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Applications of Hadamard transform-gas chromatography/mass spectrometry to the detection of acetone in healthy human and diabetes mellitus patient breath

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ABSTRACT

The Hadamard transform-gas chromatography/mass spectrometry (HT-GC/MS) technique was successfully employed to detect acetone, a biomarker for diabetes mellitus (DM) prediction, in human breath. Samples of exhaled breath were collected from four DM patients (one type-I and three type-II) and eight volunteers (nondiabetic healthy subjects), respectively. The gas samples, without any pretreatment, were simultaneously injected into a GC column through a Hadamard-injector based on Hadamard codes. Under optimized conditions, when cyclic S-matrix orders of 255, 1023 and 2047 were used, the S/N ratios of the acetone signals were substantially improved by 8.0-, 16.0- and 22.6-fold, respectively; these improvements are in good agreement with theoretically calculated values. We found that the breath acetone concentration levels in the four DM patients and the eight volunteers ranged from 1 to 10 ppmv and 0.1 to 1 ppmv, respectively.

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1. Introduction

As a non-invasive glucose monitoring technique, the analysis of breath samples has the potential for the monitoring of early stages of DM [1–4]. Normally, when the blood glucose levels are elevated, insulin is released from the pancreas to normalize these levels. However, in patients with DM, the absence or insufficient production of insulin results in hyperglycemia. The diagnosis of DM can be established including any of the following criteria, glycohemoglobin A1c (HbA1c) ≥6.5%, plasma glucose ≥126 mg/dL after an overnight fast, symptoms of DM and a random plasma glucose level of ≥200 mg/dL. Symptoms of diabetic ketoacidosis can be detected by the smell on a person's breath because acetone is produced as a direct byproduct of the spontaneous decomposition of acetoacetic acid. This is the reason for why it is often described as smelling like fruit or nail polish remover. It should be noted that mild acidosis may result from prolonged fasting or when following a ketogenic diet or a very low calorie diet [5,6]. HbA1c provides an integrated measure of blood glucose values over the preceding 2–3 months. Chronic hyperglycemia that persists, even in fasting states, is most commonly caused by DM and can be diagnosed by an analysis for HbA1c; there are two main types of DM. Type 1 DM results from the body's failure to produce insulin, and currently the patient requires to inject insulin or wear an insulin pump, whereas Type 2 DM, noninsulin-dependent DM or "adult-onset DM", results from insulin resistance, a condition in which β cells fail to use or produce insulin properly, and is sometimes combined with an absolute insulin deficiency. Since the concentration of acetone in the breath is very low, it is very difficult to accurately measure it in human breath. Furthermore, little information is available on correlations of acetone breath with diabetic diagnostic parameters, namely, blood glucose (BG), HbA1c and ketoacidosis (in urine and blood, respectively). Consequently, a novel and more sensitive method for detecting and quantifying acetone in exhaled breath would be highly desirable. Traditionally, gas chromatography–mass spectrometry (GC–MS) is currently one of the most popular, widely used and powerful techniques for the analysis of volatile organic compounds (VOCs) and analogs thereof. When a solid-phase microextraction with on-fiber derivatization was applied, it is possible to reach a detection limit of 0.05 ppbv for acetone [7]. An in-needle preconcentration method was also reported [8] for detecting acetone from urine and air samples. Various methods for the determination of acetone

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were also reported, including the uses of a light emitting diode (LED)-based photometric method [9], ion mobility spectrometer [10], and solid-phase microextraction [11]. Each method mentioned above has a unique advantage and disadvantage regarding sensitivity, precision and simplicity of use. The Hadamard transform (HT) technique has been applied to many fields, including time-of-flight mass spectrometry [12–15], Raman spectrometry [16–18], fluorescence imaging [19–22], ion mobility spectrometry [23,24], NMR [25,26] and capillary electrophoretic separations [27–31]. The application of a multiplexing technique such as a Hadamard transformation has also been demonstrated to be useful in GC [32–35] and liquid chromatography [36–41]. Trapp reported on the use of high-throughput multiplexing GC using the HT method [42]. We also previously reported on applications of the Hadamard transform-gas chromatography/mass spectrometry (HT-GC/MS) method [43–47]. In this study, we report on a HT-GC/MS method that permits the rapid and sensitive detection of acetone in the breath at the sub-ppmv levels. The use of HT-GC/MS to detect trace levels of acetone, the degree of enhancement in S/N ratios and details of the experimental conditions are described herein. Furthermore, the use of headspace solid-phase microextraction SPME–GC–MS for the analysis of acetone in human breath was also performed and compared.

