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Short Report

Fast Analysis of Taurine in Energetic Drinks by Electrospray Ionization Mass Spectrometry

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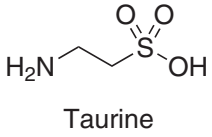
Espectrometria de massas sequencial usando infusão direta de amostra e ionização por *electrospray* no modo de ions negativos foi utilizada para quantificar taurina em bebidas energéticas com alta seletividade e sensibilidade, O metodo é simples e rápido (menos de 2 min de corrida) e apresenta alta repetitividade e recuperação. Bebidas energéticas comerciais apresentaram quantidades de taurina em concentrações muito diferentes (menor ou maior) das quantidades declaradas.

Direct infusion electrospray ionization tandem mass spectrometry in the negative ion mode with single reaction monitoring is show to allow high selectivity and sensitivity in the quantification of taurine in energetic drinks. The method is also simple and rapid (less than 2 min run time), with high recovery and repeatability. Commercially available energetic drinks were found to contain taurine in concentrations quite different (lower or higher) from the declared amounts.

Keywords: electrospray mass spectrometry, taurine, energetic drinks, food control

Introduction

Taurine (2-aminoethane sulfonic acid, Figure 1), is a major free β-amino acid in humans. Taurine exists free in the intracellular fluid, and is widely distributed in many tissues including the myocardium, liver, skeletal muscle, brain, retina, platelets and leukocytes. Taurine is not a protein constituent and for a long time it was considered a nonessential nutrient for humans. In recent years, however, it has become clear that taurine is an important amino acid involved in a large number of metabolic processes and that it becomes essential under certain circumstances. Taurine plays also an important role in the maintenance of physiological functions.[1-3] The functions of taurine include osmoregulation,[4] cell membrane stabilization,[5] antioxidation,[6] detoxification,[3] neuromodulation,[7,8] and brain and retinal development.[9] Moreover, some pharmacological functions of taurine have also been reported against congestive heart failure,[10] liver disease,[11] hyperlipidemia[12] and epilepsy.[13]



Taurine

Figure 1. Taurine amino acid.

Taurine occurs naturally in food, especially in seafood and meat. The mean daily intake from omnivore diets was determined to be around 58 mg (range from 9 to 372 mg) and to be low or negligible from a strict vegan diet.[14-16] Taurine intake has been also estimated to be generally less than

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200 mg *per* day, even in individuals eating a high meat diet.[15] There has been concern about the effects and amount of taurine intakes mainly from "energetic" drinks.[17,18] Positive effects of taurine-containing drinks such as on hormonal responses which led to a higher athletic performance have been reported.[19] High performance liquid chromatography (HPLC) preceded by extraction procedures has been the main approach applied to quantify taurine.[20,21] Altogether, these steps are time-consuming and not so easy to automate. With the main objective to simplify and minimize the time of analysis, whereas keeping a satisfactory level of selectivity and sensitivity, we tested a direct infusion electrospray ionization tandem mass spectrometry approach to determine taurine in energetic drinks.

Experimental

Five different brands of "energetic" drinks, all of them containing taurine, were purchased from supermarkets in Brazil (São Paulo State) from January to April 2010. Three lots were analyzed, differentiated by fabrication dates for each of the five trade marks. All samples were within the validity period and without any visible damages. All determinations were conducted in triplicate.

The standard of taurine was purchased from Sigma (T-0625, 61K0127). HPLC grade methanol was purchased from Merck. The water used for sample and mobile phase preparation was purified using the Milli-Q system (Millipore). The mobile phases were filtrated by Millipore filters with 0.45 μm size pores.

ESI-MS^n analyses were performed on a API 2000 QTrap (Applied Biosystem) mass spectrometer. The tandem mass spectrometric (MS/MS) experiments were performed using single reaction monitoring (SRM) for the dissociation of the precursor ion of *m/z* 124 to its most abundant fragment ion of *m/z* 80. The best mode of ion detection for taurine was the negative ion mode. The major conditions were as follows: scan range, *m/z* 50-200; heater temperature, 100 °C; flow of the nitrogen carrier gas, 20 L min^{-1}; sheath gas, 0.0 L min^{-1}; curtain gas, 20 L min^{-1}; nebulizer potential, −3500 V; declustering potential, −46 V; and entrance potential, −7 V.

