveolar breath collected (as opposed to whole breath) in a sample; levels of major endogenous compounds are shown to be influenced by physical activities and subsequent recovery periods; and environmental exposures to xenobiotic VOCs can be characterized by assessment of post-exposure breath elimination curves. The instrumentation and methodology are described and example chromatograms and quantitative data plots demonstrating the utility of the methods are presented.

1. Introduction

The volatile organic compounds (VOCs) in exhaled breath have long been an area of interest to the health profession as a non-invasive indicator of the status of a variety of health parameters. For instance, a breath odor of acetone is an indicator of uncontrolled diabetes and the odor of dimethyl sulfide is related to liver disease [1]. Other uses of exhaled breath as a diagnostic medical tool for coronary artery disease, schizophrenia, tissue rejection, etc. have been discussed in the literature [2–5]. Upon detailed analysis of exhaled breath, the presence of xenobiotic compounds or excess endogenous compounds can indicate recent exposures through inhalation, ingestion, or dermal absorption because the breath concentrations are directly related to blood levels [6], and the blood levels are indicative of the absorbed dose. A classic example of the utility of breath analysis is the "breathalyzer" test as an indicator of ingestion of ethanol [7]. In more recent work, the presence of tetrachloroethylene and/or trichloro-

* Corresponding author. Address for correspondence: U.S. EPA, Mail Drop 56, AREAL, Research Triangle Park, NC 27711, USA.

SSDI 0378-4347(94)00545-1

ethylene in the breath has been linked to dry cleaning exposure [8], the presence of chloroform has been related to exposure to chlorinated municipal or swimming pool water [9,10] and Wallace et al. [11] used breath sampling to demonstrate that environmental cigarette smoke was a major source of residential benzene exposure. In such cases, the subject's breath becomes the indicator of his own exposure to the respective source.

Breath can be collected into various sampling containers [12], onto adsorbent cartridges [13], or directly into analytical systems [14]. Though all these sampling methodologies are useful and have their distinct advantages, for the purposes of this paper analytical methods were developed to characterize the exhaled breath matrix as captured directly in stainless steel SUMMA canisters; the specific sampling methodology employed here is presented in detail in the literature [15]. SUMMA is a trademark electropolishing/passivation technique applied to the interior of the canister to remove active absorption sites and leave behind a smooth, inert surface. Gas samples collected in SUMMA canisters have been shown to be stable for a variety of VOCs for periods of 30 days or longer without any appreciable degradation [16].

Standard methods for measurement of VOCs in an ambient air matrix [16] are not directly applicable to exhaled human breath for a number of reasons: the breath matrix is very moist (water condenses from it at room temperature), contains about 4.6% CO_2 (outdoor air levels are

