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ORIGINAL RESEARCH PAPER

OPTIMIZATION OF A HPLC ANALYSIS METHOD FOR TAURINE AND CAFFEINE IN ENERGY DRINKS

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Abstract: This paper presents the optimization of a rapid, inexpensive, reliable and selective isocratic high performance liquid chromatographic (HPLC) method for the simultaneous determination of caffeine and taurine in energy drinks with two common detectors in series: evaporating light scattering detector (ELSD) and an ultraviolet (UV) detector. Satisfactory analysis results were obtained on an Astec apHera NH2 column using methanol/water (30:70 v/v) as mobile phase. The optimized method was used for the analysis of commercial energy drinks containing large amounts of carbohydrates (100 $g \cdot L^{-1}$) and considerably lower amounts of taurine and caffeine (4 and 0.6 $g \cdot L^{-1}$, respectively). The advantages of this method consist of its lack of preliminary samples treatment and also the fact that basic LC instrumentation was employed.

Keywords: *Caffeine, Energy Drink, HPLC, Taurine*

INTRODUCTION

Food safety represents a major concern in the entire world. Liquid chromatography proved to be a use full tool for the food risks determinations. Thus it can be used to determine the presence of different contaminants [1] or verifying the labeled concentration of the components [2].
Lately, stimulant drinks have become very popular especially among the young customers and represent an important percentage of the soft drinks sales. The energy drinks increasing popularity is connected to their reputation of improving physical and mental performances due to their composition (amino acids, methylxanthines, vitamins and high amounts of carbohydrates). Recent studies [3] suggest that the association of energy drinks and alcohol (popular practice among adolescents) can induce extends motor impairment and ataxia in adolescent mice.
Taurine (2-aminoethanesulfonic acid) is a sulfur-containing amino acid that is non-essential for most living organisms (except for cats [4]). It is present in the composition of most energy drinks; it also functions as a neurotransmitter [5]. The muscle production and maintenance is correlated with the amino acids content, especially that of taurine [4]. Nevertheless, the excess of taurine has been associated with cardiomiopathy and hepatic toxicity [6]. The other component of importance in the energy drinks compositions is caffeine (1,3,7 trimethyl-xanthine), that presents stimulatory effects on different body systems (eg. the central nervous and cardiovascular system) [7]. Caffeine excesses can give rise to toxic symptoms that can go from tremors and tachycardia to seizure and even death. Furthermore, combined exposure to different methylxanthines may increase the toxic effects of other drugs and information on the possible interactions in humans of caffeine with other constituents of energy drinks, such as taurine, is very limited.
Caffeine was determined in various food by numerous techniques including spectroscopic [8, 9] and chromatographic methods, like high performance thin layer chromatography (HPTLC) [10, 11] and liquid chromatography (HPLC) most often using ultraviolet (UV) detection [12 – 16]. There are papers that described the direct determination of taurine in food or medical samples by HPLC with evaporative light scattering detection (ELSD) [17] or mass spectrometric (MS) detection [17 – 19]. The simultaneous determination of these two compounds was described by Aranda et al. [11], the authors determined in energy drinks by HPTLC with multiple detection: caffeine, taurine, pyridoxine, nicotinamide and riboflavin. If caffeine can be directly detected by UV detection, taurine can only be detected after a ninhydrin derivatisation step. Marchei *et al.* [20] presented the validation of a HPLC-MS method for the simultaneous determination of methylxanthines and taurine in dietary supplements using a C_{18} reversed phase column. Yet, for routine analysis, mass spectrometry represents a rather expensive technique and thus a simpler method is needed.
However, for the simultaneous analysis of caffeine and taurine the choice of common detection systems remains limited. Sensitive determination of caffeine can be achieved by UV detection, whoever this type of detection was not appropriate for a sensitive determination of taurine as this compound has only very low extinction coefficients in the accessible UV range until 210 nm. For taurine determination ELSD was the most appropriate detector [2, 21, 22], as it is generally considered to be a very convenient LC detector for analytes without UV chromophore and furthermore, this detection mode has