Analytical methodology

Aliquots of 100 μL from the energetic drinks were quantitatively transferred to a 1.5 mL volumetric flask. After the homogenization, the solutions were first filtrated two times with a common filter paper and next with a Durapore membrane (HVLP 01300 Millipore) with 0.45 μm size pores. Subsequently, the samples were automated injected into the ESI source of the mass spectrometer (10 μL) by using an Aliance HPLC system (Waters). Taurine was eluted by an isocratic elution system using a 1:1 methanol/water solution.

Method validation: Limits of detection and quantification

A preliminary evaluation of the limit of detection (LOD) was performed by automatic successive dilutions of taurine using an Aliance HPLC system (Waters), determining the lower detectable amount that was, approximately, two to three times the average value of noise. After determining the LOD, taurine was quantitated in samples of energetic drinks. Amounts of the standard taurine, equivalent and higher than established for LOD curves using pure standard, were added to the drink matrixes. The limit of quantification (LOQ) was considered as three times the LOD.[22] The ESI(-)-MS of pure taurine (Figure 2) shows mainly the deprotonated molecule $[M - H]^-$ of *m/z* 124 as well as the dimer of *m/z* 249, the trimer of *m/z* 374 and the tetramer of *m/z* 499. The ion of *m/z* 80 was attributed to SO_3^- formed via in-source collision-induced dissociation of $[M - H]^-$.

Standard recovery

The accuracy of the method was evaluated through recovery tests by adding taurine standard to the energetic drinks using two different concentration levels: 8.5 mg/100 mL and 85.0 mg/100 mL for 100 μL of sample. All determinations were conducted in triplicate.

Repeatability

The closeness of the agreement between the results of successive measurements of the same measurand carried out under the same conditions of measurement. The repeatability was evaluated by five determinations, in triplicate, using two concentration levels of taurine in standard solutions and in energetic drinks. The repeatability conditions included the same measurement procedure, the same analyst, the same measuring instrument, used under the same conditions, the same location, and repetition over a short period of time. The repeatability was calculated following Caulcutt and Boddy.[22]

Results and Discussion

The direct injection ESI(−)-MS/MS method developed using flow-injection type of setup allows rapid determination of taurine, that is, within approximately 1.3 min after injection, with a total analysis time of *ca*. 2 min. Although no pre-separation is employed, false positives are minimized

