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Rapid determination of acetone in human plasma by gas chromatography–mass spectrometry and solid-phase microextraction with on-fiber derivatization

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Abstract

Acetone is an important volatile disease marker. Due to its nature of activity and volatility, it is a difficult task to measure the concentration of acetone in biological samples with accuracy. In this paper, we developed a novel method for determination of trace amount acetone in human plasma by solid-phase microextraction technique with on-fiber derivatization. In this method, the poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) fiber was used and *O*-2,3,4,5,6-(pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) was first loaded on the fiber. Acetone in plasma sample was agitated into headspace and extracted by solid-phase microextraction (SPME) fiber and subsequently derivatized with PFBHA on the fiber. Acetone oxime was analyzed by gas chromatography–mass spectrometry (GC–MS). Quantitative analysis of acetone in plasma was carried out by using external standard method. The SPME conditions (extraction temperature and time) and the method validation were studied. The present method was tested by determination of acetone in diabetes plasma and normal plasma. Acetone concentration in diabetes plasma was found to be higher than 1.8 mM, while in normal plasma was lower than 0.017 mM. The results show that the present method is a potential tool for diagnosis of diabetes.

Keywords: Derivatization; GC; Acetone

1. Introduction

There are two sources of acetone production in mammals: the decarboxylation of acetoacetate and the dehydrogenation of isopropanol. The former compound is the major source of acetone in mammals and arises from either lipolysis or amino acid dedegradation [1]. After administration of 2-^{14}C-acetone to rats by stomach tube or by injection, the examination of animal carcasses led to a demonstrable amount of radioactivity in glycogen, urea, cholesterol, fatty acids, amino acids, heme as well as a substantial amount of ^{14}C was recovered in vivo in exhaled carbon dioxide [2,3]. Experimental results demonstrated that acetone metabolism happened through acetate and formate. In mammals, acetone metabolic disturbance makes plasma acetone level increase and the related disease is referred as acetonemia. Acetone concentration was found to elevate by at least two degrees of magnitude in the plasma of diabetic patients [4,5]. Even in treated diabetic patients, its concentration is much higher than in normal population [6]. The defect of complex enzyme of propionyl–CoA carboxylase (EC 6.4.1.3) or the methylmalonyl–CoA mutase (EC 5.4.99.2) also led to the increase of acetone concentration [7,8]. Recently, Kalapos pointed out in a review that acetone is an important volatile disease marker [1].

Over the decades, several methods were used for its determination in biological samples. In the beginning, colorimetric methods were developed and used for the determination of acetone in plasma [9–11]. These methods have common disadvantages: the lack of specificity and detection limit. In the last decades, gas chromatographs equipped with flame ionization detector or mass spectrometric detector has been developed for determination of acetone concentrations in body fluids and in expired air [12–16]. High-performance

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liquid chromatography (HPLC) with derivatization agent of 2,4-dinitrophenyl-hydrazine (DNPH) was also applied to determination of acetone in plasma samples [17]. Solid sorbents and evacuated stainless steel canisters were used as techniques for collection and preconcentration of acetone in human breath [18–20], and then the enriched acetone was analyzed by gas chromatography–mass spectrometry (GC–MS) detector or other detectors. Kundu et al. developed a simple dip-and-read color matching assay measures acetone in urine and breath [21,22]. Recently, a relative novel technique, solid-phase microextraction (SPME), combined with GC–MS was developed for determination of acetone in plasma [23] and in breath [24,25]. Acetone in plasma was headspace extracted by the fiber and desorbed at GC injector and analyzed by gas GC–MS [23].

However, due to its nature of activity and volatility, acetone is easily lost onto the surfaces of GC injectors and column. Therefore, it is very difficult to accurately measure acetone in plasma or breath by GC method. HPLC method requires complex sample preparation and is a time-consuming procedure [17].