2. Experimental

2.1. Reagents and breath samples

Acetone (liquid) and the standard acetone gas (10 ppmv in nitrogen) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and National Oxygen Pte Ltd (Singapore), respectively. All other chemicals and gases were of analytical grade and were obtained from commercial sources. Exhaled breath samples were collected from DM patients (type-I and type-II DM patient) and volunteer (nondiabetic healthy subject), respectively, with the permission of the TMU-Joint Institution Review Board 201211015.

2.2. Apparatus

An in-house fabricated Hadamard-injector (a micro-control valve that had been modified from a regular 24 V pulse electromagnetic valve), was used for the sample injection [44–46]. It was controlled via a personal computer through a PCI 6221 device (National Instruments, USA), based on a series of Hadamard codes. A gas chromatograph (GC 5890 Hewlett-Packard, Avondale, PA, USA) equipped with a mass spectrometer (Hewlett-Packard 5972 mass selective detector) was used, in which a capillary column (30 m × 0.25 mm I.D.) with an HP-5MS (cross-linked 5% PH ME siloxane) bonded stationary phase film, 0.25 μm in thickness (Agilent Technologies, USA) was employed. A commercial headspace SPME device, extraction needle (model SU-57310U) and fiber (carboxen/polydimethylsiloxane, 75 μm) were obtained from Supelco (Bellefonte, USA).

2.3. Experimental method and conditions

Fig. 1 shows a schematic diagram of the on-line HT-GC/MS system. This system consists of a Tedlar bag (human breath gas), a stainless steel container (standard acetone gas), sample reservoir, a homebuilt Hadamard-injector and a commercial GC/MS instrument. The breath sample, in a Tedlar bag either collected from the DM patients or from the volunteers (nondiabetic healthy subjects), was transferred to the sample reservoir by suction, where a vacuum pump was used to draw out the gas. When the sample reservoir was filled in the sample, the valves were closed.
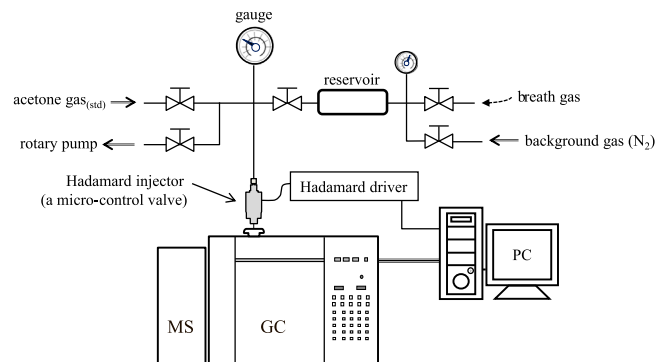

Fig. 1. Schematic diagram of the on-line HT-GC/MS system used.

Following this, the Hadamard-injector was pressurized by background N_2 gas to $2.0\ kg/cm^2$. A personal computer was used to rapidly turn the Hadamard-injector on and off, based on a series of Hadamard codes, leading to the introduction of the pressurized gas sample through the capillary into the GC column. The optimized conditions were a background pressure of $2.0\ kg/cm^2$ and an injection time of 0.02 s (actually injected volume, 1.6 μL). The inlet temperature was maintained at 180 °C and the column oven was also held at 45 °C (carrier gas: helium, flow-rate 0.8 mL/min). The temperature program was set at 45 °C (head pressure, 4.4 psi; split purge, 30 mL/min). The mass spectrometry conditions were as follows: ionization energy, 70 eV; and, ion source temperature, 260 °C. The selected ion monitor (SIM) mode was used for acetone by selecting ion peaks at $m/z=43$ and 58, respectively. The dwell value was set at 33 and 32 ms, resulting to 10 data points/second being recorded.