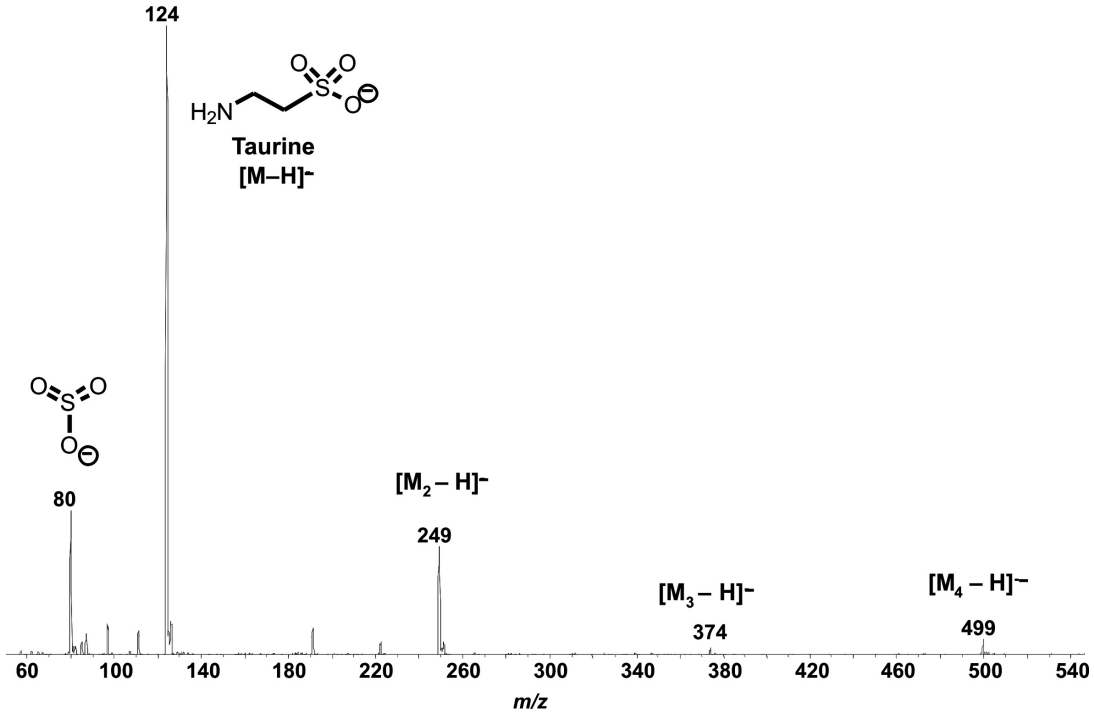

Figure 2. ESI(–)-MS of a taurine solution in H_2O:MeCN (1:1 v/v).

through the use of SRM (Figure 3 and 4) via the transition *m/z* 124 → *m/z* 80. The analytical curve for taurine, outlined by external standardization, displays good linearity within the range of pre-established concentration from 10 mg mL^{-1} to 0.1 mg mL^{-1}, with an excellent correlation coefficient of 0.999 (Figure 5). The analyses showed no matrix effect

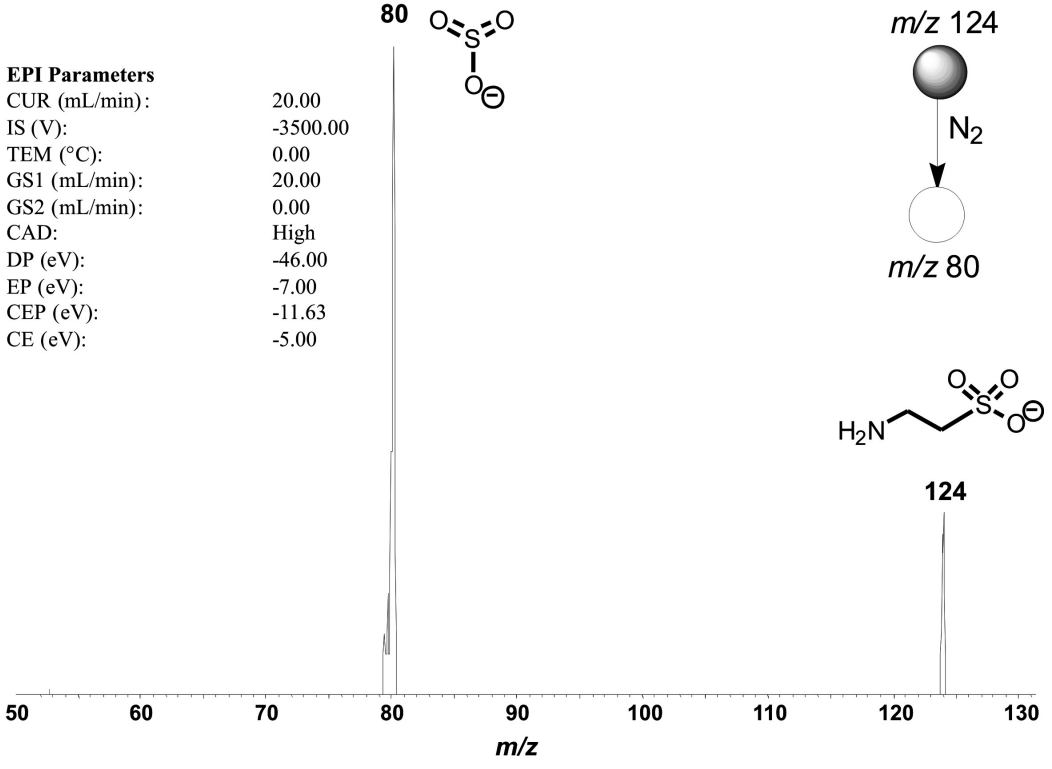

Figure 3. ESI(–)-MS/MS for deprotonated taurine of *m/z* 124. Note major dissociation by the loss of neutral molecule of C_2H_6N composition to form SO_3^- of *m/z* 80.