Recently, GC with the derivatization agent of *O*-2,3,4,5,6-(pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) was developed for accurate analysis of carbonyl compounds in air or water. In the method, carbonyl compounds including aldehyde and ketone can be converted, through derivatization with PFBHA, into chemical forms that are thermally stable and suitable for GC analysis. Cancho et al. developed GC–MS with SPME for analysis of aldehyde compounds in water [26]. Aldehyde compounds in water were first derivated with PFBHA, and then the formed oximes were extracted by SPME fiber and analyzed by GC–MS [26]. The reaction of carbonyl compounds with PFBHA could complete at ambient temperature for several seconds. A simpler method, GC–MS and SPME with on-fiber derivatization was applied to determination of aldehyde compounds in air and water [27–30]. Ho and Yu demonstrated the feasibility of collection and analysis of airborne carbonyls by on-sorbent derivatization and thermal desorption [31].

In this paper, GC–MS and SPME with on-fiber derivatization was developed for qualitative analysis of acetone in human plasma. The poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) fiber was used and PFBHA in solution was first loaded on the fiber. Acetone in plasma sample was headspace sampled by SPME and rapid derivatized with PFBHA on the fiber. Determination of acetone concentration in diabetes and normal plasma was performed by measurement of its oxime by GC–MS.

2. Materials and methods

2.1. Materials and chemicals

Acetone and *O*-2,3,4,5,6-(pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) were purchased from Sigma (St. Louis, MO, USA). Thirteen plasma samples were collected from Hospital of East China, Shanghai, China. Six normal subjects were three men (age: 23, 35 and 40 years) and three women (age: 29, 33 and 52 years), while seven diabetes patients were two women (age: 43 and 52 years) and five men (39, 46, 48, 51 and 56 years). Solid-phase microextraction holder and the fiber of 65 μm PDMS/DVB were from Supelco (St. Louis, MO, USA). The magnetic stirrer was purchased from ShiLe Company (Shanghai, China). The magnetic stirrer also allowed temperature control. Bidistillation water was used for preparation of 11.0 mg/ml of PFBHA solution.

2.2. Preparation of acetone calibration solutions for qualification

Determination of acetone in plasma was performed by external standard method. Consideration of the effect of plasma matrix, the plasma free of acetone was made for preparation of calibration solutions. Twenty milliliter of plasma sample from a normal human and a 4 cm stir bar were placed in a 40 ml glass bottle without lid. To get rid of acetone from the plasma completely, it was heated at 60 °C for 120 min, stirred at 1100 rpm. Calibration solutions of 0.00672, 0.0672, 0.672, 1.34 and 2.68 mM were made by dissolving acetone into the prepared plasma.

2.3. GC–MS conditions

All analyses were performed on HP 6890 GC system, coupled with a HP MD5973 quadruple mass spectrometer. Compounds were separated by using a 30 m, 0.25 mm i.d., 0.25 μm film HP-5MS fused-silica capillary column (Agilent, USA). The carrier gas was helium with flow rate of 1.0 ml/min. Splitless mode was used. The injector temperature was set as 250 °C. The column temperature programs were: initial temperature of 60 °C, increase to the final temperature of 270 °C at 10 °C/min, hold for 5 min. The temperature of mass spectrometer was 230 °C.

2.4. Loading SPME fiber with PFBHA

PDMS/DVB (65 μm) fiber was selected because it adsorbed PFBHA with greater reproducibility than the other fibers, such as 100 μm PDMS. 1.0 ml solution of PFBHA (11 mg/ml) in acetone–free water was placed in 4 ml PTFE capped vials with 1 cm stir bar. The solution was stirred at 1100 rpm. The PDMS/DVB fiber was placed in the headspace of the solution. To get the adsorption-time profile, the SPME fibre was exposed to headspace of the solution at ambient temperature for different time of 0.5, 1, 2, 5, 10, 15, 20 and 25 min, respectively. Chromatographic peak areas were used for adsorbed PFBHA quantification. To ensure the desorption was complete when the SPME needle was inserted into GC injector, different desorption times was tested to examine the desorption efficiencies.

