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Applications of Hadamard transform-gas chromatography/mass spectrometry to online detection of exhaled breath after drinking or smoking

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ABSTRACT

A Hadamard transform-gas chromatography/mass spectrometry (HT-GC/MS) technique was employed for the online detection of ethanol or toluene in exhaled breath after drinking or smoking, respectively. Exhaled breath samples, collected from volunteers, were directly injected into the GC inlet by a Hadamard-injector without any pretreatment. In the case of breath from a drinker, using a conventional single injection, a small ion peak (corresponding to ~0.1 ng of ethanol), the intensity of which was approximately equal to or less than the limit of detection. When the HT technique was applied, the signal-to-noise (S/N) ratio was dramatically improved. Furthermore, in the case of breath from a smoker, using conventional injection, a weak ion peak (corresponding to ~0.7 pg of toluene) was marginally detected. However, the HT technique led to an improvement in the S/N ratio, with the peak corresponding to the limit of detection. In both cases, the HT technique permitted specific components in exhaled breath to be determined, without the need for any extraction procedures.

1. Introduction

In GC (gas chromatography) separations, extraction and clean up steps are generally needed prior to the actual analysis of a practical sample [1]. For this reason, various extraction methods have been developed and are currently in use. Examples include liquid–liquid extraction, solid-phase extraction, accelerated solvent extraction and supercritical fluid extraction, etc. However, with the emphasis on green chemistry in analytical chemistry, a GC detection method that does not involve the use of solvents would be highly desirable. Hence, the use of a mathematical method is one alternative for improving the limit of detection (LOD) of an analysis and the resolution of analytical measurements. The Hadamard transform (HT) technique has been applied to many fields, including time-of-flight mass spectrometry [2–5], Raman spectrometry [6–8], fluorescence imaging [9–12], ion mobility spectrometry [13,14], NMR [15,16] and capillary electrophoretic separations [17–21]. The application of a multiplexing technique such as a Hadamard transformation has also been demonstrated to be useful in GC [22–25] and liquid chromatography [26–31]. Trapp reported on the use of high-throughput multiplexing GC using the HT method [32]. We also previously reported on applications of the Hadamard transform-gas chromatography/mass spectrometry (HT-GC/MS) method [33–35], in which the signal-to-noise (S/N) ratios of the signals were substantially improved after the inverse Hadamard transformation of the encoded chromatogram. In this study, we report on a Hadamard transform (HT) technique that permits the rapid and sensitive online detection of analytes by GC at low concentration levels with the complete elimination of any extraction step. Ethanol and toluene in exhaled breath samples, which were collected after drinking and smoking, respectively, were determined by GC/MS. The operation steps of HT-GC/MS, the levels of ethanol and toluene in exhaled breath samples, the degree of enhancement in S/N ratios and details of the experimental conditions are reported herein.

2. Experimental

2.1. Reagents

Ethanol and toluene were obtained from Acros (Belgium). Beer (alcohol content, 4.5%; Taiwan Tsing beer Corp., Ltd.) and cigarettes (Mild Seven, Japan Tobacco) used in this study were purchased from a local supermarket. All other chemicals and gases were of analytical grade and were obtained from commercial sources.

2.2. Apparatus

A gas chromatograph/mass spectrometer (GC 5890 equipped with 5972 mass selective detectors; Hewlett-Packard, Avondale, PA, USA) was used in this study. An in-house fabricated Hadamard-

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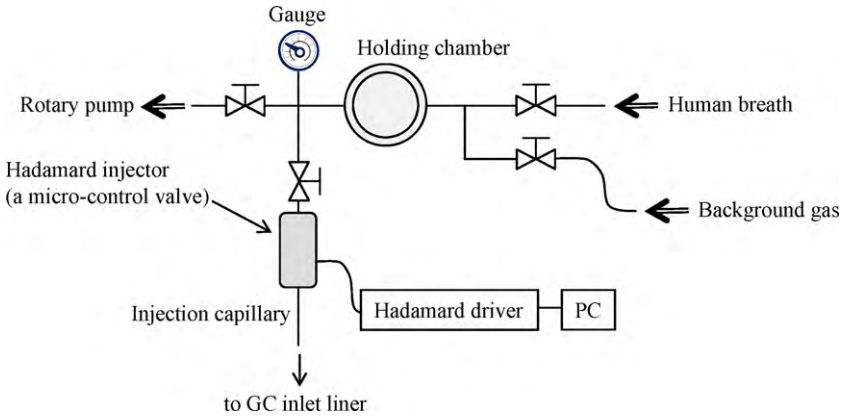

