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On-line gradient liquid chromatography–Fourier transform infrared spectrometry determination of sugars in beverages using chemometric background correction

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ABSTRACT

An on-line gradient reversed phase liquid chromatography–Fourier transform infrared spectrometry (LC–FTIR) method was developed for the determination of fructose, glucose, sucrose and maltose in beverages. Improved chromatographic resolution was obtained using a linear gradient from 75 to 55% (v/v) acetonitrile in water in 15 min. Changes in the background spectra were corrected employing "univariate background correction based on the use of a reference spectra matrix" (UBC-RSM) and using the ratio of absorbance (AR) at 2256 and 2253 cm^{-1} for the identification of the eluent spectra within the RSM. The method provided limits of detection in the order of 0.75 mg ml^{-1}. The precision (as relative standard deviation) ranged between 3.3 and 4.1% for glucose and fructose, respectively at a concentration level of 3.0 mg ml^{-1}. Quantitative recovery values on spiked samples confirmed the accuracy of the method. A set of samples from the Spanish market were analysed to test the suitability of the procedure.

1. Introduction

The term sugar is frequently used to describe monosaccharides as glucose and fructose and disaccharides as sucrose and maltose that are absorbed, digested and fully metabolized [1].

The current interest in the physiological role of carbohydrates, the technological developments in food processing and manufacturing, and the different existing mandates of nutrition labeling (e.g. Food and Drug Administration [2]) have created a need for carbohydrate analysis at different production stages in food industries like in alcoholic and non-alcoholic drinks, fruit juices, sweets or dairy products.

Liquid chromatography (LC) has often been employed for sugar analysis in different food matrices where the most commonly used detector is the refractive index detector (RID). However, it shows poor sensitivity, high instability with regard to fluctuations in mobile phase composition and eluent temperature, low selectivity and incompatibility with mobile phase gradients [3,4].

Evaporative light scattering detection (ELSD) is compatible with gradient elution and provides a significant increase in sensitivity as compared with RID, but it is also a low-selective detector.

UV detection is not directly applicable for sugar analysis, without a pre- or post-column derivatization of the analytes, due to the low UV-absorbance of these compounds. The short wavelength required for their detection in UV reduces the selectivity of the obtained chromatographic signal increasing the number of possible interferences, thus requiring extensive sample clean-up prior to the detection.

Mass spectrometry (MS) is an expensive detection technique which provides high selectivity and sensitivity levels, but its field of application focuses on compounds at trace levels and not in the percentage range as it is the case of the main sugars present in foods.

Alternatively, infrared (IR) spectrometry is a versatile analytical tool which can be used for both, qualitative and quantitative determinations regarded as a general detection tool for organic analytes. On-line hyphenated with separation techniques as LC or capillary electrophoresis (CE) increases both, the applicability and the accuracy of IR-based methods by a significant reduction of potential spectral interferences. On the other hand, with limits of detection in the high mg l^{-1} range, it is clear that this technique cannot meet the current demands of trace analysis. However, IR spectrometry has proven to be a simple and rapid technique for quantitative and qualitative determination of analytes in the percentage level.

On-line LC–IR methods carried out under isocratic conditions use a constant eluent reference spectrum to correct the eluent spectral contribution. The advantages of this approach are its simplicity and the good quality of the recovered spectra obtained.

Using a constant mobile phase composition, also known as isocratic conditions, the capability of on-line LC–Fourier transform

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infrared (FTIR) spectrometry for the determination of sucrose, glucose and fructose in aqueous samples was demonstrated by Vonach et al. [5,6]. Edelmann et al. [7] reported the use of a quantum cascade laser as mid-IR source for the direct determination by on-line LC–IR of glucose and fructose in wine samples. Later, Edelmann et al. [8] used on-line LC–attenuated total reflectance (ATR) measurements for the analysis of organic acids, sugars and alcohols in red wine using multivariate curve resolution–alternating least squares (MCR–ALS) for the quantitative analysis of overlapping compounds. Moreover, a FTIR spectrometer has been on-line coupled with CE for the separation and quantification of sucrose, glucose and fructose in fruit juices [9]. The use of micro-machined flow cells in on-line CE–FTIR enables non-destructive, real time detection of analytes. However from the instrumental point of view the complexity of the required set-up for the CE–FTIR coupling is much higher than that for LC–FTIR.

On the other hand, the use of on-line gradient LC–FTIR is still challenging, because the use of a constant reference spectrum is not suitable if the relative concentration of the different IR-absorbing mobile phase components (e.g. water, acetonitrile or methanol) is modified during the chromatographic run [10]. Therefore, in recent years much attention has been paid to background correction in order to increase both, the applicability and the peak separation capabilities of on-line LC–FTIR [11–13]. Recently, Quintás et al. proposed a strategy named "*univariate background correction method based on the use of a reference spectra matrix* (*UBC-RSM*)" to perform an automated background correction in continuous liquid flow systems [14]. This approach is based on the assumption that using characteristic absorption bands of the mobile phase, it is possible to correct the background eluent spectral contribution to the overall absorption, by using a previously measured reference spectra matrix which covers a wide range of mobile phase compositions. This background correction method has already been successfully applied using acetonitrile:water (1% acetic acid) [14] and methanol:water [15] mobile phase gradients in reversed phase LC for the determination of atrazine and diuron pesticides in aqueous solutions and for the determination of the critical conditions of polyethyleneglycol, respectively.