3. Results and discussion

All of the data (ion intensity) were recorded by Hewlett-Packard Chem-Station software with transfer to ASCII text. The average was calculated for each 10 data points that were treated as one bin to fit the HT calculation. In the beginning, the sample gas was injected by 50% duty-cycle of pulse width; where a duty cycle is defined as the ratio between the pulse duration and the period of a rectangular waveform. Thus, the code "…0101…" means that the Hadamard-injector turns "…off–on–off–on…", where the gas samples were injected into the GC inlet based on the Hadamard code. However, we found that the periods between each peaks were too close to permit them to be separated. As a result, the coded mass chromatogram was not well matched with the Hadamard code, as shown in Fig. 2A.

For this reason, we changed the duty-cycle to 12.5%. In this case, the code "…$0_{000}1_{000}0_{000}1_{000}$…" means that the Hadamard-injector turns "…off $_{off\ off\ off}$ -on $_{off\ off\ off}$ -off $_{off\ off\ off}$ on $_{off\ off\ off}$…", etc. The actual injection time was 0.02 s, corresponding to a sample injection volume of 1.6 μL. The HT-GC-chromatograms were calculated using a homebuilt LabVIEW program. Under these conditions, acetone is rapidly detected at a retention time 84 s. When a single injection was employed, the S/N ratio appeared to be poor.

However, under the same experimental conditions, when the Hadamard injection was performed (as shown in Fig. 3A; cyclic S-matrix order, $n=255$), the S/N ratio was dramatically improved by 7.9-fold after performing the Hadamard transform (as shown in Fig. 3B; total injection volume 0.2 mL, during a period of 6.4 min). This value is in relatively good agreement with the theoretical value (8.02-fold). Under optimized conditions, when cyclic S-matrix orders of 255, 1023 and 2047 were used, the S/N ratios
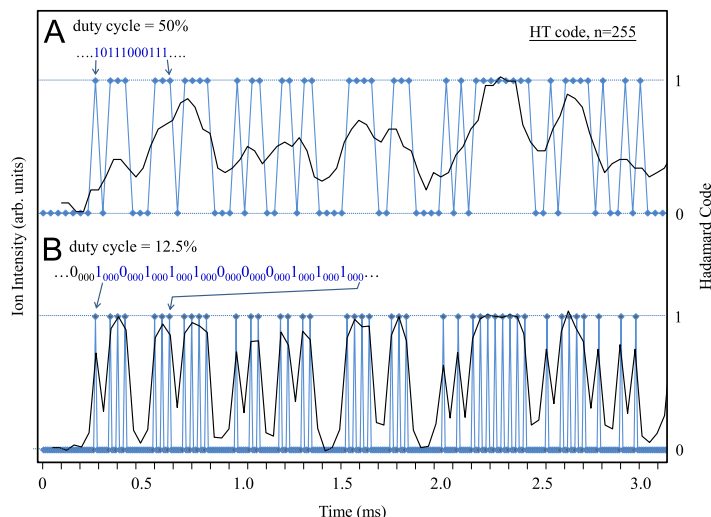
Fig. 2. Frame A, Hadamard code performed at a 50% duty-cycle. Frame B, Hadamard code performed at a 12.5% duty-cycle.
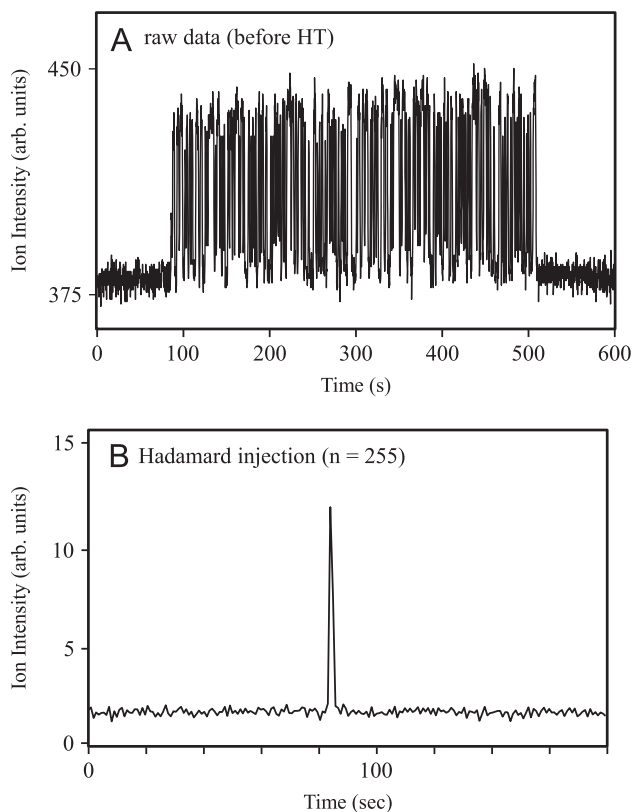