Fig. 1. A schematic diagram of the online HT-GC/MS detection system.

injector was used for the sample injection and was controlled *via* a personal computer through a PCI 6221 device (National Instruments, USA), according to a series of Hadamard codes. A capillary column (30 m × 0.25 μm I.D.) with an DB-5MS (cross-linked 5% PH ME siloxane) bonded stationary phase film, 0.25 μm in thickness (Agilent Technologies, USA), was used. The inlet temperature was maintained at 150 °C and the column oven was also held at 80 °C (carrier gas: helium, flow-rate 1.2 mL/min operating in either the splitless or split mode). The mass spectrometry conditions were as follows: ionization energy, 70 eV; and, ion source temperature, 250 °C. A selected ion monitor (SIM) mode was used by selecting ion peaks at m/z = 31, 45 and 46 for ethanol and 91 and 92 for toluene, respectively. Dwell values were set at 25 and 45 for ethanol and toluene, respectively; 8 dots/s could be recorded. Data were collected using Hewlett-Packard Chem-Station software with transfer to an ASCII text file. The average was calculated for each 12 dots that were treated as one bin to fit the HT calculation. A commercially available hand breathalyzer (Dräger Alcotest 6510, USA) was used for the comparison of the alcohol analysis.

The HT-GC chromatograms were calculated using the LabVIEW 8.6 program, as described previously [33].

3. Results and discussion

3.1. Hadamard-injector and exhaled breath collection

Fig. 1 shows a schematic diagram of the online detection HT-GC/MS system used. A stainless holding chamber (0.7 L) was used for the collection of exhaled breath. The Hadamard-injector (an in-house fabricated micro-control valve) was prepared by modifying a regular pulse nozzle, as described previously [34,35]. The injection volume of the pressurized sample was adjusted by changing the background pressure, the inner diameter of the capillary, the capillary length and the injection time. Once the exhaled breath sample was slowly and continually blown through the holding chamber, the chamber and the Hadamard-injector were pressurized by background N_2 gas. In the meantime, a personal computer was used to rapidly turn the Hadamard-injector on and, according to a series of Hadamard codes, leading to the introduction of the pressurized gas sample of exhaled breath through the capillary into the GC column. The optimized background pressure was 1.8 kg/cm^2. The injection times for the drinking and smoking experiments were 65 and 500 ms, respectively, resulting in injection volumes of 1 and 43 μL for each single injection.

3.2. Online detection of exhaled breath after drinking.

Fig. 2 shows typical GC/MS chromatograms for breath samples of an adult volunteer (weight, 73 kg), collected at 30 min (frame A) and 180 min (frame B) later after drinking 485 mL of beer (4.5%, alcohol), obtained by means of the SIM (selective ion monitoring) mode (ion peaks of m/z = 31, 45 and 46). The GC conditions are shown in the figure caption. In frame A, chromatogram (a) shows the result obtained for a single injection (injection volume of exhale breath, 1 μL). The inset (above chromatogram (a)) shows the result obtained for a 0.2 μL volume of saturated ethanol vapor at room temperature. Under these conditions, the concentration of ethanol was estimated to be ~20 ng/μL (ion intensity, 4090). Compared to this, the breath sample showed a weak ion peak for ethanol (ion intensity, 20), since the ethanol level in 1 μL of exhaled breath is extremely low (estimated to ~0.1 ng). In general, such a small amount would be difficult to detect without the use of extraction steps. However, under the same experimental conditions, when the Hadamard injection was performed (as shown in chromatogram (b); matrix order, n = 255), the S/N ratio was dramatically improved by 7.9-fold. The inset (above chromatogram (b)) shows the raw data prior to the inverse Hadamard transformation. The detected peak is completely consistent with the theoretically predicted value, suggesting that the HT-GC/MS injection device functions very well. The entire measurement can be completed within ~15 min. Furthermore, when a higher matrix order (n = 1023) was used, the S/N ratio was further improved by 11–13-fold (depending on the subject and the measurements). Frame B shows the results obtained for the same beer drinking experiment after 180 min. As can be seen from chromatogram (c), a small, marginal peak corresponding to ethanol can be seen. As expected, when the Hadamard injection was used (as shown in chromatogram (d); matrix order, n = 255), the S/N ratio was substantially improved. The inset (above chromatogram (d)) shows the raw chromatogram prior to the inverse Hadamard transformation. In fact, several studies dealing with ethanol concentrations in venous blood and expired breath have been reported [36–40]. In this study, the tendency of breath alcohol *vs.* time (h, after drinking) was investigated by HT-GC/MS as well as a breathalyzer. Fig. 3 shows the tendency for ethanol to decay in volunteers after beer drinking, as determined by the HT-GC/MS technique (A) and a hand breathalyzer (B), respectively. In the former case, the plots were obtained from the average of three runs; the order of the Hadamard matrix was set at 255. As can been seen, the concentration of ethanol in exhaled breath reached a maximum value at about 0.5–1 h (depending on the subject) after drinking. These results are also in very good agreement with the breathalyzer used in this study. It should be note that alcohol is cleared by zero order kinetics in both cases. Although the breathalyzer used in this study