2.5. Derivatization and SPME procedures

Headspace extraction was used in this study to avoid possible contamination from plasma samples and damage to the fiber that might occur through direct liquid contact [23].

Headspace extraction conditions were optimized by using a 1.0 ml calibration solution (1.34 mM). A volume with 1.0 ml of the calibration solution was placed into 8 ml PTFE-capped vial with 1 cm stir bar. The solution was stirred at 1100 rpm for 2 min to allow the equilibrium of analytes between the aqueous phase and the headspace phase. After loading with PFBHA, the SPME fibre was inserted in the headspace of the calibration solution. Adsorption time (2, 5, 10, 15 and 20 min) and temperature (25, 30, 35, 40 and 50 °C) were investigated.

The optimum adsorption conditions (25 °C, 10 min, stirred at 1100 rpm) were used for extraction of acetone in the plasma samples from normal human and diabetic patients. The acetone oxime on the fiber was desorbed at GC injector under the conditions of 250 °C for 3 min and then analyzed by GC–MS.

Calibration solutions were adsorbed and analyzed by the same procedures described above.

2.6. Precision, recovery and detection limit

Five replicate measurement of two calibration solutions (0.0134 and 1.34 mM) were carried out. Precision was assessed by calculating relative standard deviation (%R.S.D.) of the observed values.

The recovery was investigated by adding 10 μl acetone water solution (0.1 μg/μl) to 1 ml plasma samples containing known concentration of acetone. Triplicate measurement was performed.

The limit of detection (LOD) was studied by four measurement of the calibration solution with acetone concentration of 0.0672 μM. LOD was calculated based on signal-to-noise ratio of 3.

3. Results and discussion

The reaction of acetone in plasma with the derivatizing agent of PFBHA on fiber at ambient temperature is rapid and complete, and forms a very stable acetone–PFBHA oxime (Fig. 1). The amount of oxime formed on the fiber is proportional to the acetone concentration in plasma, and it can be analyzed using GC–MS.

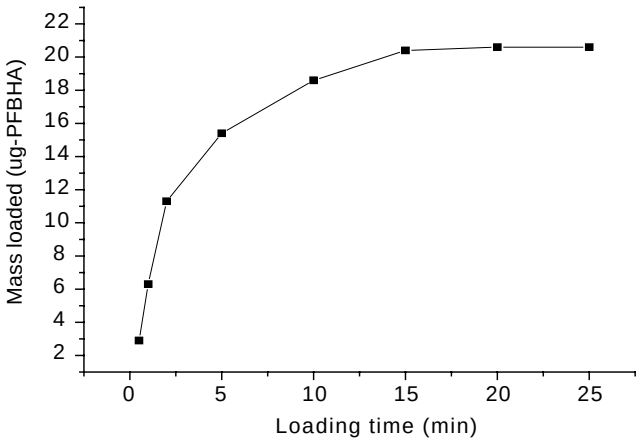

Fig. 2. PFBHA loading time vs. mass loaded.

At first, the effect of loading time on the mass of PFBHA was investigated. As shown in Fig. 2, the mass of PFBHA loaded on the fiber increased as the loading time. The equilibrium time is around 15 min. Because of low concentration of acetone in plasma, the mass of PFBHA loaded on the fiber in extraction time of 10 min is enough for derivatization of acetone. At temperature of 250 °C, the desorption efficiency was found to be 99.5% when the desorption time was 3.0 min. Therefore, SPME desorption was performed at 250 °C for 3.0 min.

The effect of time on adsorption efficiency was studied. The SPME adsorption-time profiles from the on-fiber derivatizations of acetone with PFBHA are shown in Fig. 3, which show that the amount of acetone derivative dramatically increased with extraction time and increased very slowly after 10 min. Therefore, extraction time of 10 min was selected as the optimum time.

The effect of temperature on adsorption and on-fiber derivatization was also investigated. Five temperatures in desired range (25, 30, 35, 40 and 50 °C) were used for selection of the optimum adsorption temperature. Peak areas of acetone oximes obtained at different derivatization temperatures were from 6.4×10^8 to 6.6×10^8, which shows that effect of adsorption temperature on the amount of acetone oxime was very little. The results showed that acetone were easily vaporized from plasma and rapidly reacted with PFBHA loaded on the fiber at the ambient temperature of 25 °C.

