Talanta 168 (2017) 100–104

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Dispersive liquid-liquid microextraction for a rapid determination of glyoxal in alcoholic beverages

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ARTICLE INFO

Keywords:
Glyoxal
3
4-diaminopyridine
DLLME
HPLC-fluorescence
Alcoholic beverages

ABSTRACT

Carbonyl compounds, like glyoxal, methylglyoxal, diacetyl or pentane-2,3-dione, among others, have been widely studied. Besides its endogenous origin, they are originated from foodstuffs and are related to sensorial characteristics in products such as wine and beer. Generally, for their determination, the analytes must be derivatised to adapt them for the detection system and this step takes long time. The main aim of this research was to develop a simultaneous derivatization and extraction method which takes place in only few minutes. 3,4-diaminopyridine, as derivatizing reagent, generate a fluorescent product. This reaction is selective for glyoxal. For this new dispersive liquid-liquid microextraction (DLLME) procedure combined with chromatographic determination of glyoxal, various parameters affecting the extraction were optimized and finally, a mixture of butan-1-ol as dispersant solvent and dichloromethane as extractant solvent were selected. Its chromatographic peak appears at 2.6 min. Four Spanish wines and five Spanish beers have been analysed and the results showed that the levels of glyoxal are comprised between 2.8–9.5 mg L^{-1}. The proposed DLLME method drastically reduces the reaction time from 2 or 3–20 min improving the methods found in the literature. The glyoxal concentration found in the wines and beers analysed do not suppose any health risk.

1. Introduction

Carbonyl compounds of low molecular weight, such as glyoxal (GL) and methylglyoxal (MGL), have been widely studied due to their toxic effects on the human body. They react quickly to form reactive oxygen and carbonyl species, the latter precisely being related to diabetes complications, cardiovascular disease and age-related problems [1].

Besides its endogenous origin, carbonyl compounds also have an exogenous source since they originate from foodstuffs. GL, MGL and diacetyl are the α-dicarbonyl compounds most commonly found in food, and the most widely investigated [2]. Their role in food quality is mainly related to sensorial characteristics, particularly in fermented products such as wine and beer [3–5].

The formation of these compounds occurs through the so-called Maillard reaction, which is desired to a certain extent because it is responsible of the formation of flavor and color. However, it could cause nutritional detriments and organoleptic changes in foodstuffs during storage. An intermediate stage of this reaction is the Strecker degradation, which is considered a significant source of heterocyclic flavor compounds such as pyrazines, oxazoles and thiazoles [6].

The human body has effective mechanism to cope with GL and other similar compounds, however if the contribution of α-dicarbonyl compounds in the diet is too high it is possible that these mechanisms may not work properly. Therefore, the health problems mentioned above could take place [7].

Pripis-Nicolau et al. [8] studied the reactivity of carbonyl compounds with different amino acids under mild conditions, to check which flavor components are formed in the wine. According to the Maillard reaction, the amine group of the amino acid makes a nucleophilic addition to the carbonyl group. This may occurs during bottling or during shelf-keeping because increases in temperature are favorably to this kind of reaction. They observed that in the case of glyoxal, when reacted with isoleucine and phenylalanine, produced pleasant odors. However, when reacted with cysteine or methionine, it generated compounds that have an unpleasant odor such as methanethiol (cabbage) or H_2S (rotten eggs).

HPLC is one of the most used techniques for the determination of glyoxal. Indeed, it has been extensively determined in biological, atmospheric and food samples. However, there are few methods developed for the determination of glyoxal in fermented alcoholic

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http://dx.doi.org/10.1016/j.talanta.2017.03.031
Received 29 November 2016; Received in revised form 6 March 2017; Accepted 10 March 2017
Available online 11 March 2017
0039-9140/

beverages such as wine [4,9–11] and beer [4,9,12]. Generally, the analytes must be derivatised to adapt them for the detection system [4,11,13,14].

On the other hand, GC has been rarely used for food samples [5,10,15]. Flamini et al. [16] studied the relation between the presence of glyoxal and glycolaldehyde in wine by GC-MS. They concluded that when glyoxal is present in the sample, one day after fermentation, it induces the formation of glycolaldehyde, confirming that the presence of both compounds in wine is directly correlated, probably by a redox mechanism. Also, they observed that the moderate amount of glycolaldehyde developing during malolactic fermentation may play an important role in the color stability of white wines. This highlights the importance of the glyoxal determination.

About dispersive liquid-liquid microextraction (DLLME), it is a very simple and rapid method for the extraction and preconcentration of organic compounds from aqueous samples developed by Rezaee et al. [17]. It is based on a ternary component solvent system (aqueous sample, disperser solvent and extraction solvent).

Generally, DLLME has been the preferable choice for the analysis of samples with a simple matrix, being aqueous matrices the most commonly studied. However, the most recently reported applications of DLLME have focused on more complex matrices as food and beverages [18,19], and pharmaceutical and biological samples [20]. The complexity of certain food samples as well as their degradation capacity is a drawback that has greatly difficulted the DLLME application and frequently requires a previous extraction or cleaning step, followed by a suitable reconstitution [21].

The analysis of wine and beer employing DLLME has been focused in the detection of different contaminants in the samples, as fungicides, volatile phenols as halophenols and haloanisols, ocratoxin A or sulfured compounds [18]. To the best of our knowledge, no method has been employed for the detection of inherent components in wine or beer, such as carbonyl compounds.

