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Short communication

Simultaneous determination of taurine, glucuronolactone and glucuronic acid in energy drinks by ultra high performance liquid chromatography–tandem mass spectrometry (triple quadrupole)

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ABSTRACT

In this work, we present for the first time a rapid and robust UHPLC–MS/MS method for analyzing taurine, GlcLA and GlcA in energy drinks simultaneously and without derivatization. The separation of three analytes was achieved using a Kinetex Hilic analytical column (100 mm × 4.6 mm i.d.) and a mobile phase formed by water (A) and acetonitrile (B) both with formic acid 0.1% at a flow rate of 0.8 ml min^{-1} with isocratic elution in 3.5 min. Calibration curves were calculated using the method of standard addition in a concentration range from 2 to 6 mg/100 ml for taurine ($R^2 > 0.987$), from 0.4 to 1.2 mg/100 ml for GlcLa ($R^2 > 0.997$), and from 0.2 to 0.6 mg/100 ml for GlcA acid ($R^2 > 0.998$). The validated method was applied to the analysis of nine commercial energy drinks. The level of taurine found ranged from 0.01 to 0.45 g/100 ml, and it matched with that reported in the labels of the analyzed energy drink samples.

1. Introduction

Energy drinks refer to beverages that contain, besides calories, caffeine in combination with other presumed energy-enhancing ingredients such as taurine, herbal extracts, and vitamins. In recent years, beverages denominated "energy drinks" and "sports drinks" have gained popularity among students, athletes and other active people [1]. Fully 34% of the 18–24 year olds interviewed in one study reported that they regularly consumed energy drinks, a significant indicator of the popularity of these beverages among members of the younger generation [2]. Another study reported that about half of the college students interviewed consumed at least 1 energy drink per month, either to increase their energy level in compensation for lack of sleep, or to mix with alcohol [3].

The usual composition of these drinks is based on water-soluble vitamins, carbohydrates, caffeine, taurine and glucuronolactone (GlcLA). Several investigations have been published indicating the effect of this kind of beverage on the central nervous system (CNS), showing significant improvements in mental performance (reaction time, concentration and memory) and reduction in sleepiness and sleep-related accidents, due especially to the presence of caffeine [4]. Taurine (2-aminoethane sulphonic acid) (Fig. 1), an abundant free amino acid widely distributed throughout the body and readily found in animal-derived dietary sources, is an ingredient in many energy drinks [5–7]. Studies on caffeinated taurine drink consumption have generally observed significantly shorter mean reaction times on attention tasks compared to placebo and control beverages [4,8,9]. However, it is not clear whether these results are due exclusively to the taurine, or whether they are also related to its interaction with other psychoactive ingredients in the beverage, such as glucose. Another ingredient commonly found in energy drinks is D-glucurono-γ-lactone (GlcLA), a normal human metabolite formed from glucose, that is in equilibrium at physiological pH with glucuronic acid (GlcA), its immediate precursor. Many pharmaceutical preparations containing either glucuronolactone (GlcLA) or glucuronic acid (GlcA) are used to treat bilirubinemia because they improve liver condition. Furthermore, when GlcA and GlcLA are analyzed by chromatographic methods, the equilibrium between GlcA and its lactone form (i.e., GlcLA in an aqueous solution) should be considered (Fig. 1). Suzuki et al. [10] were the only researchers that have determined GlcA and GlcLA together in pharmaceutical preparations by developing a method for the derivatization of reducing carbohydrates with

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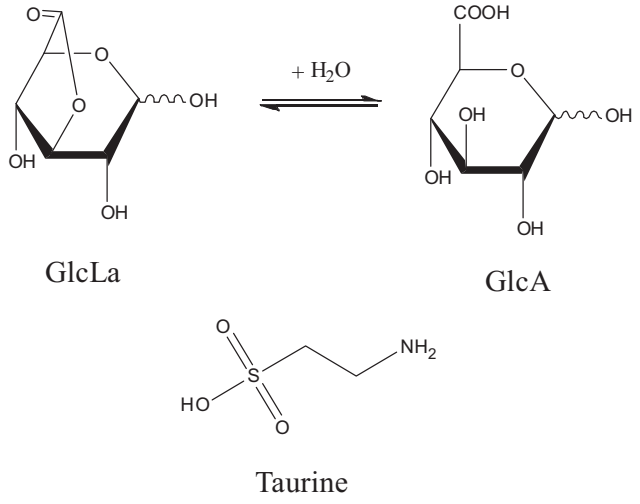

Fig. 1. The molecule of glucuronolactone (GlcLa) in equilibrium with its glucuronic acid (GlcA) analogue, and the structure of taurine.

l-phenyl-3-methyl-5-pyrazolone (PMP) using high-performance liquid chromatography (HPLC) and diode array detector (DAD). On the contrary, there are many analytical methods available in literature for taurine quantification, i.e. by using Fourier transform infrared spectroscopy (FTIR) [11] or nuclear magnetic resonance (NMR) [12] techniques. Due to absence of strong absorbing groups, taurine is quantified by means of HPLC with pre-column derivatization and fluorescent detector [13–15], or with pre-column derivatization and HPLC/MS [16,17] or with HPTLC with post-chromatographic derivatization [18]. To our best knowledge, no chromatographic analytical methods are available in literature for taurine quantification without derivatization steps.

