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Headspace generation coupled to gas chromatography–mass spectrometry for the automated determination and quantification of endogenous compounds in urine. Aldehydes as possible markers of oxidative stress

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ABSTRACT

A methodology for the determination of five aldehydes in urine has been developed based on the coupling of a headspace generation sampler with a gas chromatography–mass spectrometry system equipped with a programmed temperature vaporizer. This instrumental configuration minimizes sample manipulation and allows the determination of these compounds without the need for a derivatization step so that the methodology is largely shortened and simplified. An experimental design was carried out in order to optimize the headspace sampling. Sample matrix effect was eliminated by means of dilution of urine samples. The calibration models displayed good linearity (0.1–10 μg/L) and their validity was checked using ANOVA, and it was observed that they did not exhibit any lack of fit. The LODs obtained ranged between 0.04 and 0.08 μg/L and the LOQs between 0.12 and 0.24 μg/L. The accuracy of the method was evaluated in terms of apparent recoveries which were between 86 and 120%. The method developed was applied to the analysis of 17 samples of urine from different subjects in order to quantify these endogenous compounds.

1. Introduction

Aldehydes have been studied due to their relation with the process of oxidative stress induced by free radicals. These compounds are lipid-peroxidation products and therefore, could be considered as indicators of free-radical damage related to a great extent with several diseases [1–7], such as cancer [8–10]. Concretely, hexanal and heptanal have been studied as biomarkers of lung cancer [11–18]. Some other aldehydes such as pentanal [1], other aliphatic aldehydes and benzaldehyde have been objects of study for similar reasons [2,19,20].

These aldehydes have been analyzed in different biological matrices including blood [4,6,12–17], urine [1,4,11,18,21] and breath [8,19] for which different analytical methodologies have been proposed. Gas chromatography coupled to mass spectrometry has been applied to the analysis of blood [14–17] and urine [21]. Aliphatic aldehydes have been also determined by means of liquid chromatography coupled to tandem mass spectrometry [1,2,4,6], to a variable wavelength detector [12,13,18] or to a photo diode array detector [19]. Capillary electrophoresis with amperometric detection has been also used in recent studies; in this case, derivatization is needed to convert aldehydes in electroactive species [11]. Also, in a recent work, a colorimetric sensor array for the discrimination of a group of aldehydes has been developed [22].

For the analysis of aldehydes in biological matrices, different extraction and preconcentration techniques prior to determination have been commonly used. Between the different sample pretreatment techniques, solid-phase extraction (SPE) has been used for the analysis of pentanal and hexanal in urine [1,4] and plasma [6] samples. Solid-phase microextraction has been applied either on its own [18] or coupled with headspace generation technique (HS-SPME) for the analysis of urine [21] and blood samples [15]. Liquid-phase microextraction has been applied both as hollow-fibre liquid-phase microextraction (HF-LPME) in the case of urine samples [11] and dispersive liquid–liquid microextraction (DLLME) for blood sampling [12,13]. A novel method based on magnetic solid-phase extraction with nanoparticles as extraction sorbents has also been developed

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for the analysis of aldehydes in exhaled breath [19]. In most of the methodologies proposed, a derivatization step is used either *in situ* [1,13,14,17–19] or prior to extraction techniques [4,6,11,12,16]. The most widely used derivatization reagents are 2,4-dinitrophenylhydrazine (2,4-DNPH) [1,12,13,18,19] and o-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) [14,16,17]. Nevertheless, other compounds such as cyclohexanedione (CHD) [6], 2,4,6-trichlorophenylhydrazine (TCPH) [23], 4-(2-(trimethylammonio)ethoxy)benzenaminium halide (4-APC) [2,4] and 2-thiobarbituric acid (TBA) [11] have been tested as derivatization reagents.

Sample preparation is considered to be the most delicate and time-consuming step of the analysis. Then, whenever possible, this step should be reduced or even avoided [24,25]. The use of headspace sampling (HS) represents a good option by minimizing sample treatment [26]. Ease of sample preparation, automation, rapidity and the absence of interferences of non-volatile compounds of the matrix make static headspace the technique of choice for complex matrices [27]. With regard to biological samples, this technique has been previously used for the determination of haloacetic acids [28] and trihalomethanes [29] in urine. Headspace generation has also been applied to the determination of volatile organic compounds in the same matrix by means of its coupling with a temperature-controlled cold injection system [30]. Regarding aldehydes determination, headspace sampling base methodologies have been previously proposed for the determination of these compounds in environmental [31], pharmaceutical [32] and food [33–36] matrices.

