A New Approach for the Measurement of Volatile Organic Compounds in Human Skin Gas by Chromatography

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Citation

Abstract
This study investigated the volatile organic compounds (VOCs) in human skin gas and standard gas using modified gas chromatographic system. It devised sampling glass syringe temperature at 25°C or heating 80°C prior to injecting sampling gas into a gas chromatograph. The peak area of 6-methyl-5-hepten-2-one at 50, 100, and 250 ppb heated at 80°C was 337, 236, and 214% higher than that at 25°C, respectively. The peak area of hexanal at 50, 100, and 250 ppb heated at 80°C was 164, 159, and 135% higher than that heated at 25°C, respectively. Likewise, the peak area of acetone at 50, 100, and 250 ppb heated at 80°C was 115, 116, and 109% higher than that heated at 25°C, respectively. These results suggest that a higher VOC peak area can be obtained by heating the sampling glass syringe before injection into the gas chromatograph.

1. Introduction

Several hundred volatile organic compounds (VOCs) emanate from the breath and skin [1-3]. Acetone is formed in the liver from the spontaneous decarboxylation of acetoacetate [4], and a high correlation has been found between in the acetone concentration in exhaled air and blood glucose [5]. It has been reported that acetone is not only of interest as a marker for disease [3, 6-8], but also as a marker for human presence in rescue operations [9]. The ketones, acetone and 6-methyl-5-hepten-2-one are released at relatively high emission rates from the human body [4], and are formed from the reactive oxygen species (ROS)-induced degradation of squalene [10-12]. Hexanal, an aldehyde, is also produced by the oxidative degradation of linoleic, palmitoleic, and vaccenic acids [13]. The emission of these endogenous VOCs from the skin is a useful biomarker, and may potentially be utilized to administer clinically significant health care in a non-invasive manner [6].

Mochalski et al. [4], selected the hand or forearm for a skin gas analysis of the body, as these areas are the most easily accessible during the applied sampling protocol and, as such, are the most convenient areas for collection in the volunteers. However, because forearm skin VOCs are present at lower concentrations, the actual VOC concentration in skin gas has usually been quantified using gas chromatography with mass spectrometric
detection (GC-MS) coupled with solid phase micro-extraction as pre-concentration method (SPME) [4], selected ion flow tube mass spectrometry (SIFT-MS) [6], or selected reagent ionization time-of-flight mass spectrometry with NO+ (ERI-TOF-MS(NO) [9]. In this study, the concentrations of acetone, 6-methyl-5-hepten-2-one, and hexanal in a standard and human skin gases were measured using gas chromatography.

Tedlar (polyvinyl fluoride (PVF)) film is one of the most popular and widely accepted materials for collecting gaseous particles in the skin and breath [4, 14]. Nevertheless, it is necessary to remove any contaminants before using the sampling bags [4]. Mochalski et al. [4], have reported that the contaminants in the bags and the gas-tight glass syringe can be removed with heating. This technique implies that more VOCs can be desorbed by means of heating the sampling glass syringe or bag, thereby increasing the VOC concentration. However, there are no reports regarding the influence of heating the sampling glass syringe prior to VOC sample injection into the gas chromatograph on the peak area. Accordingly, the present study was designed to fill this gap in the literature, i.e. the effect of heating the sampling glass syringe on desorption of VOCs.

2. Experimental

2.1. Chemicals and Preparation of the Standard Gases

Acetone was purchased from Kantoh Chem (Tokyo, Japan). 6-methyl-5-hepten-2-one was purchased from Tokyo Chem (Tokyo, Japan). Hexanal, diethylether, and ultrapure water were purchased from Wako (Tokyo, Japan). Using these chemicals, 50, 100, and 250 ppb of each standard reference gas (acetone, 6-methyl-5-hepten-2-one, and hexanal) were produced.

2.2. Sampling of the Skin Gas

The subject for this experiment was a healthy male. The purposes and procedure of the study and the possible risk were fully explained to him before he signed an informed consent document. The study was approved by the Ethical Committee of the Nagoya Institute of Technology. A bag made of Tedlar (polyvinyl fluoride (PVF)) film (GL science, Tokyo) was used for sampling. The room temperature was set at 25°C, the hand were washed with running tap water for 30 sec and then with distilled water, and finally wiped with a paper towel (Kimwiper, Japan). The hand was then inserted into the sampling bag with a sealing film (Parafilm M, USA). The gas in the bag was replaced with 100 ml of nitrogen gas. The skin gas during rest was collected for 3 min into the sampling bag (Fig. 1), and 50 ml of the gas was then drawn into a glass syringe.

2.3. Analytical Conditions for the Determination

Each standard reference gas and skin gas were measured with a modified gas chromatography flame ionization detector (GC-FID) (GC-2010, Shimadzu, Japan), which was equipped with a constant temperature oven (CTO-20A, Shimadzu, Japan), gas trapping system (NIT-3, Pico-device, Japan), and temperature controller (E5LD, Omron, Japan). The sampling gas was analyzed with a cold trap gas chromatographic system [15]. A 30 ml volume of sampling gas was automatically injected into the stainless-steel tube from a 50 ml glass syringe. During this process, the sample is fed into a stainless-steel sample loop cooled with liquid nitrogen. After the sample injection valve was rotated from the trapping position to the injection position, the trap tubing was heated directly in order to improve the thermal desorption of sampling gas.