The aim of this work was to develop a sensible, rapid and robust UHPLC–MS/MS method for analyzing simultaneously taurine, GlcLA and GlcA in energy drinks without derivatization steps. The validated method was applied to the analysis of various commercial energy drinks.

2. Experimental

2.1. Materials and standards

Standards of taurine (>99%, $C_2H_7NO_3S$, molecular weight 125.15, CAS no. 107-35-7), D-glucuronic acid (>98%, $C_6H_{10}O_7$, molecular weight 194.14, CAS no. 6556-12-3), and D-(+)-glucuronic acid γ-lacton (≥99%, $C_6H_8O_6$, molecular weight 176.12, CAS no. 32449-92-6) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Individual stock solutions were prepared by dissolving 100 mg of each compound in 100 ml of water and stored in glass-stoppered bottles at 4 °C. Standard working solutions, at various concentrations, were prepared daily by appropriate dilution of aliquots of the stock solutions in water.

HPLC-grade acetonitrile >99.9% was supplied by Sigma–Aldrich (Milano, Italy), HPLC-grade formic acid by Merck (Darmstadt, Germany). Deionized water (>18 MΩ cm resistivity) was obtained from the Milli-Q SP Reagent Water System (Millipore, Bedford, MA). All the solvents and solutions were filtered through a 0.2 μm PTFE filter from Supelco (Bellefonte, PA, USA) before use.

2.2. Sample collection

Energy drink samples were bought from different supermarkets in Camerino, Italy.

2.3. Sample preparation

The energy drink samples were diluted with water (1:100, v/v), filtered on a 0.2 μm PTFE filter from Supelco (Bellofonte, PA USA) and then injected in the UHPLC–MS/MS.

2.4. UHPLC/MS/MS analysis

UHPLC–MS/MS studies were performed using an Agilent 1290 Infinity series and a Triple Quadrupole 6420 from Agilent Technology (Santa Clara, CA) equipped with an ESI source operating in negative/positive ionization mode.

Optimization of the UHPLC–MS/MS conditions was carried out by varying them in flow injection analysis (FIA) of the analytes (1 μl of a 5 mg l^{-1} individual standard solutions) by using optimizer software (Agilent).

The separation of taurine, GlcLA and GlcA was achieved using a Kinetex Hilic analytical column (100 mm × 4.6 mm i.d., particle size 2.6 μm) from Phenomenex (Torrance, CA, USA). The mobile phase for UHPLC–MS/MS analysis was a mixture of water (A, 10%) and acetonitrile (B, 90%), both with formic acid 0.1% at a flow rate of 0.8 ml min^{-1} with isocratic elution. The injection volume was 0.1 μl. The temperature of the column was 30 °C and the temperature of the drying gas in the ionization source was 300 °C. The gas flow was 12 l/min, the nebulizer pressure was 50 psi and the capillary voltage was 4000 V (negative and positive). Detection was performed by electrospray ionization (ESI)-MS in the "multiple reaction monitoring" (MRM) mode. The MRM peaks areas were integrated for quantification. To enhance the sensitivity, the acquisition time was divided into two periods. The most abundant product ion was used for quantification, and the rest of the products ions were used for qualification. The selected ion transition and the settings of the mass analyzer are reported in Table 1. All solvents and solutions were filtered through a 0.2 μm nylon membrane filter from Whatman (Dassel, Germany) before use. All samples were filtered before UHPLC analysis through a 0.2 μm single use syringe filter from Minisart RC 4, Sartorius Stedim (Goettingen, Germany).

3. Results and discussion

3.1. Chromatographic analysis and mass spectrometry

Table 1 reports the MS/MS acquisition parameters in MRM mode, i.e. time windows, precursor ions, product ions, fragmentor, collision energy (CE), retention time and polarity used for the analysis of the compounds that present more transitions. The most intense are used for the quantitative analysis and are referred to quantifier transition, while the others are employed in the identification step.

For GlcLA and GlcA the precursor ion corresponds to the deprotonated molecule $[M-H]^-$, while for taurine the precursor ion corresponds the protonated molecule $[M+H]^+$. Due to the use of HPLC Kinetex Hilic column, the developed analytical methodology showed a very good performance in terms of chromatographic separation and sensitivity. Fig. 2 reports two UHPLC–MS/MS chromatograms of (a) standard mixture of taurine, GlcLA, and GlcA and (b) an energy drink sample (no. 2).

In our case, hydrophilic interaction liquid chromatography (HILIC) was a perfect alternative to reverse-phase-high-performance liquid chromatography (HPLC) mode for separating polar compounds.

In such a complex system as an energy drink, the HILIC proved very suitable for analyzing these polar compounds, which were instead eluted near the void in reversed-phase chromatography by using other columns reported below, including the Gemini C18,