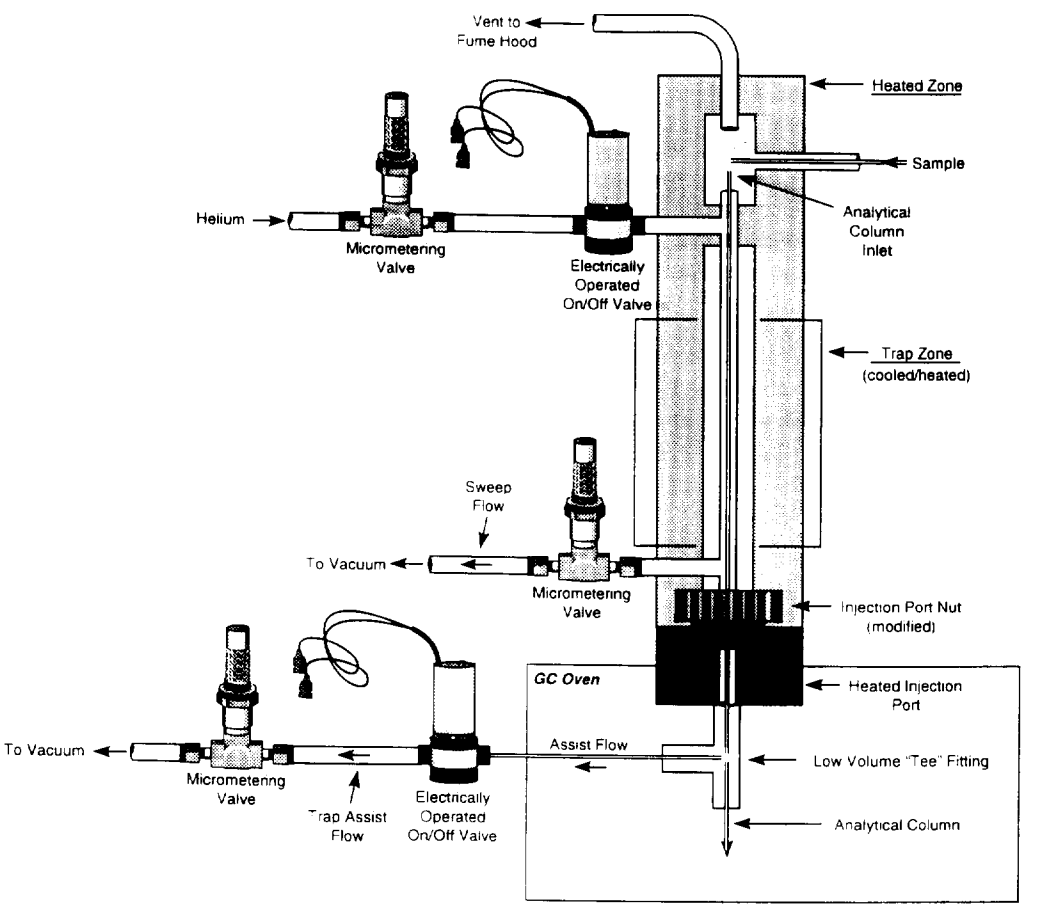

Fig. 1. Diagram (not to scale) of prototype valveless interface used for all analyses (U.S. patents are pending).

350 parts per million by volume), and contains a wide variety of types and concentrations of analytes, ranging from high levels of endogenous compounds such as acetone and isoprene to trace-level environmental contaminants such as benzene, chloroform, and tetrachloroethene. Also, many of the compounds of potential interest are polar VOCs from biological processes for which the standard methods are not designed. A second complication concerns the periodic nature of breathing. During sample collection, ambient air or "dead-space" air (that does not enter the gas exchange region of the lung) may inadvertently be pulled into the sampling system diluting the alveolar air matrix and thus affecting quantitation.

To address these issues, three distinct procedures were developed to be applied sequentially to each breath sample. The first is a rapid screening analysis wherein only the carbon dioxide level in the sample is quantified. This can serve to determine the dilution (if any) with non-alveolar air through comparison with the known levels of CO_2 in breath [15,17]. The sample is then reanalyzed specifically for the very volatile endogenous compounds: acetone and isoprene at 500–1000 parts per billion by volume (ppbv), and compounds such as methanol, ethanol, dimethylsulfide, 2-propanol, etc., in the 2–10 ppbv range. Finally, the sample is once again analyzed, this time in detail for trace-level VOCs at the sub-ppbv level. This paper discusses the three types of analyses and presents chromatographic examples and representative data illustrating the information that can be collected.

2. Experimental

All analyses were performed using an ion-trap ITS40 gas chromatographic–mass spectrometric (GC–MS) system (Finnigan MAT, San Jose, CA, USA) using cryogenic preconcentration and injection methodology developed at EPA. The sample injection scheme is based upon "valveless injection" that allows diversion of a sample gas stream into the analytical system by way of differential pressure switching as described in the literature [18]. This was modified for the present study to also allow cryogenic focussing for VOCs quantitation. In principle, the injection method is analogous to those developed for EPA Compendium Method TO-14 [16,19], with the valveless injection replacing the more commonly used six-port switching valve. An annotated diagram of the hardware used for the analyses presented in this paper is given in Fig. 1. (Note that U.S. patents for this design and applications are pending.)

