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Paper platform for reflectometric determination of furfural and hydroxymethylfurfural in sugarcane liquor

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ABSTRACT

Cachaça is the popular name of sugarcane liquor obtained from fermented sugarcane mash broth. This is one of the most popular alcoholic beverages in Brazil and is gaining ground in the global market. One of the quality parameters established by Brazilian law is the sum of the concentrations of furfural and hydroxymethylfurfural, two compounds that give the beverage an unpleasant taste and have mutagenic potential. These two substances are usually determined by chromatographic techniques that employ toxic organic solvents that can be damaging to the health of the operator and to the environment. This paper describes the development of a new methodology to determine furfural and hydroxymethylfurfural in sugarcane liquor using a diffuse reflectance technique coupled with limited-area spot-testing on a paper platform. The new method presented LOQ values of 0.74 mg L^{-1} for furfural and 1.27 mg L^{-1} for hydroxymethylfurfural. Recoveries in the ranges 89.5–108% (furfural) and 96.3–106% (hydroxymethylfurfural) indicated that there was no significant influence of the matrix in determination of the analytes. The method was applied using eleven sugarcane liquor samples from different locations in Brazil.

1. Introduction

Sugarcane liquor is one of the most common alcoholic beverages in Brazil and is increasingly popular worldwide. It is also known by the names "sugarcane spirit" or "cachaça". In 2016, it was exported to over 40 countries, generating revenues of about US$ 13.9 million [1].

This drink is obtained by distilling fermented sugarcane mash, and one of the quality parameters established by the Ministry of Agriculture, Livestock and Supply (MAPA) is the sum of the concentrations of furfural (FUR) and hydroxymethylfurfural (HMF), with a maximum limit of 5 mg in 100 mL of anhydrous ethanol [2]. Unlike many contaminants, the formation of FUR and HMF not only occurs in the fermentation step; both substances can be produced in the broth if the harvesting is preceded by the burning of the sugarcane plants, which can lead to the generation of free sugars such as pentoses and hexoses in the broth. The degradation of the free sugars then results in the formation of FUR and HMF. These compounds are markers of heating processes in many products that contain sugars in their composition [3–7], and their presence in sugarcane liquor is undesirable because it gives the beverage unwanted features such as a penetrating and nauseating aroma [8]. Furthermore, due to the planarity of their structures (Fig. 1), FUR and HMF are potentially carcinogenic/mutagenic since they can interact with DNA molecules [9–15].

Several methodologies are available for the determination of FUR and HMF in many types of samples, mostly based on chromatography [16–20]. Although these techniques provide efficient separation and determination of the analytes, with low limits of detection, disadvantages are that they usually require the use of toxic organic solvents and that the instruments employed for the analyses are expensive and require specialist operators. The method involving electrophoretic separation [21] offers an analysis without organic solvent, but the instrumentation required has higher added cost, compared to the equipment needed in the analytical method proposed here, and also necessitates a specialized operator. The methodology with digital image detection [22] is an example of an analytical procedure that reduces the use of reagents and generates lower quantities of waste, compared to conventional procedures [2]. Nevertheless, the spot method [22] used only determines the furfural concentration, rather than the sum of HMF and FUR as required by legislation [2]. Therefore, for samples in which the amount of HMF and FUR exceeds the established limit, the FUR concentration could be below this limit (as in the case of sample G), generating a false negative. With the volume required for only one determination by the digital image procedure [22], it would be possible to perform around 40 analyses using the proposed method. In addition, the waste generated in the present method is solid and readily incinerable, while in the method proposed by Franco et al. [22], the residues produced have to be converted to harmless substances.

In most cases, analytical methodologies do not conform to the principles of Green Chemistry [23], which aims to minimize (or preferably

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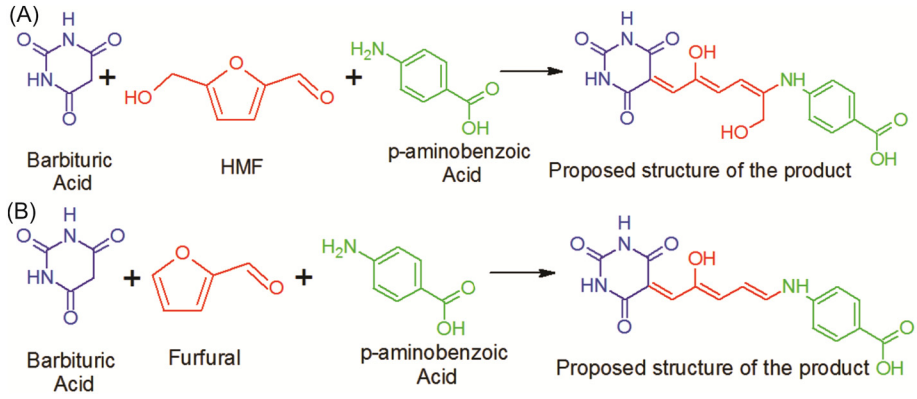

Fig. 1. (A) Possible structure of the product resulting from the reaction involving PABA, BA, and HMF; (B) possible structure of the product resulting from the reaction involving PABA, BA, and FUR.

eliminate) the use of toxic organic solvents and develop simpler and less onerous methodologies. A good alternative method for the determination of FUR and HMF is diffuse reflectance spectroscopy, which is simpler than the chromatographic techniques usually used to analyze such compounds.

