Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1066 (2005) 105–110
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Liquid chromatographic method for quantifying polyphenols in ciders by direct injection

Belén Suárez, Noemí Palacios, Natalia Fraga, Roberto Rodríguez*

Área de Tecnología de los Alimentos, Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA), 33300-Villaviciosa, Asturias, Spain

Received 18 November 2004; received in revised form 29 December 2004; accepted 14 January 2005

Abstract

An analytical method for the quantitative determination of the principal phenolic compounds (benzoic acids, hydroxycinnamic acids, 3-phenylpropionic acids, flavanols, procyanidins, dihydrochalcones, quercetin glycosides) in ciders, which successfully employs a RP-HPLC and photodiode-array detection system without prior treatment of the sample, is described. Parameters usually examined in the method validation were evaluated. Good linearity was obtained with correlation coefficients exceeding 0.999 and the detection limits ranged from 0.07 mg/L (*p*-hydroxybenzoic acid) to 2 mg/L (hydrocaffeic acid). Recoveries ranging between 90 and 104% and the reproducibility of the method was always <8% (RSD). The method was applied to a set of commercial samples and the results obtained may be helpful to establish a phenolic profile in Asturian cider.

Keywords: Phenolic acids; Flavonoids; Cider; Direct injection; HPLC.

1. Introduction

Low molecular mass polyphenols have been exhaustively studied due to their contribution to the sensory quality (colour, taste, flavour) and their use for determining adulterations in apple derived products [1–5]. During the past decade the apple polyphenols have been subjected to a number of investigations due to their presence in human diet and their biological properties and benefit effects on health. Phenolic compounds seem to protect against cardiovascular disease and have certain potential anticarcinogenic properties due to their antioxidant activity and their function as free radical scavengers [6–9].

Polyphenols analysis, in cider and apple, is generally accomplished by reversed-phase high-performance liquid chromatography with UV–vis detection. However, the determination is usually preceded by several operations, such as extraction, purification and concentration, due to the complexity of analysis. Two methods for polyphenols fractionation have been used: liquid–liquid and solid–liquid extraction [10–13]. It should be noted that sample preparation is the time determining step of whole analytical procedure, which represents 2/3 of the total analysis time, and it is the primary source of error differences in the results obtained by different laboratories [14]. Polyphenols are compounds very reactive and substantial changes in sample composition may occur due to: isomerizations under exposure to UV radiation or daylight, the oxidative transformation and hydrolysis phenomena as result of the extraction procedure [15–19]. Therefore, a direct injection of the sample could be considered as an alternative to simplify the analysis of phenolic compounds, and to prevent many errors and any polyphenols degradation during the sample handling [19–22].

In this paper, a reversed-phase HPLC method with diode array detection for the separation and quantitation of phenolic compounds in ciders, by direct injection, without any prior purification of sample, is described and validated.

* Corresponding author. Tel.: +34 985890066; fax: +34 985891854.
E-mail address: rrodriguez@serida.org (R. Rodríguez).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.01.022

2. Experimental

2.1. Reagents and standards

Polyphenol standards were supplied as follows: (+)catechin, (−)epicatechin, phloridzin, tyrosol, catechol, benzoic acids (gallic acid, protocatechuic acid and *p*-hydroxybenzoic acid), hydroxycinnamic acids (*p*-coumaric acid, caffeic acid, ferulic acid and chlorogenic acid), 3-phenylpropionic acids (hydrocoumaric acid, hydrocaffeic acid and hydroferulic acid,) by Sigma (St. Louis, MO, USA); and quercetin glycosides (hyperoside, isoquercitrin, avicularin, rutin and quercitrin) by Extrasyntèse (Genay, France). The phloretin-2′-xyloglucoside and procyanidins (B1, B2, B5, trimer C1, tetramer D and unknown trimer) were kindly furnished by Dr. A. Lea (Reading, UK). Water was purified on a Milli-Q system from Millipore (Bedford, MA, USA). Reagents and solvents were purchased from Panreac (Barcelona, Spain) and were of analytical or HPLC grade.

2.2. Samples

Eight Spanish ciders were purchased from local supermarkets of Asturias (Spain). Prior to HPLC analysis, the cider samples were degassed in an ultrasonic bath during 10 min in order to remove all carbon dioxide and filtered though a 0.45 μm cellulose acetate membrane filter from Teknokroma (Barcelona, Spain).