All chromatographic separations were performed on an RTX-5 30 m × 0.25 mm I.D. fused-silica capillary column with 1 μm phase (Restek Corp., Bellefonte, PA, USA). The MS was operated in the scan mode at 2 Hz. Quantitation was performed by integration of single-ion extracted chromatograms for the compounds of interest.

Breath collection media were 1-l and 1.8-l volume SUMMA polished stainless steel canisters (SIS, Moscow, ID, USA) using the "single breath canister" (SBC) method as described in Ref. [15]. Precleaned canisters [16] start with an initial vacuum of < 50 μmHg and are filled to ambient pressure with breath. After breath collection, the samples are further pressurized with zero grade air up to 40 psig (2800 Torr) to allow multiple analyses. The air used for dilution is tested and certified free of contamination and the dilution ratio is applied to scale the analytical results.

Specific injection and GC–MS parameters for the three analyses are as follows:

–CO_2 quantitation: isothermal injection hardware at 60°C; switch sample onto column (1.2-s pulse); isothermal oven temperature at 60°C; MS full scan (42–100 amu); ionization time 100 μs [automatic gain control (AGC) off]; multiplier 1500 V.

–Endogenous compounds: trap 3.6-ml sample at −165°C; inject onto column with trap ramp up to 100°C; oven temperature program: 5°C hold for 3 min and ramp to 80°C at 10°/min, bake out at 220°C; MS full scan (30–300 amu); AGC target 20 000; multiplier 1700 V.

–Trace VOCs: trap 72-ml sample at −120°C;

inject onto column with trap ramp up to 100°C; oven temperature program: 5°C hold for 4 min, ramp up to 220°C at 10°/min, hold for 10 min; MS full scan (30–300 amu); AGC target 20 000; multiplier 1700 V.

A variety of samples were collected and analyzed during the development of the analytical methods. These included synthetic test samples with known concentrations of CO_2 and VOCs such as chloroform, methyl chloroform, acetone, methanol and ethanol, and a variety of real exhaled breaths from various EPA programs including exposure studies for methyl-*tert.*-butyl ether (MTBE), for an indoor air study measuring irritation from the Mølhave et al. mixture [20], exposure assessment for chloroform from municipal water [15], and exposure assessment for vinyl chloride, benzene, and other VOCs from contaminated private wells.

3. Results and discussion

For the purposes of this paper, the results of the research are presented as the chromatograms and representative data demonstrating the capabilities of the three methods. The intent is to provide tools for future research into the measurement and interpretation of volatile organic constituents of breath.

3.1. Carbon dioxide method

Interpretation of breath sample data and the resulting elimination curves requires that precision in sampling and analysis be assured; one measure of the overall comparability of samples from an individual is the CO_2 concentration of the samples. Under normal conditions, the CO_2 concentration of alveolar breath for a healthy adult male at rest is 5.3% [17]. Fig. 2 shows an extracted ion chromatogram of the base peak ion (m/z 44) of CO_2. Integration of this trace and calibration with known standards gives a very precise measure of the CO_2 concentration of the breath matrix, typically with a standard deviation of 0.02% of the CO_2 concentration.

