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Analytical Biochemistry 342 (2005) 318–326
ANALYTICAL BIOCHEMISTRY
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Gas chromatography–mass spectrometric analysis of hexanal and heptanal in human blood by headspace single-drop microextraction with droplet derivatization

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Received 24 February 2005
Available online 5 May 2005

Abstract

In this work, we developed a new approach to the analysis of the lung cancer biomarkers, hexanal and heptanal in human blood that was based on headspace single-drop microextraction (HS-SDME) with droplet derivatization, followed by gas chromatography–mass spectrometry (GC-MS). Aldehydes in blood were headspace extracted, concentrated, and derivatized by a suspended microdrop solvent containing the derivatization agent *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride. The aldehyde oximes formed in the microdrop solvent were analyzed by GC-MS. The optimal HS-SDME with droplet derivatization parameters extraction solvent of decane, sample temperature of 40 °C, extraction time of 6 min, stirring rate of 1100 rpm, and solvent volume of 2.0 μL were obtained and used for analysis of hexanal and heptanal in blood. The method reproducibility, linearity, recovery, and detection limit were studied and the obtained results demonstrated the method feasibility. Finally, the proposed method was applied to the quantification of hexanal and heptanal in cancer blood and normal blood. Due to sample extraction, concentration, and derivatization being performed in a single step, the method provided a simple, rapid, low-cost, and efficient approach to analysis of aldehydes in blood samples.

Keywords: Hexanal; Heptanal; Headspace single-drop microextraction; Droplet derivatization; Blood analysis; Biomarker

Aldehyde compounds are formed by free-radical-induced reactions with cellular lipids. The presence of the aldehyde is considered a marker and evidence that free-radical-mediated reactions have taken place recently [1,2]. An increase in aldehyde concentration implies greater oxidative stress. A high level of aldehyde was found in cancer blood and the aldehydes were regarded a marker of cancer [3]. Acrolein was detected in blood from patients with breast cancer [4]. High concentrations of formaldehyde and acetaldehyde were found in the blood of tumor-bearing transgenic mice [5]. The formaldehyde level from women with breast cancer was higher than that from healthy women [6]. Recently, Kato et al. [6] detected formaldehyde in human cancer cells. Intracellular formaldehyde concentration was estimated to range from 1.5 to 4.0 mM. Hexanal, heptanal, and malondialdehyde levels in the blood samples from cancer patients were strikingly higher than those from the controls [3]. Hexanal and heptanal were found in breath from lung cancer patients [7–10]. High concentrations of hexanal and heptanal were also detected in lung cancer blood [11–14]. Elevated levels of aldehydes are considered the biomarker for enhanced oxidative stress and have been proposed as a measure to diagnose cancer status.

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0003-2697/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ab.2005.04.024

Gas chromatography–mass spectrometry (GC-MS) was applied to direct analysis of aldehydes with low molecular masses in human breath [7–10]. However, due to their volatility and activity, it is very difficult to directly measure these aldehydes in breath and blood. To overcome this problem, derivatization of aldehydes was performed prior to analysis. 2-Thiobarbituric acid was developed for derivatization of malondialdehyde in breath condensate, followed by analysis using liquid chromatography with a fluorescence detector [15]. Liquid chromatography–mass spectrometry (LC-MS) with other derivatization agents was developed for the determination of aldehydes in body fluids, air, and other matrixes [16–22]. *O*-2,3,4,5,6-(Pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA)[1] was first introduced for the derivatization of carbonyls in GC analysis by Cancilla and Hee [23]. The derivatization of carbonyls with PFBHA can be performed under mild reaction conditions (ambient temperature and aqueous solution). The reaction is very fast and the corresponding oximes form in seconds. GC-MS with PFBHA has been developed for the quantification of aldehydes in air, water, and body fluids [24–28]. Recently, a simple, rapid, and solvent-free method based on solid-phase microextraction (SPME) with the on-fiber derivatization was developed for the analysis of aldehydes in air, water, and blood [29–34]. In our previous studies, SPME with the on-fiber derivatization technique was developed for the determination of the disease biomarkers acetone and aldehydes in breath and blood [35–37]. However, these methods involving SPME have the disadvantages that SPME fiber is relatively expensive and the polymer coating is fragile and easily broken. Furthermore, sample carryover is sometimes difficult or impossible to be eliminated. A novel, low-cost, and efficient method for quantification of the cancer biomarker aldehydes in blood is very desirable.