proved to be good choice for amino acid analysis [18]. Most food samples are complex mixtures of a variety of different components; for this reason, the analytes of interest have to be separated from interfering matrix components before their determination and, when a universal thus non specific detection mode is used, baseline separation for the target analytes is required in order to minimize to sample preparation step. Most chromatographic methods proposed for caffeine determination used reversed-phase liquid chromatography on octyl- or octadecyl-siloxane-bonded silica stationary phase [20] however, these chromatographic systems are not favorable for the direct quantification of taurine in a carbohydrate-rich matrix since, under these HPLC conditions, taurine and carbohydrates are eluted in void volume. On the other hand good results have been obtained in our laboratory for the determination of taurine in energy drinks using hydrophilic interaction chromatography (HILIC) in energy drinks using [17, 23], urine [24], feces and bile [25]. The HILIC mode was developed in order to realize the separation of polar molecules like amino acids, sugars, organic acids, peptides, etc [26].
In this context, the aim of our study was to develop a HILIC method for the simultaneous analysis of taurine and caffeine in energy drinks. The method should by direct (little or no sample preparation), fast (short analysis time) and isocratic

MATERIAL AND METHODS

Taurine (Tau) was furnished by Sigma-Aldrich (Saint Quentin Fallavier, France), saccharose (sucrose) and caffeine were bought from Merck (Darmstadt, Germany). Acetonitrile (MeCN) and methanol (MeOH) were HPLC-grade and were furnished by J.T. Baker (Noisy le Sec, France). All the solutions are prepared using deionized water obtained with an Elgastat UHQ II system (Elga, Antony, France).
The HPLC system was composed of a ternary pump Merck-Hitachi L-6200 (Darmstadt, Germany), an injection valve Rheodyne 7725 (Cotati, CA, USA) with a 10 µL loop, an UV-visible HPLC Detector (Applied Biosystems) set at 272 nm (caffeine specific wavelength). ELSD detector Sedex 85 (Sedere, Alfortville, France) was coupled on-line after the UV detector and used for taurine detection. ELSD parameters were as follows nitrogen as nebulizer gas (3 bars), evaporative tube temperature set at 40 °C, and the gain set to 10. Column temperature was regulated by a Croco-Cil oven (Cluzeau, France) at 45 °C. EZChrom Server software (Merck, Darmstadt, Germany) was used for the chromatographic data handling.
The following chromatographic columns were tested: Atlantis HILIC Silica (150 × 2.1 mm I.D., 3µm) (Waters), Biobasic AX (150 × 2.1 mm I.D., 5µm) (Thermo Scientific), ZIC HILIC silica (150 × 4.6 mm I.D., 5µm) (Merck-Sequant) and Astec apHera^{TM} NH_2 polymer (150 × 2.1 mm I.D., 5µm) (Whippany). The used flow–rates recommended by the constructors were as follows: 0.2 mL·min^{-1} for Atlantis HILIC Silica and Biobasic AX, 0.5 mL·min^{-1} for ZIC HILIC and 0.15 mL·min^{-1} for Astec apHera^{TM} NH_2.
Stock standard solutions of the analytes at the concentration of 1 g·L^{-1} for taurine and caffeine and of 4 g·L^{-1} for saccharose, were prepared separately by dissolving appropriately weighed amounts of these compounds in deionised water. The solutions

were kept in the refrigerator (at 4 °C). Daily the working solutions of 100 $mg \cdot L^{-1}$ were prepared for each compound by dilution of the stock standards with mobile phase.
The energy drinks analyzed were bought from different Romanian and French markets. All the analyzed energy drinks have claimed chemical compositions that are similar: 108 $g \cdot L^{-1}$ for sugars (mainly sucrose and glucose), 4 $g \cdot L^{-1}$ for taurine and 320 $mg \cdot L^{-1}$ for caffeine. Other components are: vitamins, colorants, flavors and acidifiers without further information about their content. The beverage cans were freshly opened, degasified in an ultrasonic bath for 5 min and then diluted 1/50 in mobile phase ($MeOH/H_2O$ 30:70 v/v).

