



Determination of water-soluble vitamins in energy and sport drinks by micellar electrokinetic capillary chromatography



María Navarro-Pascual-Ahuir, María Jesús Lerma-García, Ernesto F. Simó-Alfonso, José Manuel Herrero-Martínez*

Department of Analytical Chemistry, University of Valencia, C. Doctor Moliner 50, E-46100 Burjassot, Valencia, Spain

ARTICLE INFO

Article history:

Received 15 October 2015

Received in revised form

22 November 2015

Accepted 23 November 2015

Available online 26 November 2015

Keywords:

Energy drink

Sport drink

MEKC

Water-soluble vitamins

Quality control

ABSTRACT

A method for the determination of water-soluble vitamins in several energy and sport drinks by micellar electrokinetic chromatography (MEKC) has been developed in this work. The separation of vitamins was studied in terms of background electrolyte composition (borate content, pH, surfactant type and content) and in other MEKC parameters. A study of the possible compounds found in the vitamin-enriched drinks that could interfere in vitamin determination was also performed, and a modified procedure with enhanced resolution was developed. The proposed method was successfully applied to the analysis of water-soluble vitamins in a variety of energy and sport drinks and also in fruit nectars. The method implies minimal sample preparation and reagent consumption, being environmentally sustainable. Thus, the proposed methodology could be useful for quality control purposes in the soft drink industry.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Consumption of energy and sport drinks has reached significant dimensions over the past two decades, being a constant element in diet of different social classes and age brackets. In fact, this industry has growth very much over the last years, reaching a total consumption of 4.8 billion litres in 2011 (www.zenithinternational.com, 2012).

The term of energy drink is referred to a beverage that contains, besides calories, caffeine in combination with other presumed energy-enhancing ingredients such as taurine, herbal extracts and vitamins (Heckman, Sherry, & De Mejia, 2010). On the other hand, sport drinks are specifically designed for, or marketed towards, people who are undertaking physical activity, being mainly composed by carbohydrates, electrolytes and vitamins. Thus, both types of drinks should be supported by extensive scientific and

nutritional research to back up their safety and effectiveness (Amendola, Iannilli, Restuccia, Santini, & Vinci, 2004), thus being important to establish their ingredient profile.

It is well-known that vitamins are a group of indispensable compounds for the development and normal growth of the human body, which can be classified into two main groups: water-soluble and fat-soluble. Water-soluble vitamins include B group vitamins and ascorbic acid (vitamin C). These vitamins play specific and vital functions in metabolism, and their lack or excess can cause health problems (Combs, 1992).

Water-soluble vitamins are included in energy and sport drinks for their essentiality in the normal biological functions such as co-enzymes (Aranda & Morlock, 2006). Moreover, it is claimed that the consumption of large amounts of B vitamins increases mental alertness and focus, as well as improves mood. Vitamins B2 (riboflavin), B3 (niacin), B6 (pyridoxine, pyridoxal, pyridoxamine) and B12 are the most common of the B vitamins that are incorporated into energy and sport drink formulations (Heckman et al., 2010).

Separation of water-soluble vitamins has been mainly carried out using HPLC with different detectors, such as UV (Albalá-Hurtado, Veciana-Nogués, Izquierdo-Pulido, & Mariné-Font, 1997; Almagro, San Andrés, & Vera, 2002; Heudi, Kilincü, & Fontannaz, 2005; Moreno & Salvadó, 2000; Viñas, López-Erroz, Balsalobre, & Hernández-Córdoba, 2003; Zafra-Gómez, Garbalo, Morales, & García-Ayuso, 2006), fluorescence (Woollard & Indyk, 2002; Zafra-

Abbreviations: MEKC, micellar electrokinetic chromatography; CZE, capillary zone electrophoresis; BGE, background electrolyte; HPLC, high performance liquid chromatography; NaOH, sodium hydroxide; SDS, sodium dodecyl sulphate; SC, sodium cholate; SDC, sodium dehydrocholate; MeOH, methanol; ACN, acetonitrile; IS, internal standard; EOF, electroosmotic flow; ED, energy drink; SD, sport drink; FN, fruit nectar; LOD, limit of detection; LOQ, limit of quantification.