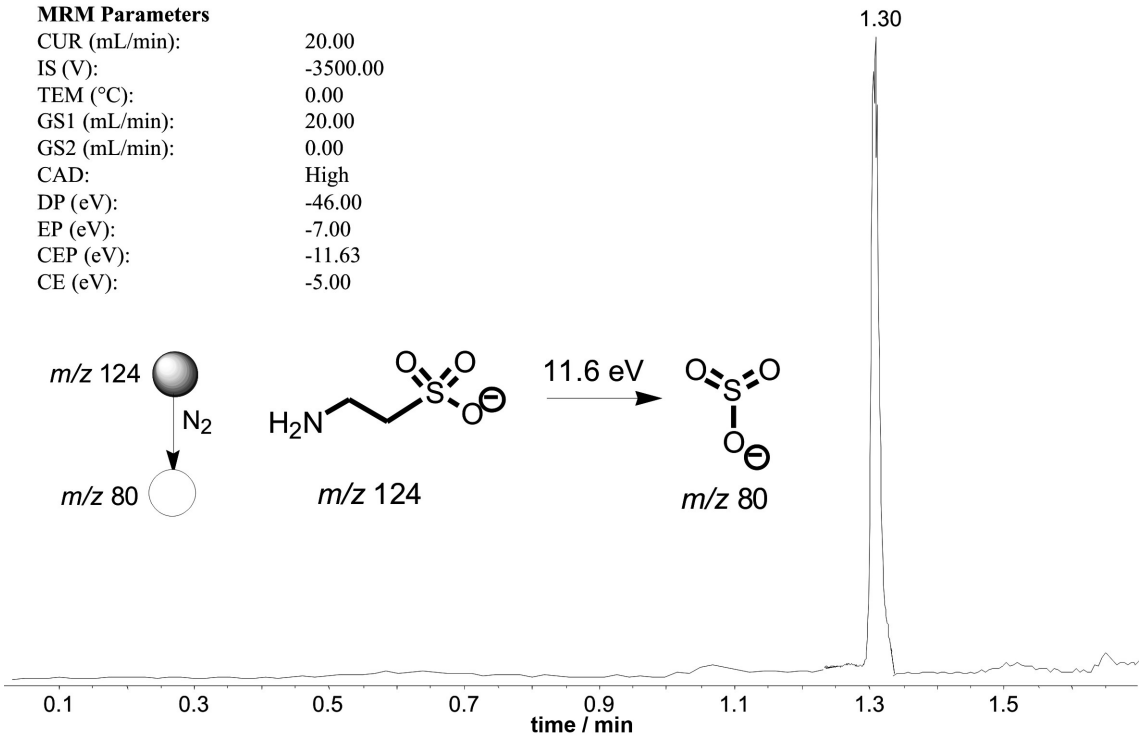

Figure 4. Cromatogram using SRM (*m/z* 124 → *m/z* 80) showing the detection of taurine after 1.3 min of sample injection.