The aim of the present work is to develop an alternative automated method for the determination of aldehydes in urine samples. The strategy is based on the separation of the volatile aldehydes by headspace sampling coupled to a programmed temperature vaporizer followed by gas chromatography–mass spectrometry determination. To the best of our knowledge, this is the first time that this approach has been proposed for the determination of these analytes in urine samples. No derivatization is required so that the methodology is largely shortened and simplified.

2. Experimental

2.1. Materials and standard solutions

The aldehyde standards (pentanal, hexanal, heptanal, octanal and benzaldehyde) employed in this study as well as the reagents needed for creatinine measurements (creatinine standard and picric acid) were supplied by Sigma–Aldrich (Steinheim, Germany). Methanol was of HPLC grade and provided by Merck (Darmstadt, Germany). Sodium chloride, sodium hydroxide and phosphoric acid reagent grade (85%) were purchased from Scharlau (Barcelona, Spain).

Stock solutions (2500 mg/L in methanol) of each aldehyde were prepared and stored at 4 °C in a refrigerator. These solutions were used to spike the water and urine samples at the different concentrations analyzed.

Optimization of the method was performed with ultra-high quality water (UHQ) and urine sample at concentrations between 400 and 1000 μg/L. UHQ water was obtained with a Wasserlab Ultramatic water purification system (Noain, Spain).

2.2. Sample preparation

NaCl (2.4 g) was added to a 10-mL headspace vial. Then, 1 mL of urine sample was added, followed by 3 mL of UHQ water. The vial was sealed hermetically and placed in the headspace sampler, where it was shaken at 84 °C for 10 min. During this time, the generation and equilibration of the headspace took place.

2.3. Creatinine measurements

Urine is a highly variable matrix. Not only urine composition can vary daily depending on the diet but also concentration of endogenous compounds varies because of the irregularity of urine volume excreted. Due to this variability, normalization of analyte concentrations to total creatinine concentration (mmol) is required. This practice allows a randomly sampling of urine instead of a 24-h collection.

For this purpose, a modification of Jaffè method has been used [37,38]. Jaffè method is based on the photometric detection of a reddish complex resulting from the reaction between creatinine and picric acid. These measurements were carried by a spectrophotometer Shimadzu UV/Vis-160, equipped with a processing and recording unit, using a quartz cell (Suprasil), 10 mm path length. Normalized concentration values are expressed as nmol aldehyde/mmol creatinine.

2.4. Headspace

HS sampling was performed with a PAL autosampler (CTC Analytics AG, Zwingen, Switzerland). This sampler is equipped with a tray for 32 consecutive samples and an oven with positions for six sample vials. Oven temperature was kept at 84 °C and the equilibration time was set at 10 min. During this time, agitation of the sample was performed at 750 rpm. A 2.5-mL syringe at 120 °C was used, fixing an injection volume of 2.50 mL. The fill speed and injection speed were fixed to 100 μL/s and 250 μL/s, respectively. After injection, the syringe was cleaned with a flow of He (99.999%, Air Liquid) for 2 min. The time between samples was set at 14 min.

2.5. Programmed temperature vaporization

All experiments were carried out with a programmed temperature vaporizer (PTV) inlet (CIS-4, Gerstel, Baltimore, MD, USA). A Gerstel CIS-4 liner (71 mm × 2 mm) was used, packed with a chemical sorbent (Tenax-TA). In the optimized method, solvent-vent injection mode was used. Cooling was accomplished with liquid CO_2 (Air Liquid).

The initial injector temperature was set at 50 °C. Vent flow was adjusted to 20 mL/min, and vent pressure to 5.00 psi. The purge time was set at 0.5 min. The initial temperature of the liner was maintained for 0.55 min as a safety mechanism so that the heating ramp would start when the split valve was closed. Once venting had finished, the split valve was closed and the liner of the PTV was flash-heated at 12 °C/s up to 250 °C. The analytes were then transferred from the liner to the capillary column (1 min). Then, the split valve was opened and the liner temperature was maintained at 250 °C, and held for 2 min.

2.6. Fast gas chromatography

To perform the gas chromatography measurements, an Agilent 6890 GC device equipped with a low polarity DB-VRX capillary column (20 m × 0.18 mm × 1 μm, working range −10 °C to 260 °C) from J&W Scientific (Folsom, CA, USA) was used. The carrier gas was helium N50 (99.999% pure; Air Liquide).

The column oven temperature programme used an initial temperature of 45 °C for 2.00 min; an increase at 60 °C/min–175 °C and then an increase at 45 °C/min–240 °C, finally holding for 0.5 min. These temperature ramps are the maximum ones permitted by the instrumental configuration employed. The total chromatographic run time was 6.11 min.