RESULTS AND DISCUSSION

Due to its principle, ELSD needs a volatile mobile phase to function [27], once this requirement has been satisfied, any compound less volatile than the mobile phase should be detected and the detection limits should depend on the volatility differences between the analyte and the mobile phase. Taurine and caffeine are suitable analytes for this type of detection as previously reported [17, 22, 28].
HILIC introduced by Alpert in 1990 [26] represents an interesting alternative to reversed-phase liquid chromatography for the separation of polar compounds. This chromatographic mode uses a polar stationary phase (silica, diol, amino or zwitterionic column) and a hydro-organic mobile phase, with high percentage of organic solvent (generally at least 50 % of the mobile phase composition). The retention mechanism is mainly based on the partition of the analytes between the water layer covering the stationary phase and the mobile phase, so that the more polar the solute, the higher the retention. Solutes retained by the HILIC column are eluted by increasing the proportion of water or, in general, by increasing the polarity of the mobile phase. This chromatographic mode is similar to normal phase chromatography since polar compounds are retained longer than non polar ones thus, this mode proved to be ideal for amino acids or peptides [29] and for taurine analysis [17, 23 – 25] but less appropriated for more hydrophobic solutes such as caffeine [2, 16].
The first challenge of our study was to obtain favorable chromatographic conditions for caffeine retention onto hydrophilic stationary phase and baseline separation between caffeine, taurine and all the other ingredients present in energy drink.
Among various kinds of HILIC columns, silica based packing materials such as Atlantis HILIC Silica, Biobasic AX or ZIC HILIC silica, did not make possible the caffeine retention under mobile phase conditions appropriate for taurine and sucrose retention (percentage of organic modifier higher than 50 %). However, taurine, caffeine and sucrose can be satisfactorily resolved on a polyamine bonded polymeric gel column (Astec apHera NH_2) with a methanol-water mobile phase.
It is clear that there is a dominant HILIC interaction for taurine and sucrose on Astec apHera NH_2 as evidenced by the observed retention time effect of increasing organic content in mobile phase: as the organic content of the mobile phase is increased from 50 % to 75 % MeOH, the retention times of taurine and sucrose are substantially increased whereas caffeine is eluted in void volume. In contrast with the other polar silica supports, and due to its polymeric backbone, the Astec NH_2 stationary phase can offer secondary hydrophobic interactions for caffeine retention. Thus, by decreasing the

organic modifier concentration in mobile phase from 55 % to 30 %, the retention of taurine and carbohydrates decreases as expected in a HILIC mode, whereas, the retention of caffeine increases as in a typical reversed-phase mechanism (from t_R = 3.6 min with MeOH/water (55:45, v/v) to t_R = 4.6 min with MeOH/water (30:70, v/v)). After optimization, a mobile phase composed of MeOH/water (30:70, v/v) was a good compromise to exclude caffeine from the void volume and to keep satisfactory retention for taurine. A column temperature regulated to 45 °C greatly improved the peak shape of taurine.

Selecting the proper sample solvent in the HILIC mode is also an important criterion for optimum chromatographic performance as reported previously [30]. Better peak shapes for taurine and caffeine were obtained for solutions prepared in mobile phase. Figure 1a presents the separation of a standard solution of saccharose, caffeine and taurine (100 mg·L^{-1} of each) under the optimized, isocratic LC-ELSD conditions.