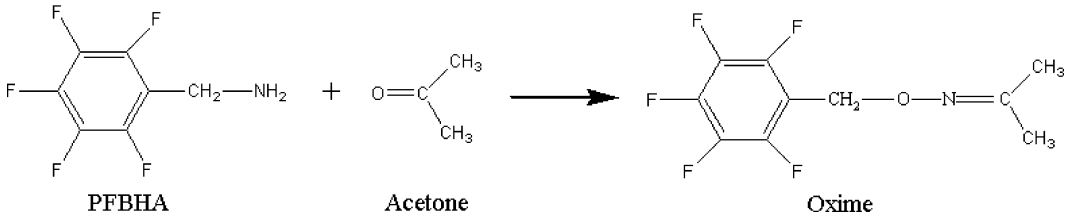

Fig. 1. Schematic of the reaction between acetone and the derivatizing agent (PFBHA) occurring on the SPME fiber.

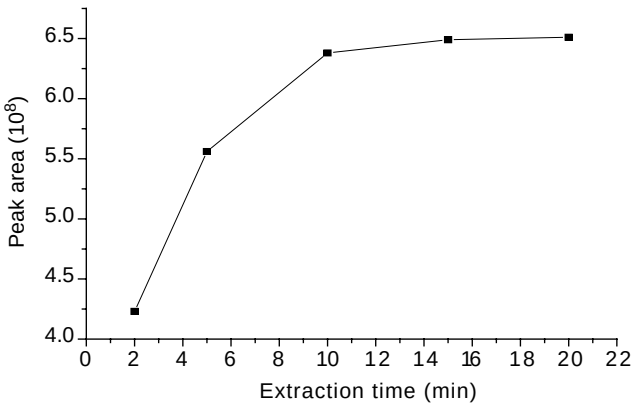

Fig. 3. Adsorption–time profiles for acetone in a calibration solution (1.34 mM) using headspace SPME with on-fiber derivatization. Sample volume was 1 ml and extraction temperature was 25 °C.

Precision of the method was studied. Five replicate measurements of two calibration solutions (0.0134 and 1.34 mM) were used to calculation of relative standard deviation (R.S.D.) value. R.S.D. value for 0.0134 and 1.34 mM acetone solutions were 9.4 and 8.9%, respectively. The R.S.D. value was very close to the value of 5.9% by Miekisch's method [23].

Recoveries were investigated by adding 1.0 μg acetone into three plasma samples with acetone concentrations of 1.94, 2.16 and 0.0156 mM. Recoveries calculated are 94% for 1.94 mM, 96% for 2.16 mM and 84% for 0.0156 mM.

The *m*/*z*, 181 ion is the base peak in the EI mass spectra of all the PFBHA derivative of carbonyl compounds. This fragment ion originates from the pentafluorobenzyl moiety. It is thus common to PFBHA and its derivatives [32]. As we know, selected ion monitoring (SIM) can decrease the