This paper reports for the first time the development of a DLLME procedure for glyoxal determination in alcoholic beverages by means of a derivatization and extraction followed by HPLC-FD. In this proposed method, the derivatization and extraction of glyoxal in wine and beer was performed in only one step, and the obtained derivative in the extraction solvent was directly injected into the chromatographic system without any further treatment. The DLLME conditions were studied, and the method validation was investigated.

2. Materials and methods

2.1. Chemicals

Aqueous solution of glyoxal (40%) was purchased from Sigma-Aldrich (Madrid, Spain). Stock analyte solution (55 mg L^{-1}) was prepared by weighting and diluting in water and stored at 4 °C in absence of light. Working analyte solutions were prepared taking the correspondent aliquot and diluting with water. 3,4-diaminopyridine (3,4-DAP) was purchased from Sigma-Aldrich, and a 160 mg L^{-1} stock solution was daily prepared by dissolving in water adequate amounts of the powder presentation of the compound.

Mono-chloroacetic acid and NaOH used to prepare the chloroacetic acid/sodium chloroacetate buffer (pH 2.0; 0.3 M) needed to fix the pH of the derivative were obtained from Panreac (Barcelona, Spain).

Acetic acid/sodium acetate buffer (pH 5.5; 60 mM) used as mobile phase was prepared by diluting in water the adequate volume of the liquid presentation of acetic acid glacial (Panreac) and adjusted the pH at 5.5 with the addition of NaOH solution. Mobile phase was filtered through a 0.22 μm membrane nylon filter (Teknokroma, Barcelona, Spain), and degassed by ultrasonication before use. Samples were also filtered through 0.22 μm membrane PTFE filters (Millipore, Madrid, Spain) before injection.

Synthetic wine solutions were prepared by dissolving 5 g L^{-1} of L-(+)-tartaric acid (Scharlau, Barcelona, Spain) in a hydroalcoholic solution 13% (v/v) ethanol (Panreac). The pH of these resulting solutions was adjusted to 3.50 with NaOH.

The organic solvents used as extraction mixture, butan-1-ol and dichloromethane, were purchased from Panreac and Scharlau, respectively. All other solvents employed were of analytical grade. Chloroform, isohexane, isobutanol, acetone and pental-2-ol were purchased from Panreac. Propan-2-ol, acetonitrile and dimethylformamide were from Sigma-Aldrich; carbon tetrachloride was purchased form Probus, and cyclohexane from Carlo Erba.

All solutions were prepared with Milli-Q water (Millipore). Methanol HPLC grade was purchased from Panreac.

The wines and beers analysed were acquired from local markets, and were kept at 4 °C, avoiding exposure to direct light. A total amount of four wine brands, all of them belonging to Denomination of Origin (D.O.) “Ribera del Guadiana” (Extremadura, Spain) and five Spanish beers were taken for this study.

2.2. Instrumentation and software

Fluorescent studies were performed using a fluorescence spectrophotometer Varian Model Cary Eclipse (Varian Australia Pty Ltd, Mulgrave, Victoria, Australia), equipped with a Xenon lamp. All measurements were done in 1 cm quartz cells at ambient temperature. The Cary Eclipse software was used for controlling the instrument, data acquisition, and data interpretation.

Chromatographic studies were performed on an Agilent Model 1100 LC instrument (Agilent Technologies, Palo Alto, CA, USA), equipped with an on-line degasser, a quaternary pump, a manual six-way injection valve containing a 20 μL loop and fluorescence detector. The ChemStation software was used for controlling the instrument, data acquisition, and data interpretation. The chromatographic separation was achieved on a Zorbax Eclipse XDB-C18 (150×4.6 mm, 5 μm; Agilent Technologies) analytical column.

A Crison Micro pH 501 m (Barcelona, Spain), equipped with combined glass/saturated calomel electrode, was used for pH measurements. The pH electrode was calibrated at the beginning of the working day.

Calibration curves and analytical figures of merit were performed by means of the ACOC program [22], in MATLAB code.

2.3. Calibration curve

To build the calibration curve, standard solutions were prepared as follows: in a centrifuge tube, 1 mL of the synthetic matrix of wine, aliquots of stock analyte solution of glyoxal in variable concentration (0.025–0.200 mg L^{-1}), 3,4-diaminopyridine (75 μL of a 160 mg L^{-1} stock solution) and water until a final volume of 1.5 mL were placed. Then, adequate volumes of a solution of NaOH (0.4 M) were added to achieve a pH of approximately 11.6. Immediately, the dispersive liquid-liquid microextraction was performed with a mixture of 750 μL of butan-1-ol and 150 μL of dichloromethane. Then, solutions were vortex for a minute and centrifuged during 5 min at 3000 rpm.

After centrifugation, the upper phase was collected with a syringe and placed in a 3,0 mL volumetric flask. After that, pH was fixed at 2.0 with a chloroacetic acid/sodium chloroacetate buffer 0.03 M and methanol (MeOH) was added to obtain a minimum concentration of 30% (v/v). Solutions were diluted with water up to the mark, homogeneized, filtered through a 0.22 μm membrane PTFE filters and aliquots of 20 μL were injected into the chromatographic system. The separation and detection was carried out under the optimized conditions. Once obtained the chromatogram, the retention time and the analytical signal (peak area) was measured using the ChemStation package.