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Short communication

Analysis of endogenous aldehydes in human urine by static headspace gas chromatography–mass spectrometry

María Serrano, Mercedes Gallego*, Manuel Silva

Department of Analytical Chemistry, Campus of Rabanales, University of Córdoba, E-14071 Córdoba, Spain

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ABSTRACT

Endogenous aldehydes (EAs) generated during oxidative stress and cell processes are associated with many pathogenic and toxicogenic processes. The aim of this research was to develop a solvent-free and automated analytical method for the determination of EAs in human urine using a static headspace generator sampler coupled with gas chromatography–mass spectrometry (HS–GC–MS). Twelve significant EAs used as markers of different biochemical and physiological processes, namely short- and medium-chain alkanals, α,β-unsaturated aldehydes and dicarbonyl aldehydes have been selected as target analytes. Human urine samples (no dilution is required) were derivatized with *O*-2,3,4,5,6-pentafluorobenzylhydroxylamine in alkaline medium (hydrogen carbonate–carbonate buffer, pH 10.3). The HS–GC–MS method developed renders an efficient tool for the sensitive and precise determination of EAs in human urine with limits of detection from 1 to 15 ng/L and relative standard deviations, (RSDs) from 6.0 to 7.9%. Average recoveries by enriching urine samples ranged between 92 and 95%. Aldehydes were readily determined at 0.005–50 μg/L levels in human urine from healthy subjects, smokers and diabetic adults.

1. Introduction

Aldehydes are intermediary or final products of the metabolism involved in a wide spectrum of biochemical and physiological processes, such as oxidative stress and cell processes [1,2]. The main source of endogenously produced aldehydes is the lipid peroxidation (LPO) process, with 4-hydroxynonenal, acrolein, crotonaldehyde and malondialdehyde being the most relevant endogenous aldehydes (EAs) [3,4]. Together with LPO, other biochemical processes contribute to the formation of different EAs, such glycation and the metabolism of amino acids, alcohol and sugar [3,5], among others. For the foregoing, accurate measurements of EAs in biological matrices, such as blood and urine are important in order to evaluate their implications for human health. Recently, urinalysis is a practical means (a non-invasive and simple assay) for acquiring information on these aldehydes as potential biomarkers of several diseases [6–9].

The determination of EAs in human urine includes a derivatization step before extraction and chromatographic analysis due to the high polarity, chemical instability and volatility of these compounds. Gas chromatography (GC) is the most useful choice for determining these carbonyl compounds in human urine after their derivatization with different labels [10–16]. The papers published have usually focused on determining one/a few aldehydes [12–16]; direct GC methods (without derivatization) have also been used but very rarely for this purpose [8,17]. Liquid chromatography (LC) has also been used to determine EAs in human urine [6,9,18–20]. The most current and relevant contributions involve the use of 2,4-dinitrophenylhydrazine (DNPH) as the derivatization reagent followed by LC analysis of the hydrazones formed by diode array [20] or mass spectrometric (MS) detection [6,9]. Although DNPH/LC–MS methods seem to be valuable options with respect to GC–MS ones, DNPH presents some drawbacks that can preclude its use for the determination of some EAs, such as formaldehyde, glyoxal and methylglyoxal [6,9,21].

Regarding urine sample preparation methods, liquid–liquid extraction (LLE) in *n*-hexane after batch derivatization of EAs has been the primary technique used for the extraction and preconcentration of derivatives prior to their determination by GC using electron-capture (ECD) [11–13] and MS [10–13] detectors. To overcome the well-known drawbacks related to LLE, simple, fast, and solvent-free microextraction techniques have been proposed as alternatives, including static headspace (HS) [14], single-drop microextraction [15] and headspace-solid-phase microextraction [17]. Recently, a HS–GC–MS methodology for the direct determination (without derivatization) of five aldehydes in urine as possible

* Corresponding author. Fax: +34 957 211 066.
E-mail address: qa1gafem@uco.es (M. Gallego).

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markers of oxidative stress has been reported [8]. However, the most relevant markers (malondialdehyde, acrolein, crotonaldehyde, glyoxal, methylglyoxal, etc.) were not included in the study because a prior derivatization step is required for their HS–GC–MS determination.

Based on the considerations mentioned above, the objective of this research was to develop a solvent-free, automated and sensitive HS–GC–MS analytical method to determine EAs in human urine. Relevant endogenously produced aldehydes used as markers of different biochemical and physiological processes have been selected as target analytes, e.g., short- and medium-chain alkanals (formaldehyde to heptanal), α,β-unsaturated aldehydes (acrolein and crotonaldehyde) and dicarbonyl aldehydes (glyoxal, methylglyoxal and malondialdehyde). To our knowledge, this is the first report on the use of HS–GC–MS for the simultaneous determination of these twelve significant EAs in human urine.

