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A novel sorptive extraction method based on polydimethylsiloxane frit for determination of lung cancer biomarkers in human serum

Hui Xu*, Shuyu Wang

Key Laboratory of Pesticide and Chemical Biology, Ministry of Education, College of Chemistry, Central China Normal University, Wuhan 430079, China

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ABSTRACT

In this study, a porous polypropylene frit was coated with polydimethylsiloxane (PDMS) as extraction medium, based on the home-made PDMS-frit, a rapid, simple and sensitive sorptive extraction method was established for analysis of potential biomarkers of lung cancer (hexanal and heptanal) in human serum samples. In the method, derivatization and extraction occurred simultaneously on the PDMS-frit, then the loaded frit was ultrasonically desorbed in acetonitrile. Polymerization, derivatization–extraction and desorption conditions were optimized. Under the optimal conditions, satisfactory results were gained, a wide linear application range was obtained in the range of 0.002–5.0 μmol L^{-1} ($R > 0.997$) for two aldehydes, the detection limits ($SN^{-1} = 3$) were 0.5 nmol L^{-1} for hexanal and 0.4 nmol L^{-1} for heptanal. The relative standard deviations (RSDs, $n = 5$) of the method were below 7.9% and the recoveries were above 72.7% for the spiked serum. All these results hint that the proposed method is potential for disease markers analysis in complex biological samples.

1. Introduction

We all know that lung cancer is the leading cause of cancer death throughout the world [1,2]. Recently increasing attentions have been paid on analysis of small molecular metabolites aldehydes in the exhaled breath, blood and urine samples of lung cancer patients. Volatile organic compounds, such as hexanal and heptanal, are supposed to be characteristic biomarker candidates for lung cancer patients [3–12]. Therefore, a simple, sensitive and reliable method to quantitative analysis of aldehyde biomarkers in biological fluids is highly desirable to early detection of the disease.

Due to the physical and chemical characteristics of aldehydes, such as lack of intrinsic chromophores, high volatility and low chemical stability, the derivatization of aldehydes prior to analysis is necessary for their quantitative analysis in real samples [9–13]. 2,4-Dinitrophenylhydrazine (DNPH) is a typical derivatization reagent of aldehydes with high-performance liquid chromatography (HPLC) analysis [9–12,14,15]. In addition, due to the complexity of biological fluids and the relatively low concentrations of metabolites in the samples, appropriate sample preparation steps are usually adopted for accurate analysis of aldehydes [13]. A very important and practical sample preparation technique, named solid-phase microextraction (SPME) was first introduced by Pawliszyn [16] and it has been successfully applied for determination of aldehydes in chicken [17], plants oils [18], water [19,20], human exhaled breath [21] and human blood [3,7]. Another widely used solid-phase extraction method called stir bar sorptive extraction (SBSE), was first described in the nineties by Baltussen et al. [22]. The method is a relatively simple, efficient technique for extraction and concentration of organic compounds from aqueous samples to a thick film of polydimethylsiloxane (PDMS). SBSE has much higher adsorption capacity than SPME for its larger solid phase volume. The principles of SBSE and the photograph of PDMS stir bar were described detailed in the literatures [23–25]. In the method, the preparation of stir bar is complicated and time-consuming, and the stirring rate cannot be too fast due to the limit of the size of stir bar and the aim of protection of the PDMS coating. Therefore, long extraction time is often required. For example, the smallest stir bar in traditional SBSE method is 1 cm in length and 24 μL in PDMS-phase volume, it often requires long extraction time (60–210 min, 500–1000 rpm) [26–28]. In addition, large amount of stripping solvent (1.5 mL of ACN) is also needed due to the bar shape of stir [28].

In this work, a porous polypropylene frit with average pore size of 20 μm (outside diameter of frits: 4.8 mm, thickness of frits: 1.6 mm) was selected as a novel support to load PDMS. Compared with the conventional stir bar, the PDMS extraction disk is simple preparation and easy-to-use, it is unattached with magnetic stir, hence common magnetic stir can be used with high stirring

Abbreviations: HPLC, high-performance liquid chromatography; PDMS, polydimethylsiloxane; DNPH, 2,4-dinitrophenylhydrazine; SPME, solid-phase microextraction; SBSE, stir bar sorptive extraction.

* Corresponding author. Tel.: +86 27 67867953; fax: +86 27 67867955.
E-mail address: huixu@mail.ccnu.edu.cn (H. Xu).

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speed, short extraction time thereby can be obtained. Low amount of stripping solvent can be used because of the disk-shaped frit. Porous frit as scaffold has been utilized to load polymeric monolith and to strengthen the mechanical strength of monolith in our previous report [11]. In this work, based on the PDMS-frit, a novel sorptive extraction method was developed for analysis of aldehydes (hexanal and heptanal) in serum samples of lung cancer patients and normal people. Derivatization, extraction and concentration were integrated in one step. After that, the target analytes on PDMS-frit was ultrasonically desorbed and the eluant was analyzed by HPLC-DAD. A series of parameters affecting the derivatization–extraction efficiency were optimized, validation of the methodology was investigated systematically, their comparison with conventional SBSE was also studied.