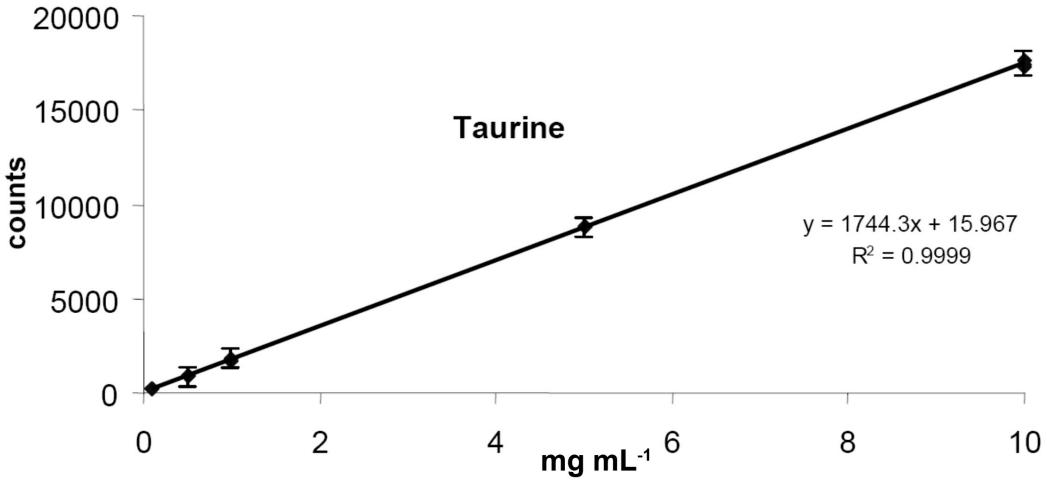

Figure 5. Analytical curve for taurine quantification in energetic drinks.

according to the use of SRM. The slope calibration curves from conventional and matrix-matched were statistically identical.

Table 1 shows the concentrations of taurine determined in several samples of energetic drinks. From the five brands of energetic drinks analyzed, three of them displayed much lower amounts of the amino acid corresponding to just 30 to 35% of the declared values. The brands RB and FH displayed amounts much higher than the ones declared, that is, 217% and 175%, respectively.

Methodology validation

The LOD found for taurine from standard solutions and energetic drink samples were the same: 0.03 mg mL^{-1}. The LOQ was therefore 0.1 mg mL^{-1}. The recovery rates varied between 99.1-99.2% in the two levels of energetic drinks (Table 2). These values reveal a good recovery rate for levels of amino acids present in the drink. Table 3 shows the repeatability ranges found from five determinations of taurine in standard solution and energetic drinks, with 95%

Table 1. Quantification data of taurine in energetic drinks by ESI-MS/MS (SRM)

Brand lot	[a]Label (mg/100 mL)	[b]TA (mg/100 mL), A ± SD	[c]CV (%)
BU		134 ± 1.2	0.9
BU	400	128 ± 0.9	0.7
BU		102 ± 1.3	1.3
Average of BU lots		121.3	
AT		147 ± 1.8	1.2
AT	400	140 ± 1.5	1.1
AT		128 ± 1.3	1.0
Average of AT lots		138.3	
BB		123 ± 1.1	0.9
BB	400	125 ± 1.6	1.3
BB		140 ± 1.2	0.9
Average of BB lots		129.3	
RB		870 ± 1.1	0.1
RB	400	880 ± 1.2	0.1
RB		850 ± 1.4	0.2
Average of RB lots		866.7	
FH		880 ± 1.2	0.1
FH	400	870 ± 0.9	0.1
FH		352 ± 1.3	0.4
Average of FH lots		700.7	

[a]Concentration of taurine declared on the label; [b]Concentration of taurine found on the samples; [c]Coefficient of variation. A = amount; SD = Standard Deviation

Table 2. Recovery of taurine in energetic drinks

Concentration in sample mg/100 mL	Concentration added mg/100 mL	Concentration found mg/100 mL	Recovery (%)
198.2	8.5	205	99.1
	85	281.1	99.2

Table 3. Repeatability of taurine in energetic drinks and standard solution

	Taurine Standard			Energetic Drink	
	C (mg/100 mL)	r		C (mg/100 mL)	r
Taurine	10.0076 10.0831 10.1041 10.1009 10.1048	0.163	Taurine	100.7654 100.2439 100.9072 100.8632 100.9681	1.145

C= concentration; r = repeatability.

confidence. The obtained value for five determinations in duplicate are therefore expected to vary within the limits provided by repeatability with the specified confidence.

Conclusions

Direct infusion ESI-MS/MS in the negative ion mode using SRM (*m/z* 124 → *m/z* 80) can be applied with high confidence, speed and selectivity to quantifty taurine in energetic drinks. As observed before,[18] the present results indicate that energetic drinks often contain taurine in concentrations quite different (lower or higher) from the declared amounts.

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