2. Experimental

2.1. Chemicals and standards

Aldehydes (≥95–99%), *O*-2,3,4,5,6-pentafluorobenzylhydroxylamine hydrochloride (PFBHA, ≥98%), internal standard (IS, 1,2-dibromopropane), creatinine (≥98%) and picric acid (≥98%) were supplied by Sigma–Aldrich–Fluka (Madrid, Spain). Methanol was purchased from Romil Chemicals (Cambridge, UK) and *n*-hexane from Merck (Darmstadt, Germany). Sodium carbonate and anhydrous sodium hydrogen carbonate were supplied by Panreac (Barcelona, Spain).

Stock standard solutions were prepared at concentrations of 1.0 g/L in methanol and stored in amber glass vials at −20 °C. More diluted individual or cumulative solutions were prepared daily in ultra-grade water. Working standards were prepared at nanogram-per-liter levels by spiking known amounts of the diluted stock solutions into 20 mL glass vials containing 10-mL reconstituted urine, which was prepared from a native human lyophilized urine control (Medidrug Basis-line U) and acquired from LCG Standards SLU (Barcelona, Spain).

2.2. Chromatographic conditions

Analyses of the samples were carried out by using an HS autosampler G1888 and an HP 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with an HP 5975C mass selective detector. Operating conditions were those recently proposed by our research group for the determination of aldehydes in treated waters [22], excepting the oven temperature for the HS autosampler, which was fixed at 85 °C. Chromatographic optimization was conducted in total ion chromatogram mode, and quantification of the target analytes was performed in selected ion monitoring (SIM) mode using *m*/*z* 181 as the quantifying ion (highest intensity). Suitable fragments of each analyte for identification are listed in table of analytical features.

2.3. Analytical procedure

Ten mL of human or reconstituted urine sample containing between 0.005–0.05 and 5–50 μg/L of each aldehyde were placed in a 20 mL glass vial containing 1 g of anhydrous sodium hydrogen carbonate and 2 g of sodium carbonate (saturated solution) for adjusting the pH (10.3) and the ionic strength. Then, 50 μL of 200 g/L of PFBHA aqueous solution and 20 μg/L of 1,2-dibromopropane were added and the vial was immediately sealed and vortexed for 30 s for homogenization purposes. Finally, the vial was placed into the autosampler carrousel. Samples were analyzed by HS–GC–MS, using the above-mentioned operating conditions.

Table 1
Selected variable values to determine aldehydes in human urine and treated water samples by HS–GC–MS.

Conditions	Human urine	Treated water[a]
Chemical variables		
Sample pH	10.3	8.4
PFBHA amount, μmol	40	30
Ionic strength, mol/L	6.3	6.0
Modifier (*n*-hexane), μL	Not required	200
Instrumental variables		
Oven temperature, °C	85	80
Equilibration time, min	20	20

[a] Results obtained from Ref. [22].

2.4. Urine samples

Three groups of subjects, following informed consent, provided urine samples: eleven healthy adult non-smokers, four adult smokers and two diabetic adults. All subjects provided first morning urine samples (after overnight fasting, to minimize dietary influence), which were collected into sterilized polyethylene bottles of 100 mL without headspace to prevent the formation of air bubbles and stored at 4 °C up to 72 h. When the time between urine collection and analysis exceeded 72 h, samples were stored at −20 °C up to 30 days to avoid storage losses. Frozen urine samples were left in a refrigerator until completely thawed. If required, thawed urine samples can be stored for 4 h prior to their analysis in a refrigerator. After gentle mixing, urine was transferred into the analysis vials.

3. Results and discussion

3.1. Method development

Although the derivatization/extraction conditions for some aldehydes were established in a previous study [22], chemical and HS conditions must be re-optimized taking into account that additional aldehydes are included in this study and the determination was carried out in a more complex matrix such as human urine. In this context, a reconstituted urine ("urine blank") prepared from a native human lyophilized urine control was used as the matrix. A pooled urine sample was not used as the matrix because target aldehydes are present to a greater or lesser extent in real urine due to their endogenous character.

Table 1 shows the optimum values of chemical and instrumental variables affecting the derivatization/extraction process of EAs as well as those corresponding to treated water for comparison purposes. The following comments can be inferred: (i) the relative peak areas for dicarbonyl aldehydes (glyoxal, methylglyoxal and malondialdehyde) increased significantly at alkaline pH (9.0–10.6) whereas those obtained for the other aldehydes were independent throughout the pH range assayed (1.1–11.3). This behavior can be attributed to a possible alkali-catalyzed reaction of the hydroxylamine group of PFBHA with these kind of aldehydes [22]. A carbonate buffer solution (pH 10.3, maximum buffer capacity) was used to adjust the derivatization sample pH, which also provided the sufficient ionic strength (ca. 6.3 mol/L) for oxime extractions. (ii) The amount of derivatization reagent and reaction temperature (oven temperature) increased slightly with respect to the procedure reported for treated water (see Table 1), which can be attributed to a probable urine matrix effect; and (iii) the addition of an organic modifier (*n*-hexane) did not enhance the volatilization of any oxime-product, so no modifier was required.