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A new configuration for bar adsorptive microextraction (BAμE) for the quantification of biomarkers (hexanal and heptanal) in human urine by HPLC providing an alternative for early lung cancer diagnosis

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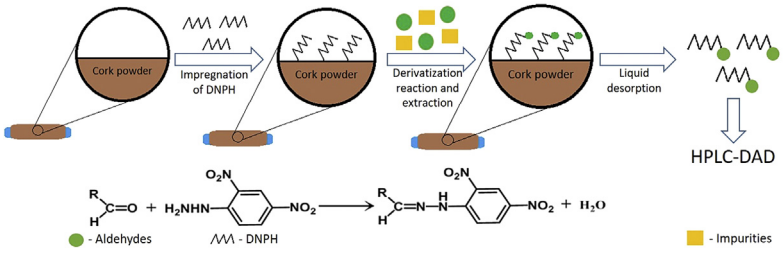
HIGHLIGHTS

- The bar adsorptive was impregnated with a derivatization reagent on its surface containing a biosorbent material.
- Derivatization reaction and the extraction occurred simultaneously on the surface of the bar under acidic conditions.
- The limits of detection for hexanal and heptanal were 0.80 and 0.40 μmol L^{-1}, respectively.
- The method is of low cost and can be used for the quantification of two lung cancer biomarkers in human urine samples.

GRAPHICAL ABSTRACT



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ABSTRACT

In this paper, a remodeling of the bar adsorptive microextraction (BAμE) technique is proposed with impregnation of the derivatization reagent on the surface of the adsorptive bar containing a biosorbent material. The derivatization reagent was 2,4-dinitrophenylhydrazine (DNPH), which was adsorbed on the surface of the bar containing cork powder as the extractor phase for the determination of two aldehydes (hexanal and heptanal) which are known as lung cancer biomarkers in human urine samples. The derivatization reaction and the extraction occurred simultaneously on the surface of the bar (length 7.5 mm) under acidic conditions. The method optimization was carried out by univariate and multivariate analysis. The optimal conditions for the method were a DNPH to aldehydes ratio of 40:1, buffer solution of pH 4.0, extraction time of 60 min and liquid desorption of 10 min in 100 μL of acetonitrile. The aldehydes were analyzed by HPLC-DAD with a simple and fast (6 min) chromatographic run. The limits of detection (LODs) for hexanal and heptanal were 1.00 and 0.73 μmol L^{-1}, respectively. The relative recoveries in urine samples ranged from 88 to 111% with relative standard deviations (RSDs) being less than 7%. The method developed is of low cost and can be successfully used for the quantification of these two lung cancer biomarkers in human urine samples, potentially providing an early diagnosis of lung cancer.

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1. Introduction

Aldehydes are organic compounds containing a carbonyl center extensively found in biological systems. These substances are by-products of cellular lipid oxidation caused by high levels of free-radicals arising from metabolism. Over the years, studies have been demonstrated a link between free radicals and some diseases, including cancer, and aldehydes are considered potential biomarkers of oxidative activity [1–4]. For this reason, many different aldehydes have been studied in distinct biological samples including saliva [5], plasma [6], blood [7,8] and urine [8–11]. The results demonstrated high levels of hexanal and heptanal in lung cancer patients [5–10] and these two compounds can thus be considered as biomarkers of lung cancer, with their detection potentially leading to early diagnosis.

The determination of aldehydes is commonly performed by high performance liquid chromatography [5,6,8,9] and gas chromatography [7,12]. Nevertheless, direct analysis of aldehydes by liquid chromatography with diode array detection presents challenges due to factors such as volatility, activity and lack of chromophore groups [13]. To overcome these obstacles a derivatization reaction is required, using a hydrazine based reagent such as 2,4-dinitrophenylhydrazine (DNPH) [14], o-(2,3,4,5,6-penta-fluorobenzyl)-hydroxylamine hydrochloride (PFBHA) [15] and *N*-methyl-4-hydrazino-7-nitrobenzofurazan (MNBDH) [16]. DNPH is the derivatization reagent most commonly employed for the determination of aldehydes by HPLC. However, the hydrazones formed from the reaction between the aldehydes and DNPH under acidic conditions can exist in E and Z stereoisomer conformations due to the C=N double bond, which can lead to analytical errors [17,18].

Different types of sample preparation for the determination of hexanal and heptanal in a variety of matrices have been reported in the literature, such as magnetic solid phase extraction in urine [9], solid phase microextraction in aqueous solution [19] and in exhaled breath [12], polymer monolith microextraction in saliva [5] and plasma [6].