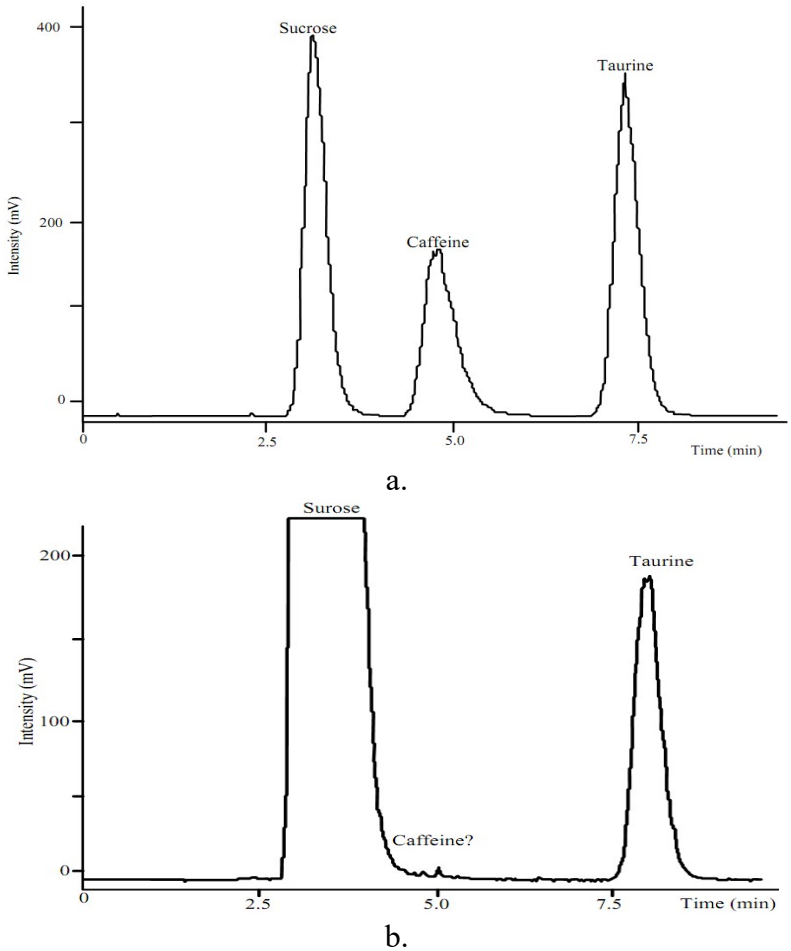

Figure 1. *LC-ELSD analysis of standard solution of saccharose, caffeine and taurine (a) analytes concentration 100 mg·L^{-1} each; (b) analytes concentration 2000, 6, 80 mg·L^{-1}, respectively*

Under these chromatographic conditions, caffeine and taurine were eluted with a retention time of 4.6 and 7.4 min, respectively and sucrose was eluted in void volume. Satisfactory baseline separation of the target analytes was observed.

Taking in account that a dilution of the sample prior to its analysis was required to avoid a column overloading due to the very high concentration of sugars (about 108 $g \cdot L^{-1}$) in energy drink, it was necessary to verify if the developed methodology allows quantifying low levels of taurine and caffeine, about 300 $mg \cdot L^{-1}$ and 4 $g \cdot L^{-1}$ respectively, in the presence of high levels of carbohydrates. Figure 1b shows the LC-ELSD chromatogram obtained for a standard solution of 6 $mg \cdot L^{-1}$ caffeine, 80 $mg \cdot L^{-1}$ taurine and 2 $g \cdot L^{-1}$ saccharose (concentration levels expected in beverage sample diluted 1/50). As can be seen in Figure 1b, the ELSD response for taurine is suitable for its quantification whereas it was not possible to detect caffeine due to its too low content close to the ELSD limit of detection. In fact, carbohydrates were eluted just before caffeine and the high concentration of carbohydrates generates an ELSD signal saturation during their elution, which prevents the detection of the low level of caffeine eluted just after.

The simultaneous determination of caffeine and taurine in energy drink was acheaved by introducing UV detector prior to ELSD and thus no modifications of the chromatographic conditions were necessary (Figure 2).

