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High-performance liquid chromatographic determination of hexanal and heptanal in human blood by ultrasound-assisted headspace liquid-phase microextraction with in-drop derivatization

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ABSTRACT

In this paper, an ultrasound-assisted headspace liquid-phase microextraction with in-drop derivatization was developed for the extraction and determination of hexanal and heptanal as the biomarkers in human blood. In the method, a polychloroprene rubber (PCR) tube was utilized as container to load extraction solvent (methyl cyanide) and derivatization reagent (2,4-dinitrophenylhydrazine, 2,4-DNPH). Volatile aldehydes were headspace extracted and simultaneously derivatized in the droplet, followed by LC-UV detection of the formed hydrazones. The stability of organic solvent and the sensitivity of the method enhanced greatly. Under the optimal conditions, good linearity was obtained in the concentration range of 0.01–10 μmol L^{-1} ($r > 0.997$) and the limits of detection (LOD) for hexanal and heptanal were 0.79 and 0.80 nmol L^{-1}, respectively. The recoveries in blood sample ranged from 75.2% to 101.1% with the inter- and intra-day precisions less than 9.8%. The method possesses the advantages such as simplicity, sensitivity, efficiency, low consumption of solvent, and little interference from sample matrix. It provides great potential for the investigation of volatile disease biomarkers (aldehydes) in complex biological samples.

1. Introduction

Aldehyde compounds such as formaldehyde, acetaldehyde, acrolein, hexanal and heptanal generated by human body have been much investigated, since elevated level of aldehydes is considered as the biomarker of different diseases [1–7]. Most of the aldehyde compounds are formed by free-radical-induced reaction with injured or dying cellular lipid [8–10]. The presence of aldehydes has been related to various pathological conditions such as atherosclerosis, carcinogenesis, and precancerous dysplasia [11–13]. High levels of hexanal and heptanal were found in the blood of lung cancer patients [14]. Therefore, it is of great importance in the determination of these aldehydes in blood analysis of the early clinical diagnosis.

However, the determination of aldehydes is difficult because these molecules are volatile, unstable, highly water-soluble and lack a chromophore or fluorophore [15]. To improve the sensitivity and precision in the determination of these compounds, samples must be derivatized usually with a substituted hydroxylamine or hydrazine. The formed derivatives are separated by chromatographic techniques coupled with sensitive detectors [16]. In addition, owing to the complexity of sample matrices and the relative low concentration of aldehydes in blood samples, sample cleanup and enrichment procedure are necessary to improve the sensitivity of method. Different analytical methods have been developed to extract aldehydes. The most extensively used methods for the extraction of aldehydes in complex samples are solid phase microextraction (SPME) [17,18], the method is attractive due to its solvent-free, simplicity and rapidity [19–27]. Recently, Lord et al. applied a solid phase analytical derivatization device to fully automate extraction, derivatization and liquid chromatography and detected malondialdehyde in human plasma [15]. Zhang et al. have presented a new method for the determination of aldehydes in plasma, which is based on polymer monolith microextraction (PMME) with in situ derivatization, followed by HPLC/DAD detection [28].

Apart from SPME, more attention has been focused on miniaturized and solvent-free liquid-phase extraction technique. Liquid-phase microextraction (LPME), also called single-drop microextraction (SDME), which was introduced by Jeannot, Cantwell [29,30], and He and Lee [31] in 1996, meet the trends of development. It is now becoming one of the most common methods of sample preparation, particularly for the extraction of organic

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compounds from environmental and biological samples. Thereinto, headspace single-drop microextraction (HS-LPME) is a good choice to analyze volatile and semivolatile compounds in different matrices without any interference of the sample matrix [32,33]. It has been extensively applied in various real samples in recent years due to its simplicity, available experimental setup, fast analysis and low consumption of organic solvent. Liu developed SDME for the analysis of anisaldehyde isomers in human urine and blood serum [34]. In 2008, SDME method was also used to determine volatile aldehydes in fresh cucumbers [35]. Fiamegos and Stalikas determined the carbonyl compounds in biological and oil samples by HS-LPME with in-drop derivatization [36]. Recently, Xie et al. analyzed small molecular aldehydes (SMAs) in single puff smoke by coupling extraction and derivatization in single drop (EDSD) with MALDI-FTICR-MS detection [37].

Nevertheless, the disadvantages of HS-SDME are obvious, and the application of the method is also restricted by some important factors. Firstly, because of the small contact area between microdrop and the tip of microsyringe needle, the surface tension is relatively low, and the volume of organic solvent is usually no larger than 5 μL. Secondly, the microdrop easily falls down from the tip of microsyringe needle at a large volume, so careful and elaborate manual operation is required in the experiment.