The glass syringe filled with the sampling gas was fixed to the constant temperature oven and set at 25°C or heated to 80°C. After stabilization of the temperature, the sampling gas was warmed for a further 10 minutes and then measured. The output of the constant temperature oven was 500 W, and as the heat capacity of the glass syringe was approximately 100 J/K, it could be completely heated in several minutes. Each standard gas was measured three times after the heating of the sampling glass syringe at 25°C and heated to 80°C.

A capillary column, DB-WAXETR (0.32 mm internal diameter and 30 m length, Agilent Technologies J&W, USA), was used to analyze the VOCs. After an initial column temperature of 40°C for 3 min, the temperature was then programmed to increase at 20°C/min up to 200°C over 8 min, and was then maintained for 5 min. Helium was used as the carrier gas and the make-up gas. The flow rate was 12.0 ml/min for the carrier gas, 20.0 ml/min for the make-up gas, 25.0 ml/min for hydrogen, and 250.0 ml/min for air.

3. Results and Discussion

Fig. 2 presents the gas chromatogram of the standard reference gases acetone, 6-methyl-5-hepten-2-one and hexanal at 50 ppb, at an oven temperature of 25°C and 80°C. The height of the chromatogram for both VOCs is greater at 80°C than at 25°C. Our results basically coincide with the results of Mochalski et al. [24], who suggests that heating a
sampling bag increases the peak area due to increase in the desorption of VOCs.

Fig. 2. Gas chromatogram of acetone, 6-methyl-5-hepten-2-one and hexanal at 50 ppb standard gas, at oven temperatures of 25°C and 80°C.

Fig. 3 presents the gas chromatogram of the skin gas acetone, 6-methyl-5-hepten-2-one and hexanal, at an oven temperature of 25°C and 80°C. The height of the chromatogram for both VOCs is greater at 80°C than at 25°C.

Fig. 4 shows the peak areas of the standard gases (acetone, 6-methyl-5-hepten-2-one, and hexanal) at oven temperatures of 25°C and 80°C. The peak area is represented by the effective carbon number (ECN) (acetone is 2.0, 6-methyl-5-hepten-2-one is 6.9, and hexanal is 5.0) in the FID [16]. Each peak area/ECN of acetone at 50, 100, and 250 ppb at an oven temperature of 80°C was 15, 16, and 9% greater than that at 20°C, respectively. Each peak area/ECN of 6-methyl-5-hepten-2-one at 50, 100, and 250 ppb at an oven temperature of 80°C was 337, 236, and 214% greater than that at 25°C, respectively. Likewise, each peak area/ECN of hexanal at 50, 100, and 250 ppb at an oven temperature of 80°C was 164, 159, and 135% greater than that at 25°C, respectively. This clearly demonstrates that the concentrations of acetone, 6-methyl-5-hepten-2-one, and hexanal increase markedly at an oven temperature of 80°C as compared to 25°C.

Fig. 3. Gas chromatogram of acetone, 6-methyl-5-hepten-2-one and hexanal in skin gas, at oven temperatures of 25°C and 80°C.
The peak area/ECN at 50, 100, and 250 ppb was lower for 6-methyl-5-hepten-2-one as compared to acetone and hexanal. The lower peak area/ECN for 6-methyl-5-hepten-2-one at 80°C might be due to the distribution coefficient $K^{14}$ at the glass syringe. When it is assumed that the standard reference gas has an extremely low concentration and that adsorption by the saturated vapor pressure can be disregarded, $K$ is calculated below.

$$K = \frac{P_m - P}{P} \quad (1)$$

$P$ is the Peak Area/ECN (mV·s) in each gas at 50, 100, and 250 ppb and $P_m$ is the common value in each gas at the same concentration. $P_m$ at 50, 100, and 250 ppb was 14.2, 25.5, and 59.4 (mV·s), respectively, with $R^2 = 1.0000$.

Fig. 5 illustrates the relationship between natural logarithm of $K$ (ln$K$) and the carbon number (3 for acetone, 8 for 6-methyl-5-hepten-2-one, and 6 for hexanal) at an oven temperature of 25°C and 80°C. The ln$K$ is lower at 80°C than at 25°C, and a positive relationship between carbon number and ln$K$ is observed at both temperatures [17]. These results suggest that desorption of VOCs is easy when the number of carbon is small and ln$K$ is low. From these results, a higher VOC peak area can be obtained by heating the glass syringe before injection into the gas chromatograph.

4. Conclusion

Our results suggest that a higher VOC peak area can be obtained by heating the sampling glass syringe before injection into the gas chromatograph and enabled to measure 6-methyl-5-hepten-2-one and hexanal in human skin gas which were difficult measurement in the previous method. The carbon number of VOC is correlation in the molecular weight and boiling point. Measurement by heating method is effective in high molecular weight and high boiling point of VOCs.

Acknowledgement

This human skin gas project was supported by the Aichi Science and Technology Foundation.

References