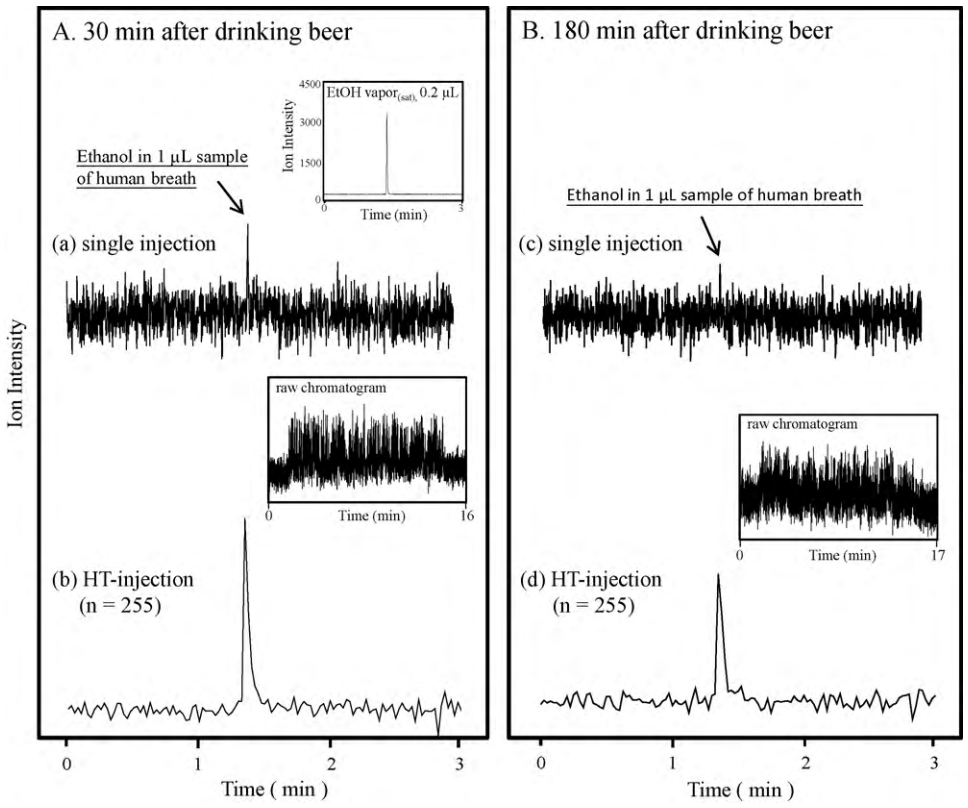

Fig. 2. Typical HT-GC/MS chromatograms of breath samples from a volunteer. The samples were collected at 30 min (frame A) and 180 min (frame B) after drinking 485 mL of beer, respectively. Chromatograms (a) and (c), single injection; (b) and (d), Hadamard injection (order of matrix, 255). GC conditions: outlet pressure, 1.8 kg/cm^2; head pressure, 8 psi; split purge, 317 mL/min; inlet, oven and detector temperatures were 150, 80 and 250 °C, respectively. The inset (above chromatogram (a)) shows the result obtained using a 0.2 μL volume of saturated ethanol vapor at room temperature; the insets (above chromatograms (b) and (d)) show the raw data prior to the inverse Hadamard transformation.