Fig. 3. (A) Hadamard encoded GC–MS chromatograms of acetone standards (10 ppmv) obtained by a single injection (chromatogram a) using the SIM mode (ion peaks at $m/z=43$ and 58 were selected for monitoring). (B) Hadamard decoded GC–MS chromatogram of acetone standard (10 ppmv).

Fig. 4. Typical GC–MS chromatograms for a breath sample obtained from a volunteer. Chromatograms a and b, results obtained by a syringe injection (injection volume, 10 μL) and Hadamard injection (order of matrix, 255), respectively. Chromatogram c, result obtained by the traditional headspace solid-phase microextraction method.

of the acetone signals were substantially improved by 8.0-, 16.0- and 22.6-fold, respectively; these improvements are in good agreement with theoretically calculated values. It is clear that the S/N ratio is significantly enhanced, as predicted from the theory. In order to evaluate the applicability of the present method for the practical detection of acetone in human breath, a complementary experiment was performed.

Fig. 4 shows typical HT-GC/MS chromatograms obtained from a gas sample obtained from a volunteer (SIM mode: $m/z=43$ and 58). The GC and MS conditions are the same as described above. As can be seen, when a single injection is used (Fig. 4) it is difficult to
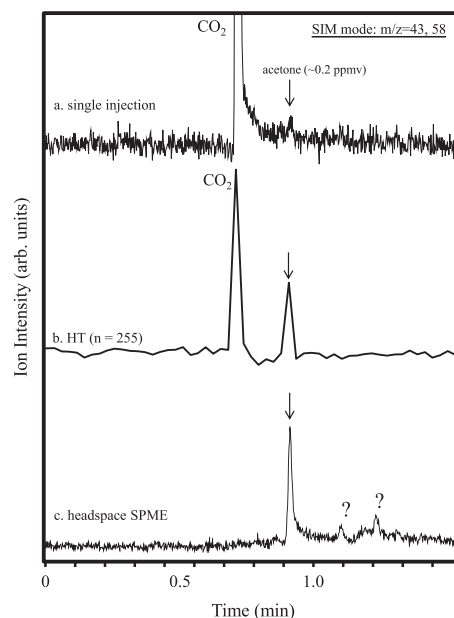
identify the peak for a low concentration; the S/N ratio appeared to be poor in chromatogram a, which involved injection via a syringe (injection volume, 10 μL). Based on a calculation curve, the concentration of acetone in the breath sample was determined to be 0.2 ppmv. It is clear that the concentration of acetone in breath from a normal subject is extremely low. In contrast to this, under the same experimental conditions, when the Hadamard injection method was used (as shown in chromatogram b; matrix order, $n=255$), the S/N ratio was dramatically improved. LOD and LOQ were determined at 0.1 and 0.3 ppmv, respectively; a linear range was found at the range of 0.1–100 ppmv. The observed improvement in the S/N ratio of the detected peak conforms to the improvement in S/N that would be mathematically expected after application of an n-order Hadamard transform, suggesting that the HT-GC/MS injection device functions very well, even for acetone at the sub-ppmv level in a sample of human breath. Thus, this

Table 1
Breath acetone, blood glucose (BG), HbA1c and ketoacidosis data for type 1 and type 2 DM patients (T1D/T2D) and nondiabetic healthy subjects, respectively.