* Corresponding author.

E-mail address: jmherrer@uv.es (J.M. Herrero-Martínez).

Gómez et al., 2006) and mass spectrometry (Kakitani et al., 2014; Nurit, Lyan, Piquet, Branlard, & Pujos-Guillot, 2015). However, electrodriven methods, such as capillary zone electrophoresis (CZE) (Fotsing, Fillet, Chiap, Hubert, & Crommen, 1999; Schiewe, Mrestani, & Neubert, 1995; Schreiner, Razzazi, & Luf, 2003; Su, Chou, Hwang, Chang, & Liu, 2001), CE-LIF (Zhao, Lu, & Cai, 2012) and micellar electrokinetic chromatography (MEKC) (Blanco-Gomis, González, & Álvarez, 1999; Delgado-Zamarreño, González-Maza, Sánchez-Pérez, & Carabias-Martínez, 2002; Fotsing et al., 1999; Hu, Zhou, Zhang, Li, & Fang, 2001; Schreiner et al., 2003; Su et al., 2001) have been also used for this purpose. Most of these methodologies have been employed to determine B-vitamins (and in some cases also C vitamin) in multivitamin pharmaceutical formulations (Almagro et al., 2002; Blanco-Gomis et al., 1999; Delgado-Zamarreño et al., 2002; Fotsing et al., 1999; Heudi et al., 2005; Hu et al., 2001; Moreno & Salvadó, 2000; Schiewe et al., 1995; Schreiner et al., 2003; Su et al., 2001) and in baby and supplemented foods (Albalá-Hurtado et al., 1997; Schiewe et al., 1995; Viñas et al., 2003; Woollard & Indyk, 2002; Zafra-Gómez et al., 2006). However, the determination of C and B vitamins in other matrices, such as energy and sport drinks, which are complex mixtures with other additives and with a large amount of sugars have been barely explored (Aranda & Morlock, 2006; Grant & Helleur, 2008; Karatapanis, Fiamegos, & Stalikas, 2009). A planar chromatography–ESI MS method (Aranda & Morlock, 2006) was developed for the determination of three B-vitamins (B2, B3 and B6), caffeine and taurine in 8 energy drinks, using UV detection for B3 and caffeine and also for taurine after post-chromatographic derivatization, and fluorescence for B2 and B6. However, it would be beneficial to develop a method able to quantify all these analytes simultaneously by one detection method. In this way, Grant and Helleur (2008) have developed a surfactant-mediated matrix-assisted laser desorption/ionization method for the simultaneous analysis of B2, B3 and B6 vitamins and caffeine in 4 energy drinks, while Karatapanis et al. (2009) have developed an hydrophilic interaction liquid chromatography (HPLC) method using an end-capped HILIC-diol column for the separation of C and seven B-vitamins in a pharmaceutical formulation and in an energy drink. However, there is still a need of a simple, less expensive techniques and rapid screening method for the determination of both B and C vitamins for quality control purposes in complex matrices, able to eliminate other matrix interferences.

In this work, a simple MEKC method for the determination of C and seven B-group vitamins in energy and sport beverages was developed. The influence of composition of background electrolyte (BGE) and other MEKC parameters on migration time separation of the vitamins were investigated. Also, a study with some matrix compounds commonly present in these beverages, which could interfere in vitamin determination, was performed. The performance of the developed method was tested with regard to sensitivity, limit of detection and repeatability. Finally, the vitamin content present in several energy and sport drinks and fruit nectars was obtained.