Recently, a fast, simple, inexpensive, and virtually solvent-free sample preparation method has been devised for extraction of analytes from water. This technique is known as liquid-phase microextraction (LPME) or single-drop microextraction (SDME) [38–42]. Recently, Liu et al. [43] developed SDME for the analysis of anisaldehyde isomers in human urine and blood serum. To analyze volatile compounds in dirty samples, in 2001, Theis et al. [44] introduced headspace single-drop microextraction (HS-SDME), where a microdrop of organic solvent was suspended in the headspace of the analyte solution and used for headspace extraction and concentration of the volatile analytes in the solution. Due to the advantages of simplicity, rapidness, low cost, and no sample carryover, the HS-SDME technique was widely applied to environmental and biomedical analysis [45–48].

In the present work, we described a novel method, HS-SDME with droplet derivatization, for quantification of aldehydes in blood. In this method, aldehyde compounds in human blood were headspace extracted and concentrated by a microdrop solvent and immediately derivatizated with PFBHA in the drop solvent. Finally, the formed oximes in the microdrop were injected into GC-MS for analysis. The parameters of HS-SDME with droplet derivatization were optimized and the method validations were studied.

Materials and methods

Chemicals and blood samples

Hexanal (98%), heptanal (98%), butanone (internal standard, 98%) and *O*-(2,3,4,5-pentafluorobenzyl) hydroxylamine hydrochloride (98%) were purchased from Sigma (St. Louis, MO, USA). Aldehyde stock standards were prepared in methanol with concentration levels of 0.6 mM for each compound and stored in a freezer at −4 °C. The internal standard (IS) solution (0.6 mM) was prepared by dissolving butanone in methanol. Decane, 1-octanol, and dodecane (HPLC grade) were purchased from Chemical Agent, Shanghai, China. PFBHA solution (20 mg/mL) was made by dissolving PFBHA into double-distilled water. PFBHA in the aqueous solution (1.0 mL) was extracted by using 1.0 mL decane. Decane containing about 16 mg/mL PFBHA was obtained and used as the extraction solvent of HS-SDME and droplet derivatization.

Whole blood from normal subjects and lung cancer patients was drawn into a heparin-containing syringe and immediately transferred into a sealed headspace vial with a 1-cm-long stirring bar. The blood samples were stored at −20 °C until use. The characteristics of normal subjects and lung cancer patients are shown in Table 1.

Preparation of calibration solutions

Blood (10 mL) from a control subject was introduced into a 25-mL bottle vial with a 4-cm stir bar. According to our previous method [14], the free carbonyls in the blood were eliminated by heating at 60 °C for 240 min, with stirring rate of 1300 rpm. Thus, carbonyl-free blood was obtained. Working solutions with concentrations of 0.06, 0.12, 1.2, 12, and 60 μM (for both hexanal and heptanal) were prepared by dilution of the 0.6 mM stock solution with the carbonyl-free blood sample. IS solution (10 μL, 0.6 mM) was added into each working solution (1.0 mL) and calibration solutions containing 6.0 μM internal standard were obtained.

[1] *Abbreviations used:* PFBHA, *O*-2,3,4,5,6-(pentafluorobenzyl)hydroxylamine hydrochloride; SPME, solid-phase microextraction; LPME, liquid-phase microextraction; SDME, single-drop microextraction; HS, headspace; IS, internal standard; RSD, relative standard deviation; SIM, selected ion monitoring.