In this study, FTIR is used as an on-line detector for the LC separation under gradient conditions of four model carbohydrates (fructose, glucose, sucrose and maltose) in beverages in order to: (i) test the suitability of the UBC-RSM approach in the presence of increasing concentrations of sugars; (ii) enable a higher sample throughput than that obtained under isocratic conditions in an equivalent LC–FTIR system and (iii) evaluate the method for the analysis of commercially available beverage samples.

2. Experimental

2.1. Apparatus and reagents

A Dionex (Sunnyvale, CA, USA) P680 high performance liquid chromatograph system, equipped with a Kromasil 100 NH_2 column (250 mm × 2 mm, 5 μm) fro, Spain) and a sample injection loop of 20 μl, was employed for chromatographic separations. Linear acetonitrile:water gradients were run from 75 to 55% acetonitrile (Merck, Darmstadt, Germany) in 15 min.

A flow cell with CaF_2 and ZnSe windows and a pathlength of 10 μm installed on a Bruker (Bremen, Germany) IFS 66/v FTIR spectrometer equipped with a liquid nitrogen refrigerated mercury–cadmium–telluride (MCT) detector, a vacuum system and a dry air purged sample compartment was employed for the FTIR spectra acquisition. The scanner for the interferometer was operated at a HeNe laser modulation frequency of 100 kHz. Spectra were recorded in the range between 4000 and 950 cm^{-1} using the spectrum of the empty sample compartment as background, with a resolution of 8 cm^{-1} and a zero filling factor of 2. Zero filling consists in adding zeros on both ends of the interferogram before the Fourier transformation so that the spectral lines have a smoother shape.

During on-line LC–FTIR gradient experiments, 25 scans per spectrum were averaged, providing a spectra acquisition frequency of 15 spectra min^{-1}.

For instrumental and measurement control and data acquisition, the OPUS program (Version 4.1) from Bruker was employed.

Background correction and data treatment were run under Matlab 7.0 from Mathworks (Natick, USA, 2004) using in-house written Matlab files available from the authors of this paper.

D(−)-Fructose, D(+)-glucose, sucrose and maltose-1-hydrate of analytical grade were purchased from Scharlab (Barcelona, Spain). Beverage samples were directly obtained from the Spanish market.

2.2. Sample preparation

A volume of homogenized sample between 50 and 500 μl was introduced in a 5 ml volumetric flask. Then 3.5 ml of acetonitrile were added and the flask was filled up to volume with water. Before the injection in the chromatographic system the solution obtained was sonicated in an ultrasound water bath for 5 min and filtered through a 0.22 μm PFTE membrane. Carbonated liquid samples were previously degassed in an ultrasound water bath for 15 min prior to their dilution.

In order to increase the applicability of the method reducing possible interferences, acetonitrile was selected for sample dilution and chromatographic separation because according to a previously published work [16] it precipitates proteins and starch present in sample matrices.

2.3. Univariate background correction based on the use of a reference spectra matrix (UBC-RSM)

A detailed description of the UBC-RSM method can be found in a previous work [14]. In brief, the eluent correction method can be divided in five steps: in Step 1 a reference spectra matrix RSM (r, c) is measured. The acquisition of the RSM is carried out in practice by measuring a LC gradient in a defined composition range. This step also include the measurement of the sample matrix SM (z, c). The eluent composition range of the SM should be within the RSM composition interval.

Step 2 involves the calculation of the absorbance ratio (AR) at two selected wavenumbers (r_1 and r_2) for each spectrum included in the SM and the RSM. The obtained AR values are characteristic for the mobile phase composition as defined in Eq. (1).

$$\mathrm{AR}_s = \frac{y^s_{r_1}}{y^s_{r_2}} \tag{1}$$

where $y^s_{r_1}$ and $y^s_{r_2}$ are the absorbance values at the wavenumbers r_1 and r_2 (cm^{-1}) measured in the spectra $s = (1, \ldots, z)$ for spectra included in the SM and $s = (1, \ldots, r)$ for the RSM.In Step 3, for each of the z spectra included in the SM, the most appropriated background spectrum ($S_{\gamma,s}$, $s = 1, \ldots, z$) included in the RSM is located.

Step 4 consists of the calculation of a correction factor (KF) which is determined for each sample spectrum. The objective of the KF is to correct slight changes in the spectral intensity of the eluent during the run. The KF is defined as the ratio of absorbance of the sample S_s at wavenumber φ ($y^{S_s}_{\varphi}$) and the previously selected background spectrum $S_{\gamma,s}$ at a defined wavenumber φ ($y^{S_{\gamma,s}}_{\varphi}$) using the following expression:

$$\mathrm{KF}_s = \frac{y^{S_s}_{\varphi}}{y^{S_{\gamma,s}}_{\varphi}} \tag{2}$$