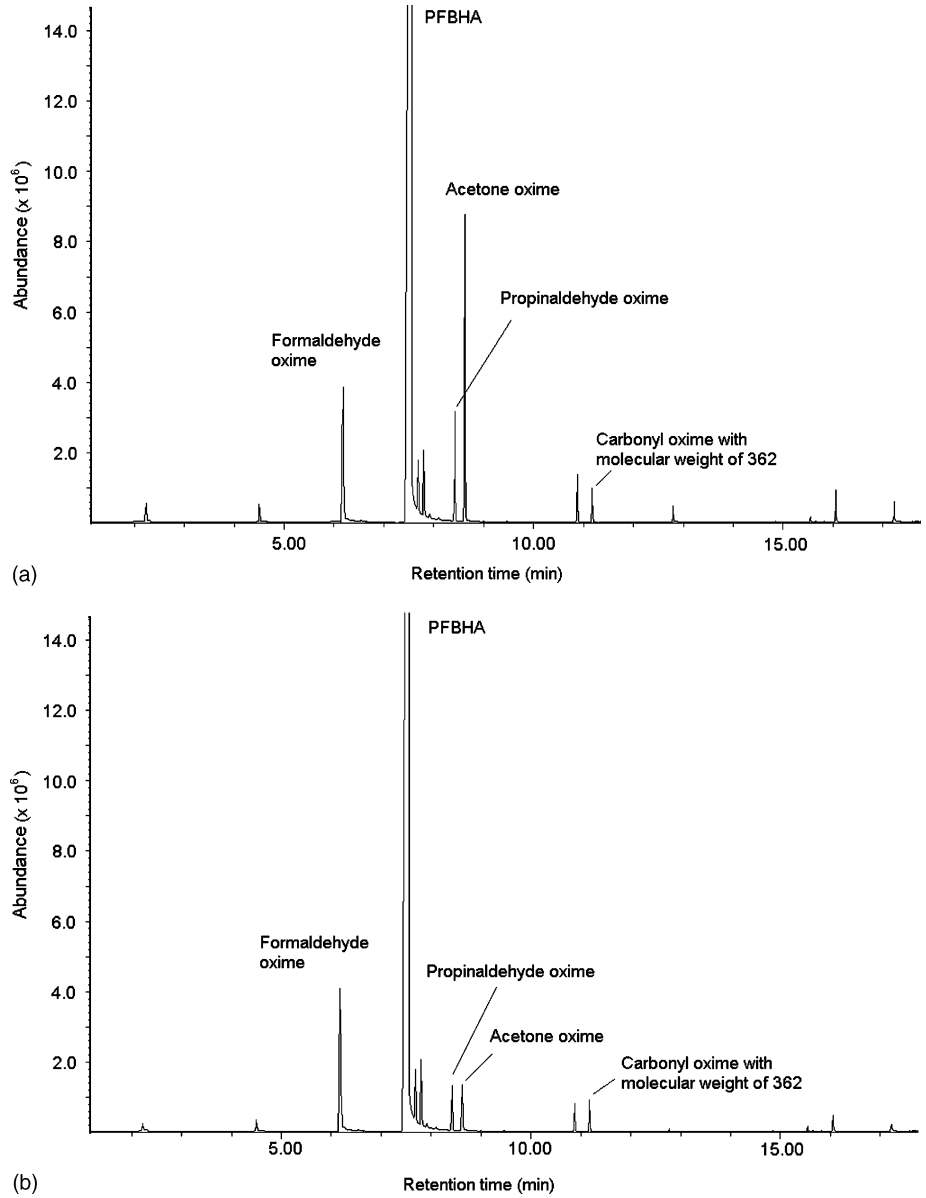

Fig. 4. Total ion chromatograms of diabetic plasma (a) and normal plasma (b) by SPME with on-fiber derivatization.