2. Experimental

2.1. Materials and chemicals

Hexanal (98%) and heptanal (97%) were obtained from ABCR GmbH & Co. KG (Germany). 2,4-Dinitrophenylhydrazine (2,4-DNPH, 99.6%) was purchased from CHEM SERVICE (West Chester, PA, USA) and it was recrystallized once in acetonitrile–water (1:5) solution before use. HPLC-grade methanol and formic acid (96%) was purchased from TEDIA Company Inc. (Tedia Company, Inc., Fairfield, OH, USA). HPLC-grade methyl cyanide was purchased from Fisher Chemicals (Fisher Chemicals, Fair Lawn, NJ, USA). Carbon tetrachloride, hexane, isopropanol and sodium chloride were all of analytical grade and also purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Toluene was of analytical reagent grade and was purchased from Shanghai Chemical (Shanghai, China). Slygard 184 Silicone Elastomer Base and Slygard 184 Silicone Elastomer Curing Agent were bought from Dow corning Corp. (Dow Corning, Midland, Michigan, USA).

The polypropylene frits with diameter of 4.8 mm, thickness of 1.6 mm and average pore size of 20 μm were bought from Haohailinfeng Company (Wuhan, China). The water used was obtained from a Millipore Simplicity 185 system (Millipore Corp., Billerica, MA, USA).

2.2. Preparation of standard solutions

Standard stock solution was prepared separately in methanol at the concentration of 5 mmol L^{-1} and stored at 4 °C. The daily standard working solution containing hexanal and heptanal was prepared by mixing and diluting with ultrapure water according to demand.

2.3. Sample preparation

The serum samples of 8 healthy subjects and 7 lung cancer patients were obtained from Hubei Cancer Hospital, Wuhan, China and the 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. Serum samples were stored at −20 °C in the freezer. Before the serum analysis, 300 μL acetonitrile was added into 200 μL serum to deposit protein, and then the mixture solution was centrifuged at 12,000 rpm for 3 min. After that, all of the supernatant was diluted by water for the determination of aldehydes according to demand.

2.4. Preparation of polydimethylsiloxane frit

The pre-polymerization mixture solution was prepared by mixing Slygard 184 silicone elastomer base and curing agent at a weight ratio of 10:1, and 200 μL toluene was added in the solution to dilute the viscous mixture. Then 20 μL of the mixture solution was added carefully on the frit and the loaded frit on a slide was immediately placed in a heating and drying oven (DHG-9070A Heating and Drying Oven, Shanghai JingHong Laboratory Instrument Co., Ltd., Shanghai, China) at 120 °C for 2 h. The photogram of PDMS-frit are shown in Fig. 1.

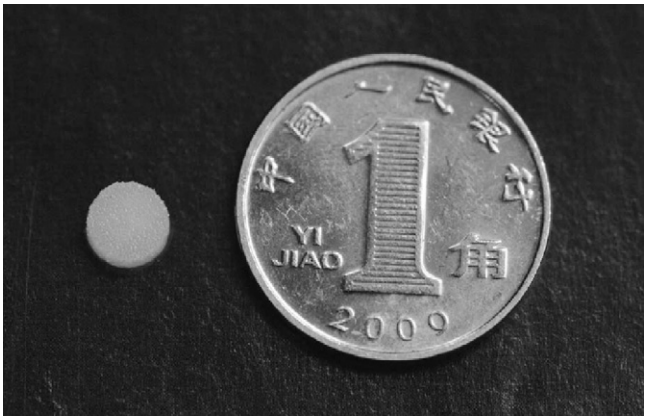

Fig. 1. The photogram of PDMS-frit.

2.5. In situ derivatization and sorptive extraction procedure

At first, 30 μL formic acid, 80 μL DNPH (5 mmol L^{-1}) and 4 mL sample solution (containing 2 μmol L^{-1} hexanal and heptanal) were introduced into a 8 mL sample vial, then the PDMS-frit was put into the mixture solution, and the vial was sealed immediately. Subsequently, the sample vial was placed on a magnetic stirrer for in situ derivatization–extraction in a hot water bath (40 °C). After 20 min, the PDMS-frit was taken out, water droplets on the frit was removed gently by a clean paper tissue. Then the frit was ultrasonically desorbed by immersing in 70 μL of acetonitrile for 3 min (40 °C), 10 μL of acetonitrile was injected for HPLC analysis. KQ218 ultrasonicator (KunShan, China) was used for desorption with the frequency of 60 Hz.

2.6. Apparatus and chromatographic conditions

The chromatographic analysis were performed on Shimadzu LC-2010A system (Shimadzu Corporation, Tokyo, Japan) equipped with a LC-20AT quaternary pump, SIL-20A autosampler, CTO-10ASVP column oven and a SPD-M20A photo diode Array detector. A personal computer provided with a LC solution program for LC operation was used to process chromatographic data. The analytes were separated on Venusil, XBP C18 column (250 mm × 4.6 mm, 5 μm), which was obtained from Agela Technologies Inc. (Beijing, China). The mobile phase was methanol/water (89/11; v/v) and was filtered through a 0.45 μm micropore membrane and the flow rate of mobile phase was kept at 1 mL min^{-1}. The column temperature was 30 °C, the detection wavelength was set as 360 nm and the injection volume was 10 μL.

3. Results and discussion

3.1. Optimization of polymerization conditions

In the polymerization reaction, the amount of stationary phase (PDMS) was evaluated for its influence on the extraction efficiency. The volume of PDMS mixture solution for each frit varied from 10 to 25 μL. As Fig. 2 indicated, with the increase of PDMS volume, the response signal of the aldehyde derivatives increased. However, there was no difference for analytes adsorption between 20 and