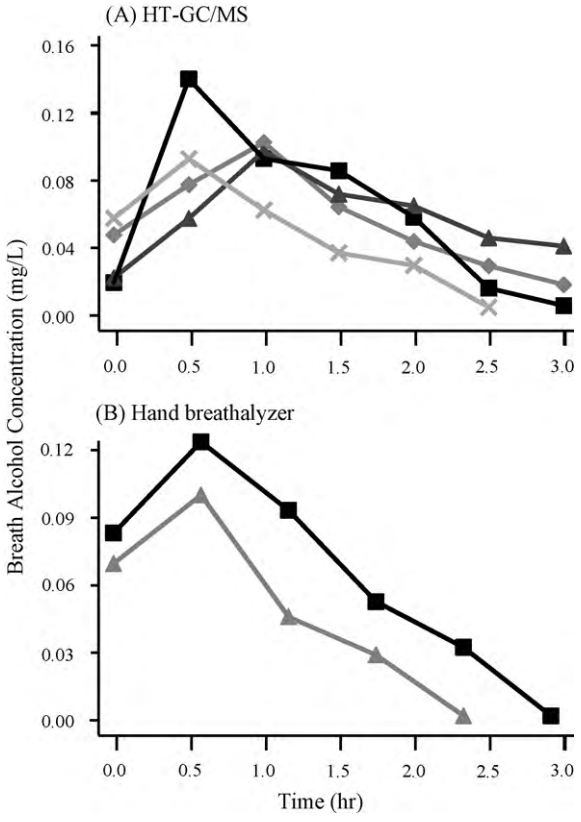

Fig. 3. The trend for breath alcohol level *vs.* time (h, after drinking) obtained by HT-GC/MS and a breathalyzer, respectively.

is portable and can be easily used for estimating blood alcohol content based on a breath sample, the detectable range for breath alcohol is 0.00–2.5 mg/L (ethanol mass per breath volume at 34 °C and 1013 hPa). It should be noted that the breathalyzer respond to a variety of in addition to ethanol, such as methanol, *iso*-propanol, acetone, formic acid and acetic acid. In contrast to this, as a complementary method, the HT-GC/MS provides not only better LOD values (~0.002 mg/L; the order of Hadamard matrix, $n = 255$) but also higher accuracy and selectivity.

3.3. Online detection of exhaled breath after smoking

Breath air is a complex mixture of volatile organic compounds (including alcohols, aldehydes, hydrocarbons, etc.), non-volatile organic compounds and inorganic compounds (such as water vapor, carbon dioxide, oxygen and nitrogen) [41–44]. Some of these compounds are even recognized as biomarkers. However, in all of the exhaled breath studies, pre-concentration processes were necessary, involving either the use of solid-phase microextraction methods [41,42], a microwave desorption device [43] or a capillary microtrap thermal desorption module [44], respectively, since the online detection of exhaled breath of exhaled breath is still difficult.

In this study, we selected toluene as an indicator of smoking. Fig. 4 shows the results obtained from breath samples of a smoker before (frame A) and after (frame B) smoking, respectively. Employing the SIM mode (ion peaks of $m/z = 91$ and 92), the HT-GC/MS chromatograms were obtained from the breath samples before smoking (frame A) and 15 min after smoking (frame B), respectively. Since the concentration of toluene in breath is extremely low, the injection volume was increased to 43 μL. As can be seen, in a single injection (chromatogram (a)) the toluene