While individuals may vary from each other in breath CO_2 concentration, a time-dependent series from one individual should yield very

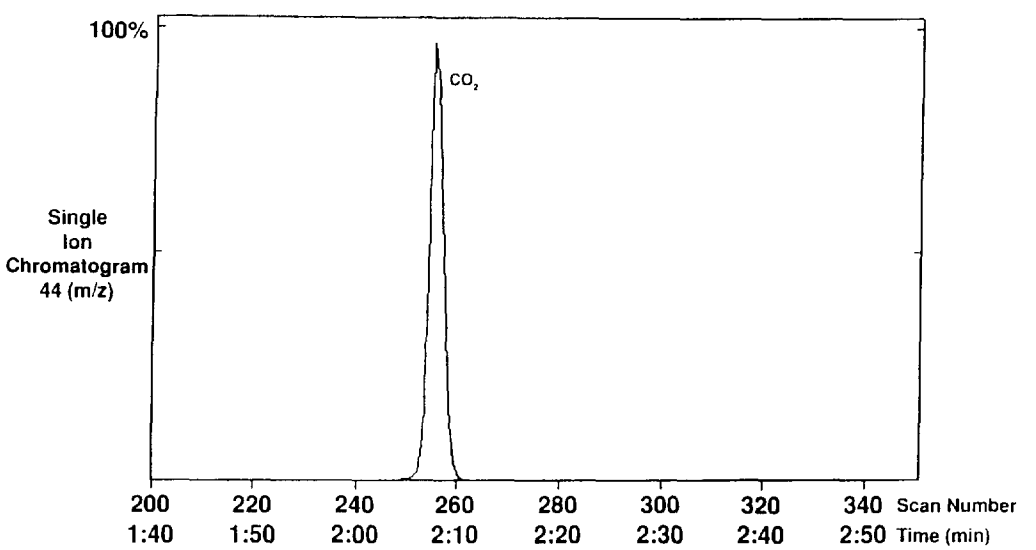

Fig. 2. Extracted ion chromatogram (m/z 44) of specific CO_2 method. Typical run-to-run precision is 0.02% CO_2 at 5% CO_2 sample level.

similar CO_2 concentrations if the sampling conditions are consistent; the changes in concentration of other species over time can then be interpreted as true elimination or exposure phenomena. If the CO_2 concentrations vary, this could be interpreted as a dilution effect from inappropriate sample collection (e.g. mixing with ambient air, mixing of alveolar and dead-space air, etc.) or as variability in the breathing pattern or metabolic rate of the subject. To demonstrate how and to what extent CO_2 can vary, a simple "breath holding" test was performed, as well as a test of different depths and rates of respiration. Results showed that the CO_2 concentration was a function of these parameters and that the variability can be significant.

Fig. 3 demonstrates the variability due to residence time in the lung with a graph of CO_2 concentration (dependent variable) plotted against length of time the breath is held prior to collection. A similar dependence of CO_2 concentration has been observed when collecting whole breath (that includes the tracheal and bronchial dead volume) vs. alveolar breath (measured as 3.9% and 4.6% CO_2, respectively), and collecting breath after 1 min of hyperventilation (pant breath at 3.1% CO_2) as shown in Fig. 4. These results show that CO_2 concentration can vary easily from 3.0 to 5.5% depending upon the subject's activities, breathing patterns, and consistency during sampling. As such, CO_2 measurements can be an excellent indicator of the consistency of the sample set.

3.2. Acetone/isoprene method

Two major organic constituents found in breath are acetone and isoprene, each typically in the range 500–1000 ppbv. To keep these compounds on scale required a separate GC–MS method. In addition, the method also yields quantitative data for other very volatile species such as alcohols. Though it is beyond the scope of this paper to speculate what this information on endogenous compounds in breath might