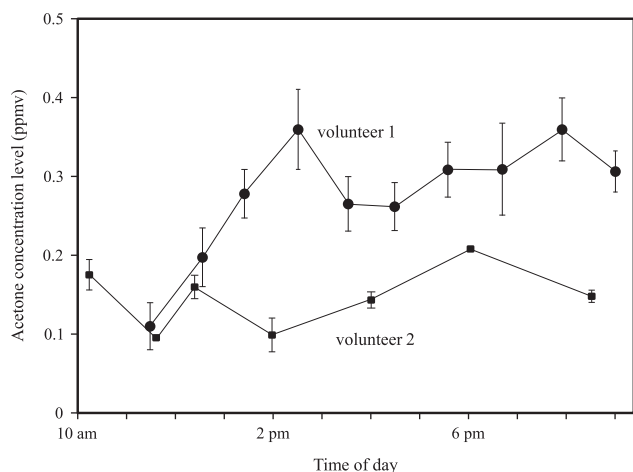| Subject no.	Breath acetone (ppmv)	BG (mg/dl)	HbA1c (%)	Ketoacidosis (urine)	Ketoacidosis (blood)
DM patients					
T1D					
1	2.2 +	230	–	1+	Negative
1[a]	–	179	10.7	1 –	Negative
T2D					
2	2.4	76	9.7	Negative	Negative
3	2.9	240	9.7	Negative	Negative
4	9.4	226	–	Negative	Negative
4[a]	3.3	180	–	Negative	Negative
Nondiabetic healthy subjects					
1	0.2				
2	0.5				
3	0.3				
4	0.5				
5	0.7				
6	0.1				
7	0.2				
8	0.2				

Fig. 5. The relationship between acetone concentration and sampling time for two volunteers during a 12 h period.

[a] After lunch.

method would be useful for screening patients who are at risk for DM at early stage. As an alternate procedure, traditional headspace solid-phase microextraction (HS-SPME) was also employed for comparison. For the case of a single measurement, the extraction process was completed within 4 min. As shown in chromatogram c, in addition to two unknown peaks, the peak corresponding to acetone was obviously improved; the SPME fiber (carboxen/poly-dimethylsiloxane) does not absorb CO_2 and this is the reason for why a CO_2 peak was not observed. The use of the headspace SPME–GC–MS method was also useful for improving the limit of detection. It should be noted that the Hadamard transformation is suitable for the analysis of a wide variety of gas samples, whereas, in the case of SPME, it is necessary to select the appropriate microextraction syringe, depending on the types of gases being analyzed, in order to achieve desired results.

Table 1 summarizes the results including breath acetone, blood glucose (BG), HbA1c and ketoacidosis data obtained for the type 1 and type 2 DM patients (T1D/T2D) and nondiabetic healthy subjects, respectively. The breath acetone concentration levels in the four DM patients and the eight volunteers ranged from 1 to 10 ppmv and 0.1 to 1 ppmv, respectively. Both blood glucose and HbA1c levels were related to the concentration of breath acetone. Furthermore, no

obvious difference was found for breath acetone levels between type-I and type-II patients; during the process of gas collection, and deep- and shallow-breathing did not result in any change in the acetone concentration. In order to evaluate the applicability of the present method to an actual sample, breath samples obtained from two volunteers were investigated during 12 h periods.

Fig. 5 shows the relationship between acetone concentration and sampling time. Although the acetone concentrations varied during the day, the low values were maintained in the range of 0.1 to 0.4 ppmv. The results indicate that the present HT method and the use of a Hadamard-injector, with a simple design, permits precise multiple injections for actual sample determination. As a non-invasive glucose monitoring technique, this method had the potential for the detection and monitoring of DM at early stages

4. Conclusion

We successfully demonstrated that the HT-GC/MS method using a Hadamard-injector is applicable to the sensitive detection of acetone in human breath samples by GC, where acetone is present at very low levels. The enhancement factors for the S/N ratios were in good agreement with theoretical values, even at a high matrix order ($n = 2047$). The present technique was applied to the monitoring of samples of exhaled breath collected either from DM patients (type-I and type-II diabetic patients) or normal subjects (nondiabetic healthy subjects), respectively. Thus, the present method has a variety of applications and could potentially be used in practical trace analysis.

Acknowledgment

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