2.3. HPLC analysis

Analyses were performed with a Waters system, equipped with a 717 automatic injector, provided with a column oven, two pumps (model 510), a diode array detector (model 996) and Millennium software v.2.1 data module. Separation of polyphenols was carried out on a reversed-phase Nucleosil 120 C_{18} (250 mm × 4.6 mm I.D., 3 μm) column from Teknokroma (Barcelona, Spain). The column was thermostated at 25 °C and a flow rate of 0.8 mL/min was used. The elution solvents were aqueous 2% acetic acid (solvent A) and 100% methanol (solvent B). The samples were eluted according to the following gradient: a linear step from 0 to 45% of solvent B in 55 min and a final isocratic step of 20 min. Injection volume was 50 μL.

Identification of compounds was achieved by comparing their spectra and retention times with those of standards when available. Detection was performed at 313 nm for the hydroxycinnamic acids, at 355 nm for the flavonol glycosides and at 280 nm for the rest of phenolic compounds (benzoic and 3-phenylpropionic acids, flavanols, procyanidins and dihydrochalcones). The spectra were acquired from 200 to 400 nm with a sampling rate of 1.0 and the highest scanning resolution (1 nm).

Quantitation was performed according to an external standard method. For the compounds lacking of standards, or those which the amount at our disposal was too small, the quantification was achieved from similar compounds: thus, the procyanidins were quantified as procyanidin B1, the phloretin-2′-xyloglucoside as phloridzin, the flavonol glycosides as quercitrin and the *p*-coumaroylquinic acid as *p*-coumaric acid.

3. Results and discussion

3.1. Separation and identification

Five families of phenols were taken as references for the analytical optimisation process in ciders: flavanols, hydroxycinnamic and 3-phenylpropionic acids, dihydrochalcones and flavonols.

Initial HPLC working conditions were selected on the basis of previously published works [12,23]. Two mobile phases

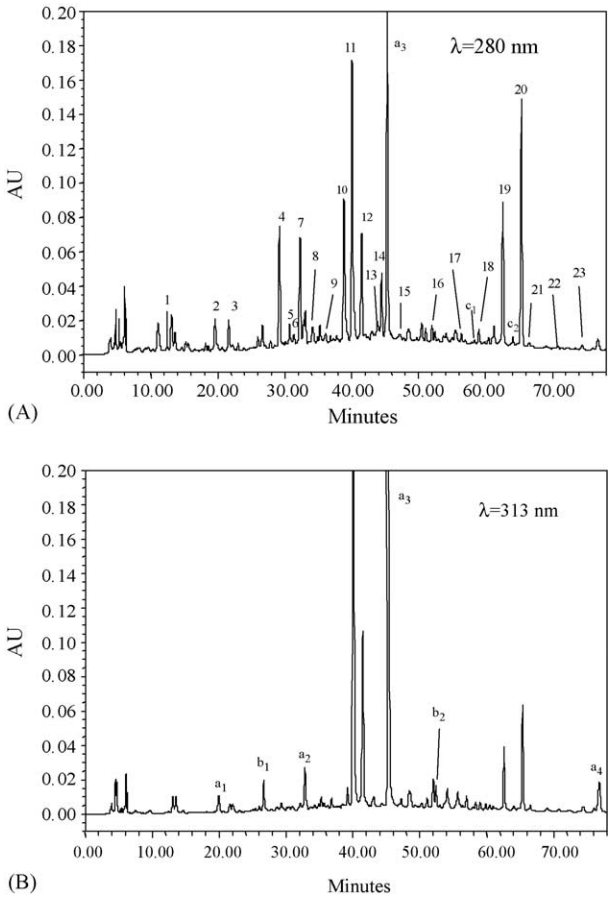

Fig. 1. Separation of phenolic compounds in cider at (A) 280 nm and (B) 313 nm. Peaks: 1, gallic acid; 2, catechol; 3, protocatechuic acid; 4, tyrosol; 5, procyanidin B1; 6, *p*-hydroxybenzoic acid; 7, hydrocaffeic acid; 8, (+)catechin; 9, unknown trimer; 10, procyanidin B2; 11, chlorogenic acid; 12, caffeic acid; 13, tetramer D; 14, trimer C1; 15, hydroferulic acid; 16, *p*-coumaric acid; 17, ferulic acid; 18, procyanidin B5; 19, phloretin-2′-xyloglucoside; 20, phloridzin; 21, hyperin; 22, avicularin; 23, quercitrin; a_1–a4, *p*-coumaric derivatives (λ_{max} = 312.0 nm); b_1 and b_2, hydroxycinnamic derivates (λ_{max} = 326.3 nm); c_1 and c_2, phloretin derivates.