Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 108 (2008) 1114–1121
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical Methods

Chiral nano-liquid chromatography–mass spectrometry applied to amino acids analysis for orange juice profiling

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Received 12 April 2007; received in revised form 20 November 2007; accepted 24 November 2007

Abstract

Determination of amino acid enantiomers is a very important topic in food analysis, since the presence of D-isomers may indicate, e.g., adulteration, microbiological contamination, uncontrolled fermentation processes, etc. In fact, the D- and L-enantiomers contents can be a useful marker for several elements such as quality control, contamination detection, processing monitoring, etc. Here we studied the potentiality of nano-liquid chromatography (nano-LC) coupled with mass spectrometry for the enantiomeric separation of several D- and L-amino acids that can be found in food products. Analytes were derivatized with fluorescein isothiocyanate (FITC). The mixture was injected and compounds focused on a C18 cartridge, then nano-LC analysis was carried out in a capillary column (75 μm i.d.) packed with vancomycin-modified silica–diol particles. The effect of some experimental parameters, such as pH and buffer concentration on enantioresolution and retention factors, was studied for method optimization. The chromatographic separation system was coupled with an ion-trap mass spectrometer through a nano spray interface. It provided a final evaluation on analytes detected in all investigated samples with LOD values as low as 8 ng/mL. That method was applied to the comparative analysis of two different orange juice samples (fresh natural vs. commercial one). Obtained profiles confirmed expected high quality standards. In fact, they mainly contained L-amino acids forms and not their antipodes.

Keywords: Amino acids; Derivatization; Chiral; Nano-liquid chromatography; Mass spectrometry

1. Introduction

During the last decade, separation techniques coupled with mass spectrometry became an essential tool in molecular biochemistry, especially in the analysis of chiral compounds of pharmaceutical, clinical, environmental and/or agro-chemical interest (Fanali, Aturki, Kašicka, Raggi, & D'Orazio, 2005). The use of enantioselective separation procedures can be very important in Food Science and Technology for several purposes such as : (i) identification of adulterated foods and beverages, (ii) a more precise control and monitoring on fermentation processes and products, (iii) microbial contamination's evaluation and identification, (iv) treatment and storage effects, (v) a more precise evaluation of some flavours and fragrance components, (vi) fingerprinting complex mixtures, and (vii) analysis of chiral metabolites of many foods and beverages' chiral and prochiral constituents (Armstrong, Chang, & Li, 1990; Simó, Barbas, & Cifuentes, 2003).

On the other hand, fruit juice industry has become one of the most important agricultural business in the world. Trade amounts to $10 billions per year being dominated by citrus juice (Robards & Antolovich, 1995). Because of this economic impact, orange juice's adulteration is an

Abbreviations: nano-LC, nano-liquid chromatography; CSP, chiral stationary phase; GABA, γ-aminobutyric acid; FITC, fluorescein isothiocyanate.

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doi:10.1016/j.foodchem.2007.11.062

important issue that demands development of new analytical procedures which are able to detect the increasingly sophisticated adulteration procedures tailored to defeat detection methods.

In this context, analysis of amino acid enantiomers is a valuable tool that provides relevant information about food and beverages' quality including orange juices (Gandolfi, Palla, Dossena, Puelli, & Salvadori, 1994; Ooghe, Kasteleyn, Temmerman, & Sandra, 1984; Robards & Antolovich, 1995). Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are the techniques used until now to carry out that type of separations (Gandolfi et al., 1994; Ooghe et al., 1984). In fact, they provide, unequivocal results in many cases. On the other hand procedures for sample preparation prior to GC analysis are frequently laborious and time consuming (Gandolfi et al., 1994) and in HPLC expensive chiral columns are used. In addition, in GC procedures the derivatizing reaction cannot be applied for some basic amino acids (Bruckner & Lupke, 1991). New actions have been recently developed. They are mainly based on capillary electrophoresis' use (Cifuentes, 2006; Simó, Barbas, & Cifuentes, 2002; Simó, Martin-Alvarez, Barbas, & Cifuentes, 2004; Simó, Rizzi, Barbas, & Cifuentes, 2005). By the way, new analytical strategies have to be implemented in order to help assessing citrus juice's quality.

