Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1085 (2005) 285–292
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Comparison of shikimic acid determination by capillary zone electrophoresis with direct and indirect detection with liquid chromatography for varietal differentiation of red wines

Claudia Mardones, Antonieta Hitschfeld, Alejandra Contreras, Karla Lepe, Luis Gutiérrez, Dietrich von Baer*

Departamento de Análisis Instrumental, Facultad de Farmacia, Universidad de Concepción, Casilla 160-C, Concepción, Chile

Received 25 November 2004; received in revised form 2 June 2005; accepted 7 June 2005

Abstract

Two capillary zone electrophoretic (CZE) methods for determination of shikimic acid in Chilean red wine were developed and compared with a HPLC method. Both electrophoretic methods were carried out by using a reversed electroosmotic flow induced by trimethyl(tetradecyl)ammoniumbromide (TTAB) with indirect detection at 260 nm using *p*-aminobenzoic acid as a UV-absorbing co-ion or by direct detection at 213 nm. In both cases, the separation was carried out in a 50 μm I.D. uncoated capillary with an effective length of 48 cm, a negative power supply of 30 kV, using a buffer based on bis[2-hydroxyethyl]imino-tris[hydroxymethyl]methane (Bis-Tris), pH 7.0 or 7.5 and hydrodynamic injection. The chromatographic separations were carried out on a C-18 reversed phase column followed by a sulfonyl-styrene-divinylbenzene (S-DVB) ion exclusion column at 70 °C with H_2SO_4 0.02 M as isocratic mobile phase and a flow rate of 0.5 mL min^{-1}. The three methods allowed the quantification of shikimic acid with quantification limits between 1.0 and 12.0 mg L^{-1} and precision between 7.3 and 10.1%, however, only the concentrations obtained by CZE with direct detection were statistically similar to those of HPLC. This parameter was evaluated as analytical tool to verify varietal authenticity of red wines. In all cases, the Cabernet Sauvignon wines presented higher concentrations of shikimic acid, compared with Merlot or Carmenère wines.

Keywords: Shikimic acid; Red wine; HPLC; Reserved phase chromatography; Ion exclusion chromatography; CZE with direct detection; CZE with indirect detection

1. Introduction

Red wine contains organic acids, which are product of sugar oxidation (tartaric, citric and malic acid) or of alcoholic fermentation during the winemaking process (succinic and lactic acid) [1,2]. Shikimic acid (3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid) is another carboxylic acid that comes from grape skin and is always present in musts and wines [3]. It is an intermediate molecule produced in the shikimate pathway, the biosynthetic pathway of benzoic and cinnamic acid, aromatic amino acids (phenylalanine, tyrosine) and anthocyanidins, flavonoids, tannins and other compounds present in the grape, which are transferred to wine during the winemaking process [4,5].

Organic acid determination in wine is normally performed to monitor fermentation processes, product stability and organoleptic properties [6–8]. Shikimic acid does not have an important organoleptic effect in wine, and due to its low concentration in comparison with another acids, quantification of this compound has been limited in this matrix [3,5,9]. However, considering the participation of shikimic acid in the biosynthesis of antocyanines, Holbach et al. [5] proposed its determination as a tool for to differentiate between

* Corresponding author. Tel.: +56 41 203749; fax: +56 41 226382.
E-mail address: dvonbaer@udec.cl (D. von Baer).

0021-9673/$ – see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.chroma.2005.06.022

different red wine varieties. On the other hand, the profile of anthocyanines, specifically the ratio between acetylated and coumarylated anthocyanines (Ac/Cum), is actually used by official organisms in Germany to differentiate red wine varieties [10,11] and the concentration of shikimic acid has been proposed as an alternative in this context [5].

Ion exclusion chromatography is frequently employed to separate organic and aromatic acids in wine and others matrices [5,12–14]. The chromatographic methodology described by Holbach et al. correspond to this mode. They used a RP-18 column in series with a sulfonyl-styrene-divinylbenzene (S-DVB) for separation of shikimic acid in wine. This method was recently accepted by the International Organization of Vine and Wine (OIV) as method for determination of shikimic acid in wine [15], including it in the compendium of international methods of analysis of wines and musts.

Capillary zone electrophoresis (CZE) has emerged as one of the most efficient methods for charged compound separation. Organic acid separation acids in grapes, wine and other beverages has been described [9,16–21]; however, only Klampfl et al. proposed the separation of organic acids, including shikimic acid, in white wine by CZE and indirect UV detection [9].