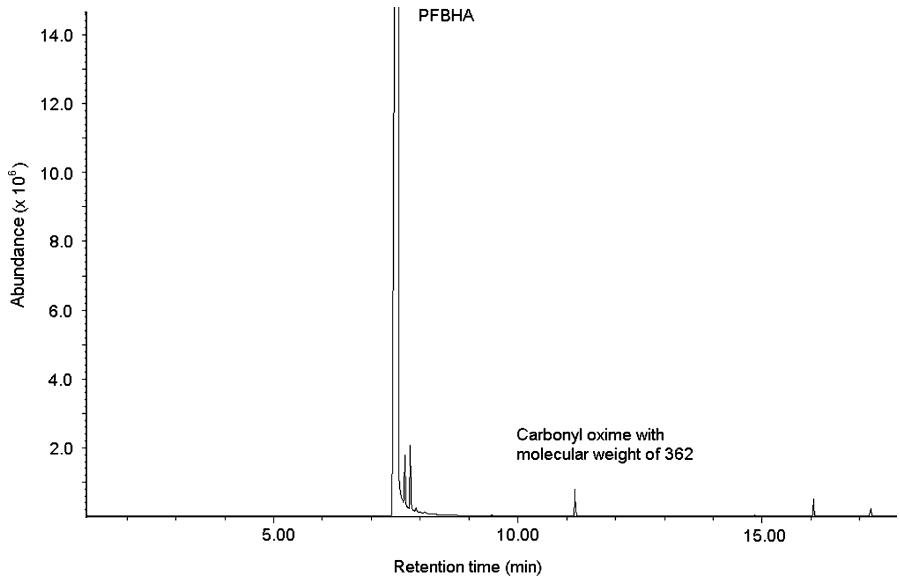

Fig. 5. Total ion chromatogram of the plasma prepared by heating at 60 °C and 120 min stirred at 1100 rpm by GC–MS–SPME with on-fiber derivatization.

detection limit. In this method, the fragment ion (*m*/*z*, 181) of acetone oxime was used as monitoring ion and applied to determination of the detection limit of acetone. A very low detection limit value of 0.004 nM was achieved on basis of S/N = 3, which is very lower than to the value (0.08 nM) obtained by analysis of acetone in human and animal plasma by SPME without derivatization [23]. This shows that using derivatization of PFBHA can improve the sensitivity for analysis of acetone in plasma.

Five milliliter plasma was needed in Miekisch's method [23], while only 1 ml plasma was used in the present method. In addition, it has a lower detection limit. Whole analysis time in the present method (about 40 min) was close to that in SPME method in literatures [23–25]. These results show that it is a rapid, simple and sensitive method for determination of acetone in plasma.

The present method was applied to analysis of acetone in diabetic plasma and normal plasma. The optimum extraction conditions (25 °C and 10 min) were used for extraction and analysis of acetone in diabetic plasma and normal plasma. Total ion chromatograms of diabetic plasma and normal plasma by GC–MS–SPME with on-fiber derivatization are shown in Fig. 4a and b, respectively. Acetone, formaldehyde and propinaldehyde oximes were detected in both normal and diabetic plasma. Among the four carbonyl compounds, only acetone has been regarded as biomarker molecule for diabetes [1]. It is seen from Fig. 4 that acetone concentration in diabetic plasma was much higher than that in normal plasma. The prepared plasma used for calibration solutions (seen to Section 2.2) was analyzed by GC–MS–SPME with on-fiber derivatization under the same conditions. Total ion chromatogram of the prepared plasma is shown in Fig. 5. No acetone oxime was detected. This demonstrated that acetone could be completely removed from normal plasma at 60 °C for 120 min, stirred at 1100 rpm. Therefore, it is feasible that calibration solutions were made by dissolving acetone into the prepared plasma. The quantitative curve for acetone was obtained by three replicate analyses of calibration solutions ranged from 0.00672 to 2.68 mM. The regression linearity and the equation for acetone tested is $y = 4.77 \times 10^8 x + 9.41 \times 10^6$, $r^2 = 0.9951$, respectively, which showed an excellent relationship between the signal (peak area of acetone oxime, *y*) and acetone concentration in plasma (*x*, mM). Concentration of acetone in normal plasma and diabetic plasma were measured by external standard method. Acetone concentration values in seven diabetic plasma samples were from 1.86 to 2.51 mM, while in six normal plasma samples were from 0.00921 to 0.0165 mM. These results show that acetone concentration in diabetic plasma was higher than 1.8 mM, while in normal human lower than 0.017 mM. These results were consistent with those in the literatures [1,4–6]. This suggests that diagnosis of diabetes might be performed by analysis of acetone in human plasma by the present method.

4. Conclusions

The research shown here demonstrated that the determination of acetone in human plasma by SPME with on-fiber derivatization provided a good precision and an excellent sensitivity with simple and fast procedures. The present method is a potential tool for diagnosis of some diseases related with acetone metabolism, such as diabetes.

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