Considering that the sample matrices generally used in the determination of aldehydes are extremely complex, a step known as sample preparation, which is of fundamental importance to the success of the method, is required [20]. This step is usually dependent on the matrix and the analyte characteristics, requiring a proper optimization of its different parameters of influence [21]. Some of the most sought after characteristics of a sample preparation step are speed, simplicity, low cost, high selectivity and specificity, potential for automation and minimal (or no) use of organic solvents, aimed at minimizing harm to the environment [22,23]. In recent years, the search for simplification, miniaturization, automation and the use of free-solvent techniques and other environmentally-friendly procedures has gained a lot of attention [20]. The development and improvement of miniaturized extraction techniques, notably solid phase microextraction and liquid phase microextraction, have gained great importance [21].

In recent years, bar adsorptive microextraction (BAμE) as a new method of sample preparation has attracted much attention. The main advantage of using this technique in relation to other sorption techniques is that the most appropriate material for the extractor phase can be selected for a specific analyte or a group of compounds [24,25]. In this regard, the use of cork as a biosorbent material was introduced by our group and has been applied in various types of analysis [26–28]. This technique is a type of solid phase microextraction, in which a sorbent material is fixed onto the surface of an adhesive tape coupled to a polypropylene tube of cylindrical shape. This technique is based on fluctuation, since the devices have low density, allowing their fluctuation above of the vortex caused by the agitation achieved with a magnetic stirrer. The experimental procedure associated with this technique consists of two main stages: the extraction and pre-concentration of the analytes in the sorbent phase and desorption into a liquid to be injected into the analytical instrument. These two steps require optimization to achieve the best results in the analysis [24,25].

In a previous study by our group, bar adsorptive microextraction (BAμE) using a cork coating was applied to the determination of benzophenone, triclocarban and parabens in aqueous samples by high performance liquid chromatography – diode array detection (HPLC-DAD) [27]. In this study, this microextraction technique was remodeled for the determination of hexanal and heptanal in human urine samples. The impregnation of the derivatization reagent (DNPH) on the surface of the bar adsorptive containing the biosorbent material was also tested and a stainless steel wire was used to maintain the fluctuation of the bar.

2. Experimental

2.1. Materials and reagents

Analytical standards of hexanal and heptanal were purchased from Sigma-Aldrich and they were used to prepare stock solutions of 1000 μg mL^{-1} in methanol obtained from J.T. Baker (Mallinckrodt, NJ, USA). The stock solutions were used to prepare a working solution containing a mix of the standards, each in a concentration of 50 μg mL^{-1} in methanol. An analytical standard of 2,4-dinitrophenylhydrazine was purchased from Sigma-Aldrich and a stock solution of 2080 μg mL^{-1} was prepared in acetonitrile obtained from J.T. Baker (Mallinckrodt, NJ, USA). This solution was used to prepare a 1000 μg mL^{-1} working solution in acetonitrile. The buffer solution was prepared using analytical standards of sodium phosphate dibasic and citric acid, both obtained from Vetec (Rio de Janeiro, Brazil). A sandpaper and a granulometry sieve (200 mesh) were used to prepare the cork powder from used cork stoppers. A stainless steel wire of 10 cm and a magnetic stir bar (star shaped) were used in the extractions. The extractions were performed in 22 mL capacity vials with covers. Glass inserts (Agilent, CA, USA) were used in the conditioning and liquid desorption steps. The conditioning of the adsorptive bar and the liquid desorption were carried out with an ultrasonic device (Ultrasomik). A 50 Hz vortex mixer (model 9033 EEQ, Edutec) was employed for the impregnation of the derivatization reagent on the surface of the adsorptive bar. The ultrapure water used in the experiments was purified in an ultrapure system (Mega purity, Billerica, USA).

2.2. Urine samples

The human urine samples used in the optimization and validation experiments of the proposed method were collected in 40 mL vials during the first passing of urine in the morning from non-smoking volunteers aged 22–23 years old.

2.3. Instrumental and chromatographic conditions

The chromatographic analyses were performed in a Shimadzu Prominence LC, 20AT series, HPLC system (Shimadzu, Kyoto, Japan) equipped with a diode array detector (SPD-M20A series) with a 20 μL loop and Rheodyne 7725i manual injection system (Rohnert Park, CA, USA). The chromatographic separation was performed in reverse phase mode in a C18 column (250 mm length x 4.6 mm i.d., 5 μm film thickness; Thermo, USA). The flow rate was 1 mL min^{-1} in isocratic mode. The solvents used as the mobile phase were acetonitrile:water (88:12) and the sample injection volume was 20 μL. The maximum wavelength monitored was 360 nm.