Recently, a great attention was paid in developing miniaturized liquid chromatographic systems that can provide higher sensitivity than classic HPLC. In fact, sensitivity will increase reducing the i.d. of the column and keeping constant all other experimental parameters. It has been reported that sensitivity is raising in proportion to the square column's radius. When it passes from 4.6 mm to 0.100 mm sensitivity will increase of about 2000 (Chervet, Ursen, & Salzmann, 1996).

This effect can be ascribed to both a reduction of analyte chromatographic dilution (Vissers, 1999) and an increase of efficiency (Hsieh & Jorgenson, 1996; Kennedy & Jorgenson, 1989). Because of the low column volumes due to the small i.d. (<100 μm), in nano-LC the injected sample volume is as low as 10 nL. Therefore, the system cannot offer high sensitivity, so large volume injection methods have been successfully investigated and applied. This technique is based on peak compression both on-column and extra-column. In the first approach, solutes are dissolved in a solvent of lower eluting power compared to the mobile phase (Claessens & Kuyken, 1987; Heron, Tchapla, & Chervet, 2000; Mills, Maltas, & Lough, 1997; Vissers, de Ru, Ursem, & Chervet, 1996). The second solution consists of using micro pre-columns combined with a switching system (Meiring, Van der Heeft, ten Hove, & de Jong, 2002; Saarinen, Sirén, & Riekkola, 1995).

An alternative/complementary approach used to increase sensitivity in nano-LC, is the coupling of separation system with mass spectrometry. Hyphenation is easy to obtain because of the relatively low flow rate involved in the separation process. Indeed, when the electrospray ionization (ESI) is used as the continuous-flow ionization technique, a decrease of the flow rate will increase ions' number in the gas phase and as a consequence sensitivity will increase (Legido-Quigley, Smith, & Mallet, 2002).

Different chiral selectors have been used in separation science for enantiomeric resolution of many compounds. Among them macrocyclic antibiotics firstly introduced by Armstrong et al. (1994) have been used for that aim.

The high resolution capability of this type of chiral selectors is due to the presence of a large number of chiral centres and functional groups that allow useful interactions for chiral enantioresolution. Several studies testify the wide applicability of these chiral stationary phases (CSPs) to the chiral resolution of different amino acids by liquid chromatography (Berthod, Liu, Bagwill, & Armstrong, 1996; Cavazzini et al., 2004; Chen, 2006; Ilisz, Berkecz, & Peter, 2006; Petrusevska et al., 2006).

Amino acid enantiomers' separation in their native form by nano-LC presents same drawbacks encountered in classical HPLC. It is mainly due to: (i) absence/reduced chromophores in the structure, and (ii) reduced interactions with the chiral selectors. Therefore, derivatization procedures can be helpful to overcome all the drawbacks. They will provide better results in terms of sensitivity and enantioselectivity. Several derivatizing reagents have been used for LC analysis of amino acid enantiomers. Among them, fluorescein isothiocyanate (FITC) resulted to be useful for LC separations.

This paper describes our results on enantiomeric separation of amino acid enantiomers after derivatization with FITC by using a capillary column of 75 μm i.d. packed with a CSP containing silica modified with vancomycin. A column-switching system was used before the nano-LC column. It was equipped with a pre-column cartridge, in order to achieve both focusing and clean up. After optimization, the method was applied to the analysis of fruit juice samples.

2. Materials and methods

2.1. Chemicals and samples

All chemicals were of HPLC grade and they were used as received. Methanol (MeOH), acetonitrile, acetone, acetic and formic acids were purchased from Carlo Erba reagenti Spa (Rodano, Milano, Italy). Ammonia solution (30%) was from Riedel-de Häen (Seelze, Germany), while sodium hydroxide, boric acid and sodium hydrogen carbonate were from Sigma-Aldrich (St. Louis, MO, USA). Distilled water was deionized by using a Milli-Q system (Millipore, Bedford, MA, USA).

γ-Aminobutyric acid (GABA), fluorescein isothiocyanate (FITC) and the enantiomers (D- and L-isomers) of arginine (Arg), proline (Pro), alanine (Ala), leucine (Leu), serine (Ser), phenylalanine (Phe), asparagine (Asn), glutamic acid (Glu), aspartic acid (Asp) were from Sigma-Aldrich (St. Louis, MO, USA). Standard solutions at