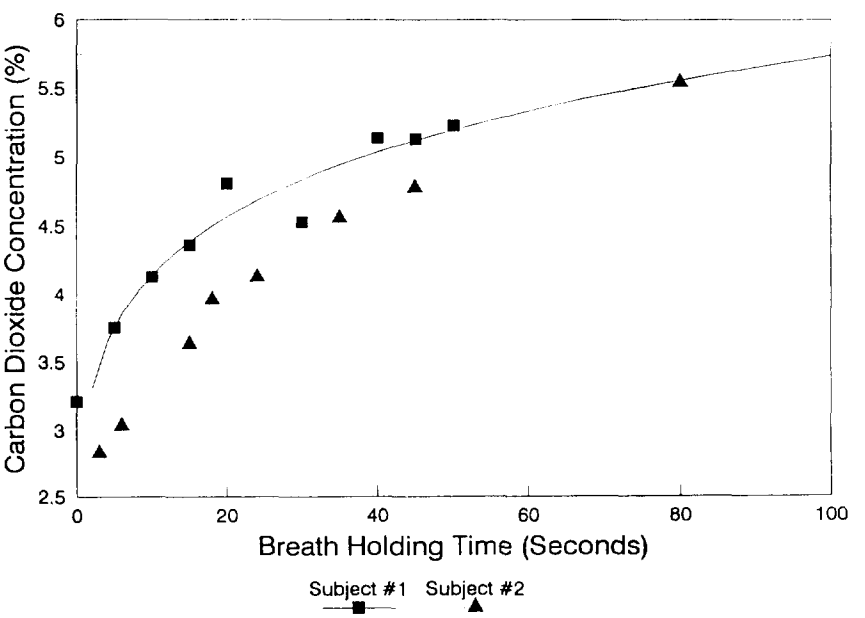

Fig. 3. Measured CO_2 concentration in whole breath as a function of time breath is held prior to sample collection. Both subjects exhibit an increasing trend in the exhaled CO_2 level as the gas-exchange time in the lung increases.

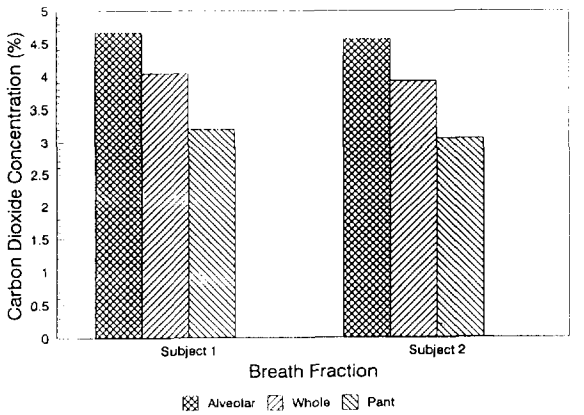

Fig. 4. Measurement of CO_2 concentration under different collection conditions. Both subjects show the dilution effect from the dead-space air (alveolar vs. whole) and the effect of hyperventilation (panting) that depletes CO_2 available in the lung.

mean, results show that there is variability in the relative amounts of these compounds between individuals, and that physical activity can also affect the quantitation of these compounds. A typical annotated chromatogram of a real exhaled breath is shown in Fig. 5. The variability of these compounds in response to exercise stress is graphically shown in Fig. 6A,B. Here a series of samples were taken before, during, and after running activity by two subjects. The data were normalized to the "before" values (100%) and plotted as a function of time/activity. The two most striking behaviors are the depression of isoprene and the elevation of CO_2 concentrations during physical exercise for both subjects (Fig. 6A). Note that similar data for methanol and acetone, as shown in Fig. 6B, do not exhibit consistent patterns.

3.3. Trace VOCs method

Due to the high levels of CO_2 in exhaled breath, the temperature for trace-level VOCs cryogenic concentration is set at −120°C (rather than at −165°C as generally used for conventional VOCs analysis). This was chosen empirically as the optimal temperature for trapping as many VOCs as possible without disrupting the GC–MS analysis with excess collected CO_2. With this method, it is possible to quantify VOCs with a typical sensitivity of 0.2 ppbv. The most volatile