The aim of this work was to present two electrophoretic methods for determination of shikimic acid in red wine and to compare these results with those obtained by application of the HPLC method developed by Holbach et al. [5]. Also, the evaluation of these methodologies as analytical tool to verify the varietal authenticity of red wine, is applied to Cabernet Sauvignon, Merlot and Carmenère wines produced in Chile.

2. Experimental

2.1. Chemicals

All solutions were prepared in 18 MΩ deionized water from a Millipore Milli-Q water purification system. HPLC grade acetonitrile and methanol were purchased form J.T. Baker (Phillipsburg, USA). *p*-aminobenzoic acid (PABA) >99%, $CaCl_2$, LiCl, LiOH, sulfuric, formic, tartaric, citric, succinic, lactic, fumaric and acetic acid were obtained from Merck (Darmstadt, Germany). Shikimic acid, malic acid, bis (2-hydroxyethyl)imino-tris(hydroxymethyl)methane (Bis-Tris) 98%; and trimethyl(tetradecyl)ammonium bromide (TTAB) 99% were obtained from Sigma (St. Louis, MO, USA). Stock standard solutions containing between 0.5 and 10 g L^{-1} of each organic acid were prepared in purified water and stored in a refrigerator at 4 °C for one week. Separation buffer for CZE methods were prepared by appropriate dilution of Bis-Tris 115 mM, PABA 25 mM, TTAB 10 mM, LiCl 600 mM and $CaCl_2$ 110 mM stock solutions and pH was adjusted with 0.1 M of LiOH or 0.1 M of HCl. HPLC mobile phase and CE buffer were prepared daily, filtered and degassed trough a 0.45 μm membrane filter.

2.2. Equipment

An Agilent capillary electrophoresis instrument (Palo Alto, CA, USA) equipped with a diode array detector set at 260 or 213 nm and a Hewlett-Packard Chemstation data processing station (Rev. A.09.01[1206]) were used to perform the electrophoretic analyses. The HPLC analyses were carried out on a Shimadzu HPLC system (Kyoto, Japan) equipped with a quaternary LC-10AD*VP* pump, FCV-10AL*VP* elution unit and DGU-14A degasser unit. The column temperature was maintained at 70 °C in a CTO-10A*VP* oven. A SPD-M10A*VP* UV/VIS spectrophotometric detector was used and data was processed with a CLASS-*VP* Shimadzu Chromatography Data System.

2.3. Electrophoretic separation conditions

Electrophoretic separation for the indirect detection method was performed on a fused-silica capillary of 56 cm (length to detector, 48 cm) × 50 μm I.D. from Bio-Rad (CA, USA). Separation was carried out by using a power supply of −30 kV (current of −7 μA) at a temperature of 22 °C and indirect detection at 260 nm. The separation buffer consisted of 10.5 mM Bis-Tris; 7.5 mM PABA; 0.2 mM TTAB; 0.53 mM $CaCl_2$, pH 7.5. The capillary was conditioned by passing 0.1 M NaOH for 30 min, water for 15 min and the run buffer for 15 min. The injection protocol began with 3 min of water, 4 min of NaOH and 5 min of the separation buffer, followed by hydrodynamic injection of the standards and samples at 25 mbar during 12 s.

The direct detection mode was performed on a capillary with the same characteristics by using a power supply of −30 kV (current of −80 μA) at 18 °C and 213 nm for detection. The separation buffer consisted of 200 mM Bis-Tris; 1.1 mM TTAB; 16 mM LiCl, pH 7.0. The capillary was conditioned by passing methanol for 30 min, 1.0 M NaOH for 30 min, water for 30 min and the run buffer for 30 min. The injection protocol began with 5 min of buffer with 2.0 mM TTAB followed by 10 min of running buffer. The injection was hydrodynamic at 50 mbar for 4 s. Analyte peaks were assigned by comparison of their retention times with those of reference compounds. Also, a co-injection of each standard with the sample was made in all cases.

2.4. Chromatographic separation conditions

HPLC separation was carried out on a C-18 of 15 cm × 4 mm I.D. and 5 μm particle size as pre-column (Institut Heidger, Kesten, Germany) and a sulfonyl-styrene-divinylbenzene (S-DVB) 30 cm × 4 mm I.D. column (Institut Heidger, Kesten, Germany) as main column. The mobile phase was isocratic 0.02 M H_2SO_4 with 0.1 mL min^{-1} as flow rate in the preconditioning phase and 0.5 mL min^{-1} in the working phase. The detection was at 225 nm and the injection volume 6 μL. Analyte peaks were assigned by comparison of their retention times with those of reference compounds.