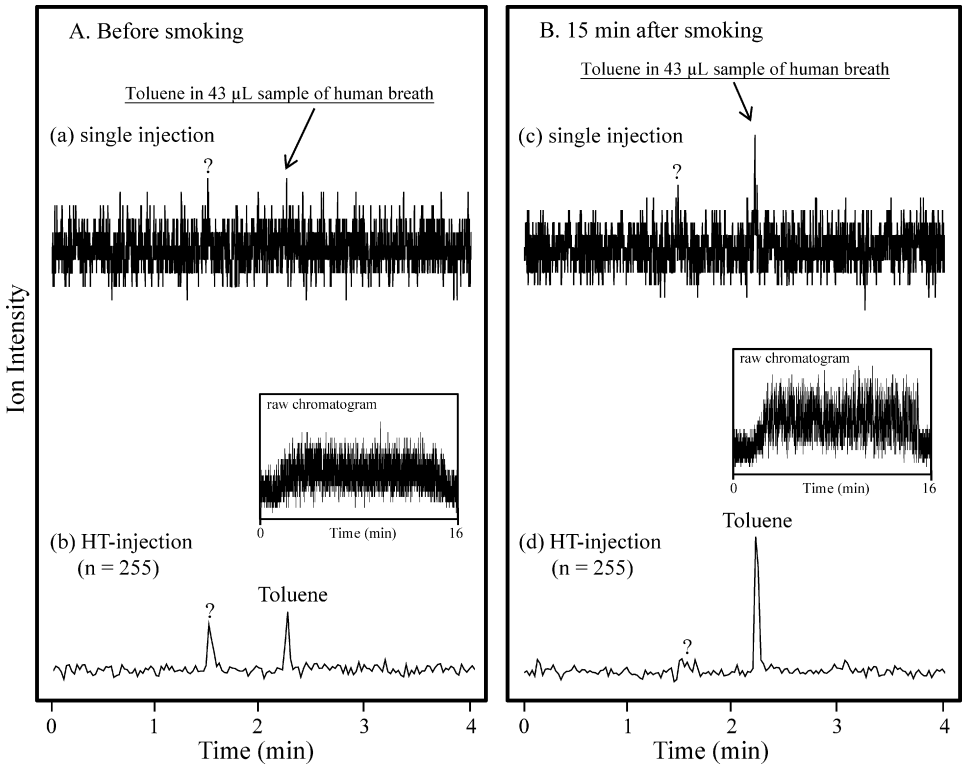

Fig. 4. Typical HT-GC/MS chromatograms of breath samples, before (frame A) and after smoking (frame B). Chromatograms (a) and (c), single injection; (b) and (d), Hadamard injection (order of matrix, 255). GC conditions: outlet pressure, 1.8 kg/cm^2; head pressure, 8 psi; split purge, 17 mL/min; inlet, oven and detector temperatures were 150, 80 and 250 °C, respectively. The insets (above chromatograms (b) and (d)) show the raw data prior to the inverse Hadamard transformation.

Table 1
Relationship between the enhancement in S/N ratios for the analyses in drinking and smoking experiments.

Matrix order	Enhancements of S/N ratios		
	Theoretical	Drinking-experiments	Smoking experiment
255	8.02	7.5–8.0	7.9
1023	16.01	10.8–12.8	15.7

The enhancement in S/N ratio was calculated as the ratio of S/N values obtained in the chromatograms, measured by HT-GC/MS and a single injection method.

peak (indicated an arrow) is difficult or impossible to identify. In contrast to this, the S/N ratio is substantially improved when the Hadamard injection method is used (chromatogram (b); matrix order, $n = 255$). Consequently, it was possible to determine low levels of toluene from the breath air sample, even before smoking. The inset (above chromatogram (b)) shows the raw chromatogram prior to the inverse Hadamard transformation. Frame B shows the results obtained from the same volunteer after smoking. As can be seen from chromatogram (c), although the toluene peak appears stronger (estimated as ~0.7 pg), when the Hadamard injection was performed again (as shown in chromatogram (d); matrix order, $n = 255$), the S/N ratio was improved; the inset above chromatogram (d), the raw data prior to the inverse Hadamard transformation. Furthermore, when a higher matrix order ($n = 1023$) was used, the S/N ratio was further improved by 15.7-fold, although the total time required for the run was longer at ~50 min. Table 1 summarizes the results and, it can be clearly seen that the S/N ratio is significantly enhanced, as predicted from theoretical observations.

4. Conclusion

In this study, we demonstrate the applicability of HT-GC/MS method, using a Hadamard-injector, for the rapid and sensitive online detection by GC, where the analytes are present at very low levels and adsorption/desorption steps are omitted. Low levels of ethanol and toluene were successfully determined in exhaled breath samples after drinking or smoking, respectively. The enhancement factors for the S/N ratios were in good agreement with the theoretical values. Furthermore, the present technique would also be applicable for the monitoring of air and exhaust samples with respect to green chemistry in analytical chemistry. Thus, the present method has a variety of potential applications and could be used in practical trace analysis.

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