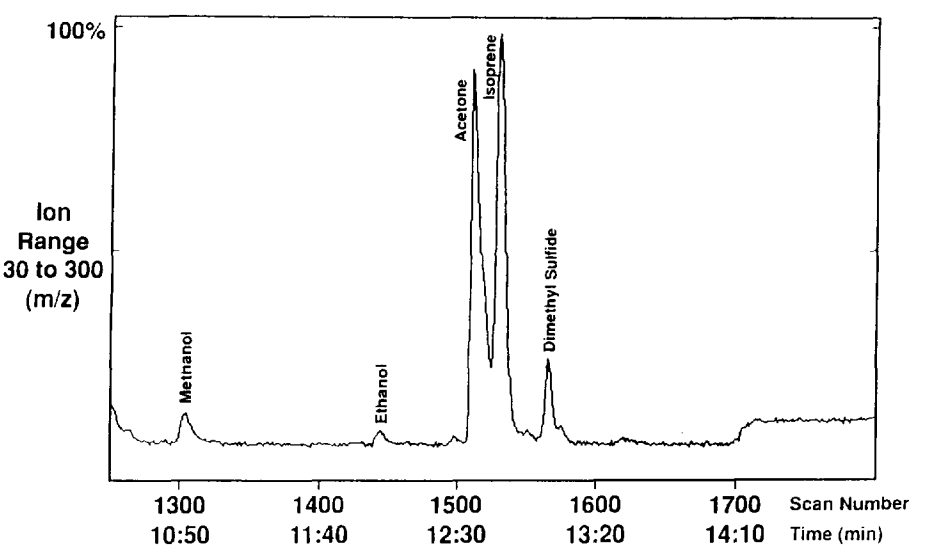

Fig. 5. Chromatogram from the acetone/isoprene analysis; other endogenous compounds can also be detected.

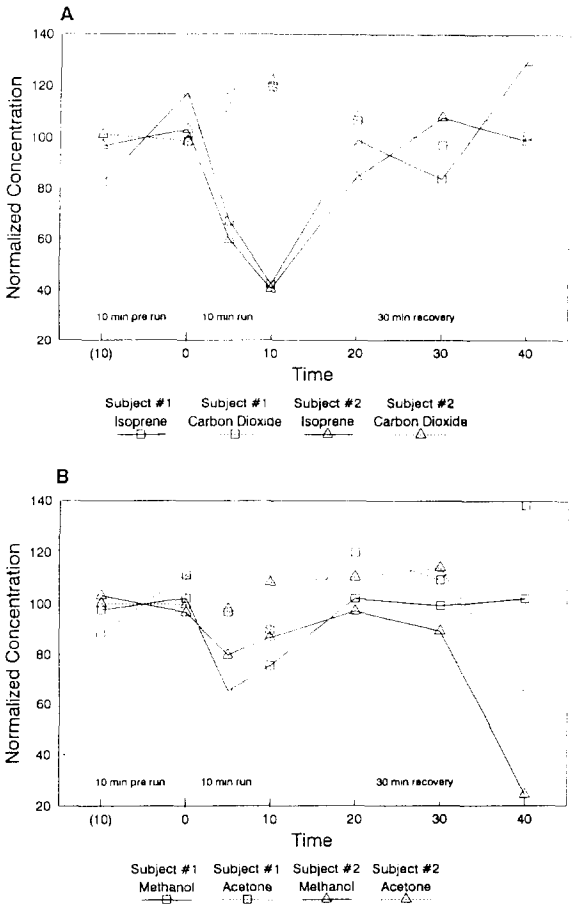

Fig. 6. Endogenous compound concentrations can be affected by physical activity. (A) There is a marked decline in isoprene concentration and a moderate increase in CO_2 concentration as a response to running for both subjects. (B) No consistent changes are observed for methanol and acetone.

quantifiable compounds are in the range of 2-propanol and dichloromethane, more volatile compounds such as some Freons, chloromethane, vinyl chloride, etc. are sacrificed to accommodate the matrix. An annotated VOCs chromatogram of a typical exhaled breath is given in Fig. 7.

The trace-level analysis is particularly useful for documenting the time-dependent elimination curve of specific VOCs after an environmental exposure. For example, the elimination of *cis*-1,2-dichloroethene subsequent to exposure from a 10-min shower with contaminated well water is given in Fig. 8. Though beyond the scope of this paper, these data can be used to calculate the minimum absorbed dose, residence time in various body compartments, and relative importance of exposure routes (inhalation vs. dermal) as has been shown previously in the literature [6,10].