2. Material and methods

2.1. Chemicals and samples

The following analytical grade reagents were used: sodium tetraborate, sodium hydroxide (NaOH), aminobenzoic acid (internal standard, IS), sodium dodecyl sulphate (SDS), sodium cholate (SC), sodium dehydrocholate (SDC), metaphosphoric acid and hydrochloric acid from Sigma–Aldrich (St. Louis, MO), and methanol (MeOH) and acetonitrile (ACN) from VWR Chemicals (Fontenay-sous-Bois, France). Deionized water (Barnstead deionizer, Sybron,

Boston, MA) was also used. The vitamin and other analytical standards were: ascorbic acid (C), thiamine (B1), riboflavin (B2), nicotinamide and nicotinic acid (B3), pantothenic acid (B5), pyridoxine (B6), cobalamin (B12), caffeine, benzoic acid, sorbic acid and acesulfame K (Sigma–Aldrich). The chemical structures and dissociation constants of the investigated vitamins are shown in Fig. 1. Individual stock solutions of B vitamins (except vitamin B2) were prepared in 5% ACN in 0.01 M HCl solution at $1000 \mu\text{g mL}^{-1}$ (Su et al., 2001), while C vitamin was prepared in ice-cold 2% metaphosphoric acid (Davey, Bauw, & Montagu, 1996; Herrero-Martínez, Simó-Alfonso, Deltoro, Calatayud, & Ramis-Ramos, 1998). Vitamin B2 was dissolved in basic media and had enough stability over 24 h. All stock solutions were stored at -20°C in amber vials. The rest of B vitamins were found to be stable for at least a week. The vitamin C solution was stable for three days at -20°C (Davey et al., 1996; Herrero-Martínez et al., 1998). Working standard solutions were prepared daily by appropriate dilution from the concentrated stock solutions.

A total of 10 commercially available samples were purchased from a local supermarket: 4 energy drinks (ED1–4), 4 sport drinks (SD1–4) and 2 fruit nectars with added vitamins (FN1–2). Energy and sport drinks were filtered directly through $0.45 \mu\text{m}$ pore size nylon filter (Scharlau, Barcelona, Spain) and injected in the CE system. For carbonated samples, a previous sonication for 20 min to remove dissolved gases was performed. Fruit nectars with fortified vitamins were centrifuged at 10,000 rpm for 10 min, being the supernatant filtered and subsequently injected. Samples containing vitamin C were diluted 1:1 (v/v) with ice-cold metaphosphoric acid (up to a final concentration of 2%), filtered and injected.

2.2. Instrumentation and procedures

An HP^{3D} CE system (Agilent, Waldbronn, Germany) equipped with a diode array spectrophotometric detector and uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ) of 58.5 cm length (50 cm effective length) \times $75 \mu\text{m}$ id ($375 \mu\text{m}$ o.d.) were used. New capillaries were successively flushed with 1 and 0.1 M NaOH and water at 60°C for 10 min each. Between runs, the capillary was flushed with 5 min BGE. Samples were injected hydrodynamically at 50 mbar \times 8 s. Separations were performed at 20 kV at 25°C . Detection was performed at 214 nm for all vitamins except for vitamin C that was 265 nm. Before injection, all solutions were filtered through $0.45 \mu\text{m}$ pore size nylon filters. CZE data acquisition was performed with ChemStation Software (Rev.A.10.01, Agilent).

3. Results and discussion

3.1. Optimization of separation conditions

Vitamin separation was firstly optimized using CZE. For this purpose, a test mixture containing the eight vitamin standards (at $100 \mu\text{g mL}^{-1}$) described in the Chemicals section was used. Most CE studies related to vitamin separation have been mainly performed using borate buffers at different concentrations (20–50 mM) and at pH values comprised between 8.0 and 9.5 (in order to assure that analytes are in their ionic form) (Blanco-Gomis et al., 1999; Fotsing, Fillet, Bechet, Hubert, & Crommen, 1997; Schreiner et al., 2003; Su et al., 2001). For this purpose, different borate contents (ranged from 10 to 60 mM) at a pH of 8.5 were first tried. The results obtained are shown in Fig. 2. As it can be observed, in general, analysis time increased when the borate content increased, which could be explained in terms of an ionic strength effect, giving as a result an EOF decrease. The migration order of vitamins was similar to that found in literature (Nishi, Tsumagari, & Kakimoto, 1989; Su et al.,

Download English Version:

<https://daneshyari.com/en/article/4559140>

Download Persian Version:

<https://daneshyari.com/article/4559140>

[Daneshyari.com](https://daneshyari.com)