For many years, the use of reflectance spectroscopy was limited to paints and pigments, paper, textile areas, ceramics, dye-stuffs and printing inks to evaluate properties such as color, whiteness, gloss, covering power, and so on [24]. Recently with the development of optical devices such as integrating sphere assemblies, diffuse reflectance spectroscopy is rapidly gaining in acceptance in analytical chemistry. Application of diffuse reflectance spectroscopy especially associated to spot test has been reported in the literature indicating the potential of this technique for quantitative analysis [25–27].

In the present work, we describe a new spot test/diffuse reflectance spectroscopy method employing a paper platform delimited with hydrophobic barriers. The combination of diffuse reflectance spectroscopy and spot testing is eco-friendly because it uses minimal quantities of reagents and consequentially generates only minor amounts of waste, while the environmental and health risks are very low [23]. Filter paper is obtained from renewable sources and provides an excellent platform for spot tests due to its cellulose fiber composition and its white color, which provides a bright and high contrast background [24].

The use of hydrophobic barriers for impregnation of the filter paper platforms used in spot tests greatly improves the analyses by preventing the analyte and reagent solutions from eluting beyond the area defined by the barrier [28], hence increasing the concentration of the colored product and the magnitude of the analytical signal. The first report of the use hydrophobic barriers was in the work of Yagoda in 1937 [29], for determination of metal ions, and since then several papers have described the use of hydrophobic barriers in inexpensive and portable methodologies [30–32].

There are many ways to impregnate the hydrophobic barriers in the paper platform [31]. One method is wax printing, where a wax-based printer prints patterns of solid wax on the surface of the paper, followed by heating in an oven or on a hotplate [28]. When the wax ink is heated, it penetrates through the porous paper, creating the hydrophobic barriers that prevent the solution eluting beyond the delimited area.

2. Experimental

2.1. Apparatus

Diffuse reflectance measurements were made using a portable spectrophotometer (USB2000, Ocean Optics) controlled with OOIBase32 software (Ocean Optics). The spectrophotometer was coupled to an integrating sphere using an optical fiber. The comparative method employed a Shimadzu UFLC-20A HPLC system with a DAD detector [17]. A mass spectrometer (Thermo Scientific LCQ Fleet Ion Trap LC/MS^n) was used to determine the product structure.

2.2. Materials, reagents, and solutions

Whatman No. 1 qualitative filter paper was used as the solid support in the spot tests. All the reagents employed were analytical grade and were used without any prior purification. Analytical standards of furfural and hydroxymethylfurfural were obtained from J.T. Baker. Ultrapure water (18 MΩ cm, Milli-Q system, Millipore) was used to prepare the solutions.

The reagent solutions were composed of a mixture of p-aminobenzoic acid (Henrifarma, Brazil), barbituric acid (Merck), and hydrochloric acid (Merck), at different concentrations for the determinations of HMF or FUR.

Stock standard solutions of 0.00550 mol L^{-1} HMF and 0.00723 mol L^{-1} FUR were freshly prepared in aqueous 40% (v/v) solutions of HPLC grade ethanol (J.T. Baker). Working solutions of FUR and HMF were prepared daily by appropriate dilutions of the stock solutions in aqueous 40% (v/v) ethanol.

2.3. Samples

Eleven sugarcane liquor samples were used to evaluate the performance of the new method proposed here. The liquor samples were either sugared (B, C, D, F, I, and J) or non-sugared (A, E, G, H, and K), and were also classified as aged (G and H) or non-aged (A, B, C, D, F, I, J, and K). The samples originated from the states of São Paulo (A, B, C, D, E, F, G, and H), Paraná (I), Pernambuco (J), and Ceará (K).

2.4. Procedure

2.4.1. Paper platform for spot tests

CorelDRAW ×5 was used to design hydrophobic barriers that were 15 mm in diameter and 0.75 mm in thickness. The design was printed onto Whatman No. 1 filter paper with wax toner (Genuine Xerox Solid Ink Black) using a wax printer (Xerox Phaser 8560), as described by Carrilho et al. [28]. After printing, the paper was heated for 120 s at 120 °C for formation of the hydrophobic barriers.

2.4.2. Reagent solution

The reagent solution, described in the work of Castoldi et al. [33], was based on the Winkler method for determination of HMF in honey samples [34]. This solution contained p-aminobenzoic acid (PABA), barbituric acid (BA), and hydrochloric acid (HCl). The analytes were quantified separately using two different reagent solutions, both composed of PABA, BA, and HCl, but at different concentrations, using a single spot test device for each analysis. The results were calculated as the sum of FUR and HMF.