4. Conclusions

The nature of the exhaled breath matrix requires at least three different GC–MS analyses to fully characterize the sample. The CO_2 quantitation is useful to assess the representativeness of the sample with respect to proper sampling or dilution error. The capability for measuring the endogenous compounds and their variability may be of use to the medical community in assessing health, and the detailed trace-level VOCs method is useful for studying previous exposures to chemicals as they are released from the body into the exhaled breath.

Future work with this method will include relating CO_2 concentrations to VOCs concentrations, documenting VOCs uptake and depletion for a variety of VOCs, and investigating the relationships among VOCs, sampling parameters, and various human activities.

Acknowledgements

The authors wish to thank Sharon Reiss of Graseby Nutech Corporation and Robert Whiton of ManTech Environmental Technology for valuable technical assistance and advice. The research described in this article has been performed, in part, with analytical equipment developed under a Cooperative Research and Development Agreement between the U.S. Environmental Protection Agency and Graseby Nutech Corporation. The information in this document has been funded wholly by the United

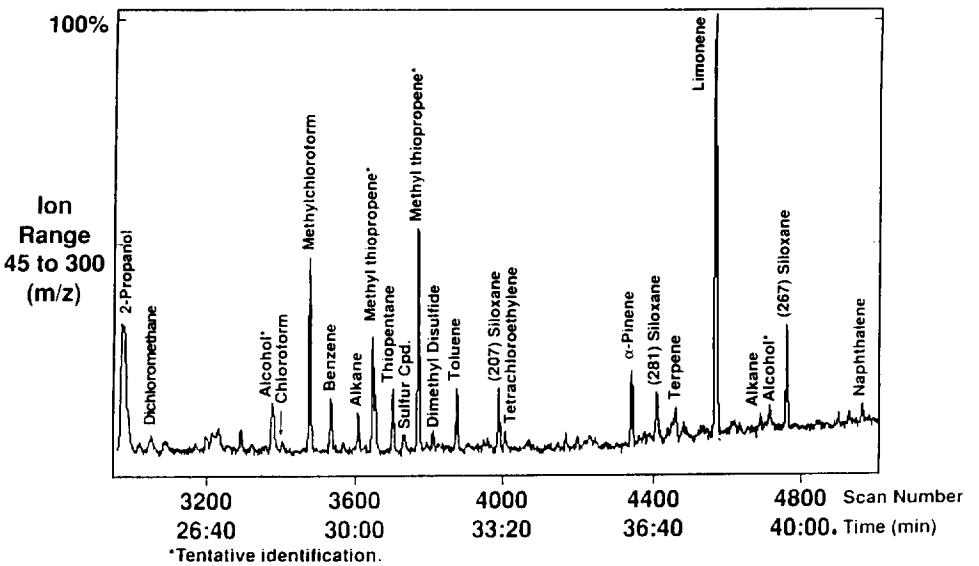

Fig. 7. Chromatogram from trace-level VOCs analysis. Typical result from a healthy adult male. For scale, the toluene concentration is approximately 1.5 ppbv.

States Environmental Protection Agency. It has been subjected to Agency review and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

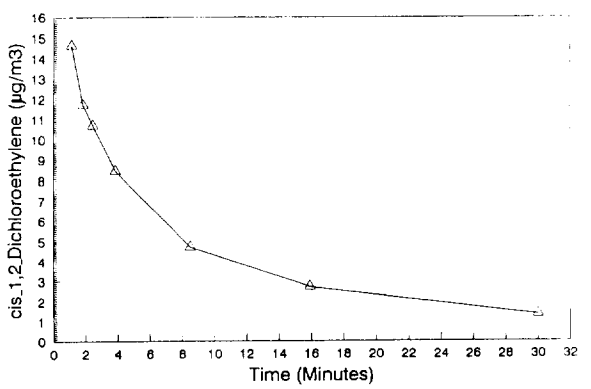

Fig. 8. Example of a time-dependent elimination curve as measured in a sequence of breath samples collected immediately after the subject showered for 10 min in water from a contaminated private well. The example compound, *cis*-1,2-dichloroethylene was one of the major VOCs measured in water analyses and in indoor-air analyses.