In our previous studies, we developed an ultrasound-assisted headspace liquid-phase microextraction (UAHS-LPME) method, with a cone-shaped polychloroprene rubber tube (PCR) as extraction solvent holder to overcome the above-mentioned problems. It has been successfully applied in the analysis of volatile organic compounds in real aqueous samples [38,39]. In this work, in-drop derivatization and simultaneous extraction was introduced into the method. The feasibility of the new method was tested, and it was applied in the analysis of volatile cancer biomarkers (hexanal and heptanal) in complex biological matrix.

2. Experimental

2.1. Reagents and chemicals

Hexanal (98%) and heptanal (97%) were purchased from ABCR GmbH & Co. KG (Germany). 2,4-dinitrophenylhydrazine (2,4-DNPH, 99.6%) was obtained from Chem Service Inc. (West Chester, PA, USA) and it was recrystallized once in acetonitrile–water (1:5) solution before use. Formic acid (96%) was purchased from TEDIA Company Inc. (Tedia Company, Inc., Fairfield, OH, USA). HPLC-grade methanol and methyl cyanide were obtained from Fisher Chemicals (Fisher Chemicals, Fair Lawn, NJ, USA). n-Heptane, cyclohexane, carbon tetrachloride, hexane, isopropanol and sodium chloride were all of analytical grade and also purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, P.R. China). The water used was ultrapure water (Millipore Simplicity 185, Corporation, Billerica, MA, USA).

2.2. Preparation of standard solutions

The individual stock standard solution was prepared in methanol at a concentration of 5 mmol L^{-1}. The daily standard working solutions of different concentrations were obtained by diluting the stock solutions with distilling water. All solutions prepared were stored at 4 °C.

2.3. Instruments

The chromatographic analysis was performed on an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a quatpump, a manual injector (20 μL injection loop), a variable wavelength detector (VWD). A personal computer equipped with an Agilent ChemStation program for LC was used to process chromatographic data. The analytes were separated on Venusil, XBP C18 column (250 mm × 4.6 mm, 5 μm), which was bought from Agela Technologies Inc. (Beijing, P.R. China). The mobile phase was a mixture of methanol–water (87:13, volume ratio) and the flow rate was 1.0 mL min^{-1}. The column temperature was 30 °C and the detection wavelength was 360 nm. An ultrasonicator (KQ-100DE ultrasonicator, KunShan, China) was used for extraction and a 25 μL LC microsyringe bought from Shanghai GaoGe Industrial and Trading Co., Ltd. (Shanghai, P.R. China) was used for injection.

2.4. UAHS-LPME procedure

The schematic diagram of the novel UAHS-LPME apparatus is shown in literature [39] with minor modification. Sample solution (5 mL) and different amounts of salt (sodium chloride, 0–0.2 g/mL) were added into a 10 mL sample vial. The PCR tube loaded with organic solvent containing 2,4-DNPH and formic acid was foisted into the rubber stopper of sample vial, then it was covered with the stopper and sealed with parafilm. Afterwards, the sample vial was placed into an ultrasonicator with hot water of constant temperature for extraction. After ultrasound-assisted headspace extraction was completed, 2 μL ammonia was added into the PCR tube to adjust pH value of the extractant, then organic solvent loaded with the derivatives of hexanal and heptanal was removed from the PCR tube by a microsyringe and the entire microdrop was injected into HPLC for analysis.

2.5. Blood sample preparation

The blood samples from 5 healthy people and 12 lung cancer patients were obtained from Hubei Cancer Hospital, Wuhan, China. Ethical approval for the study was obtained from the Ethnics Committee of Hubei Cancer Hospital prior to the collection and analysis of human blood samples. The blood samples were stored at 4 °C in the refrigerator and were not further pretreated before use. In the serum analysis, 500 μL of serum was diluted tenfold by ultrapure water for the determination of aldehydes. The derivatization and extraction procedure was the same as that described above for UAHS-LPME.

3. Results and discussion

3.1. Selection of extractant

Selecting an appropriate extractant is essential for HS-LPME method. It should have a relatively high boiling point, a high affinity to the analytes, and high surface tension to keep it suspending at the bottom of the PCR tube. In this work, several organic solvents were tested as extraction solvent, including n-heptane, cyclohexane, carbon tetrachloride, methyl cyanide, hexane, and isopropanol, and the results are shown in Fig. 1. It can be seen that good extraction efficiency is obtained when methyl cyanide is used as organic extractant. Therefore, methyl cyanide was chosen as extractant in the following experiments. The effect of extractant volume on the extraction efficiency was studied in the range of 5–25 μL. The chromatographic peak areas of aldehyde derivatives in relation to the volume of organic solvent are shown in Fig. 2. As indicated by Fig. 2, the peak areas decreased with the increase of the extractant volume. The decrease can be explained by the diluting effect of large volume of extractant. The maximum peak response was obtained when 5 μL methyl cyanide was used. However, the sampling after extraction was difficult to carry out when the extractant volume was less than 10 μL. Therefore, a 10 μL of methyl cyanide