References

[1] M. Phillips, *Sci. Am.*, July (1992) 74–79.
[2] M. Phillips, *Int. Arch. Occup. Environ. Health*, 64 (1992) 119–123.
[3] M. Phillips, M. Sabas and J. Greenberg, *J. Clin. Pathol.*, 46 (1993) 861–864.
[4] T.H. Risby, W. Maley, R.P.W. Scott, G.B. Bulkley, M. Kazui, S.S. Sehnert, K.B. Schwarz, J. Potter, E. Mezey, A.S. Klein, P. Colombani, J. Fair, W.T. Merritt, C. Beattie, M.C. Mitchell, G.M. Williams, B.A. Perler, R.T. Donham and J.F. Burdick, *Surgery*, 115 (1994) 94–101.
[5] M. Refat, T.J. Moore, M. Kazui, T.H. Risby, J.A. Perman and K.B. Schwarz, *Pediatr. Res.*, 30 (1991) 396–403.
[6] L. Wallace, E. Pellizzari and S. Gordon, *J. Exp. Anal. Environ. Epidemiol.*, 3 (1993) 75–102.
[7] M.F. Mason and K.M. Dubowski, *J. Forensic Sci.*, 21 (1976) 9–41.
[8] L.A. Wallace, E.D. Pellizzari, T.D. Hartwell, V. Davis, L.C. Michael and R.W. Whitmore, *Environ. Res.*, 50 (1989) 37–55.
[9] G. Aggazzotti, G. Fantuzzi, E. Righi, P. Tartoni, T. Cassindri and G. Predieri, *Arch. Environ. Health*, 48 (1993) 250–254.
[10] W.K. Jo, C.P. Weisel and P.J. Lioy, *Risk Anal.*, 10 (1990) 575–580.
[11] L.A. Wallace, E.D. Pellizzari, T.D. Hartwell, C. Sparacino, R. Whitmore, L. Sheldon, H. Zelon and R. Perritt, *Environ. Res.*, 43 (1987) 290–307.

[12] K.W. Thomas, E.D. Pellizzari and S.D. Cooper, *J. Anal. Toxicol.*, 15 (1991) 54–59.
[13] S.M. Gordon, L.A. Wallace, E.D. Pellizzari and H.J. O'Neill, *Atmos. Environ.*, 22 (1988) 2165–2170.
[14] S.M. Gordon, D.V. Kenny and T.J. Kelly, *J. Exp. Anal. Environ. Epidemiol. Suppl.*, 1 (1992) 41–54.
[15] J.D. Pleil and A.B. Lindstrom, *Am. J. Ind. Med.*, in press.
[16] W.T. Winberry, Jr., N.T. Murphy and R.M. Riggan, Method TO-14, *EPA-600/4-89-017, Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air*, U.S. Environmental Protection Agency, Research Triangle Park, NC, June 1988.
[17] A.C. Guyton, *Basic Human Physiology: Normal Function and Mechanisms of Disease*, W.B. Saunders Co., Philadelphia, PA, 1977, pp. 397–400.
[18] J.D. Pleil and M.L. Stroupe, *J. Chromatogr. A*, 676 (1994) 399–408.
[19] W.A. McClenny, J.D. Pleil, M.W. Holdren and R.N. Smith, *Anal. Chem.*, 56 (1984) 2947.
[20] L. Mølhave, B. Bach and O. Peterson, *Environ. Int.*, 12 (1986) 167.