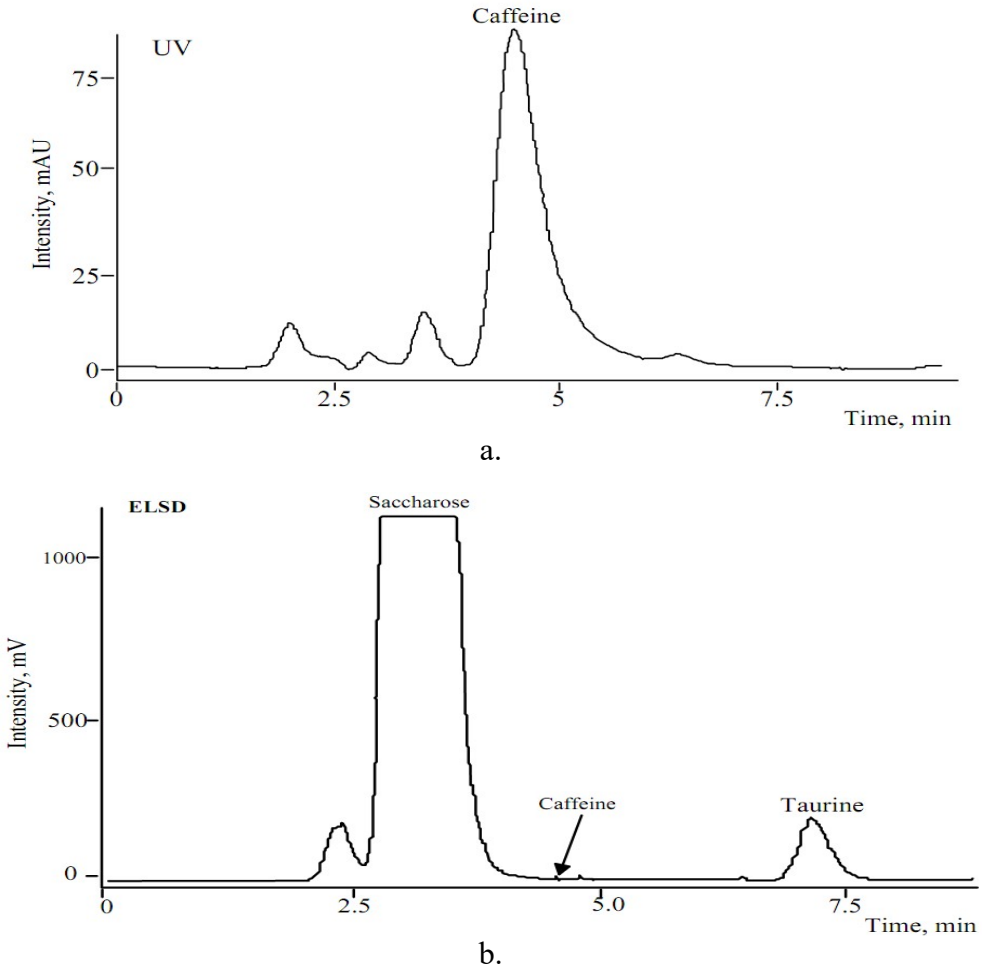

Figure 2. *Diluted (1/50) energy drink analysis.*
(a): UV detection response at 272 nm; (b): ELSD response

As can be seen in Figure 2a, caffeine can be now detected in the sample by UV at 272 nm without other compounds present in the beverage absorbing at this specific wavelength. The chromatographic peak is well-established for caffeine and the UV signal is sufficiently intense to allow caffeine quantification. On the other hand, the taurine content in sample was considered adequate to investigate its direct quantification with ELSD (Figure 2b).

The specificity of the method was confirmed by spiking the diluted energy drink sample with known concentrations of caffeine and taurine (50-100-150% of the labeled content). As can be seen from Figure 3, no peak distortion was observed when supplementary amounts of analytes were added. This latter observation leads us to conclude there is no interference with matrix components (no coelution) in the region of interest where the analytes were eluted.

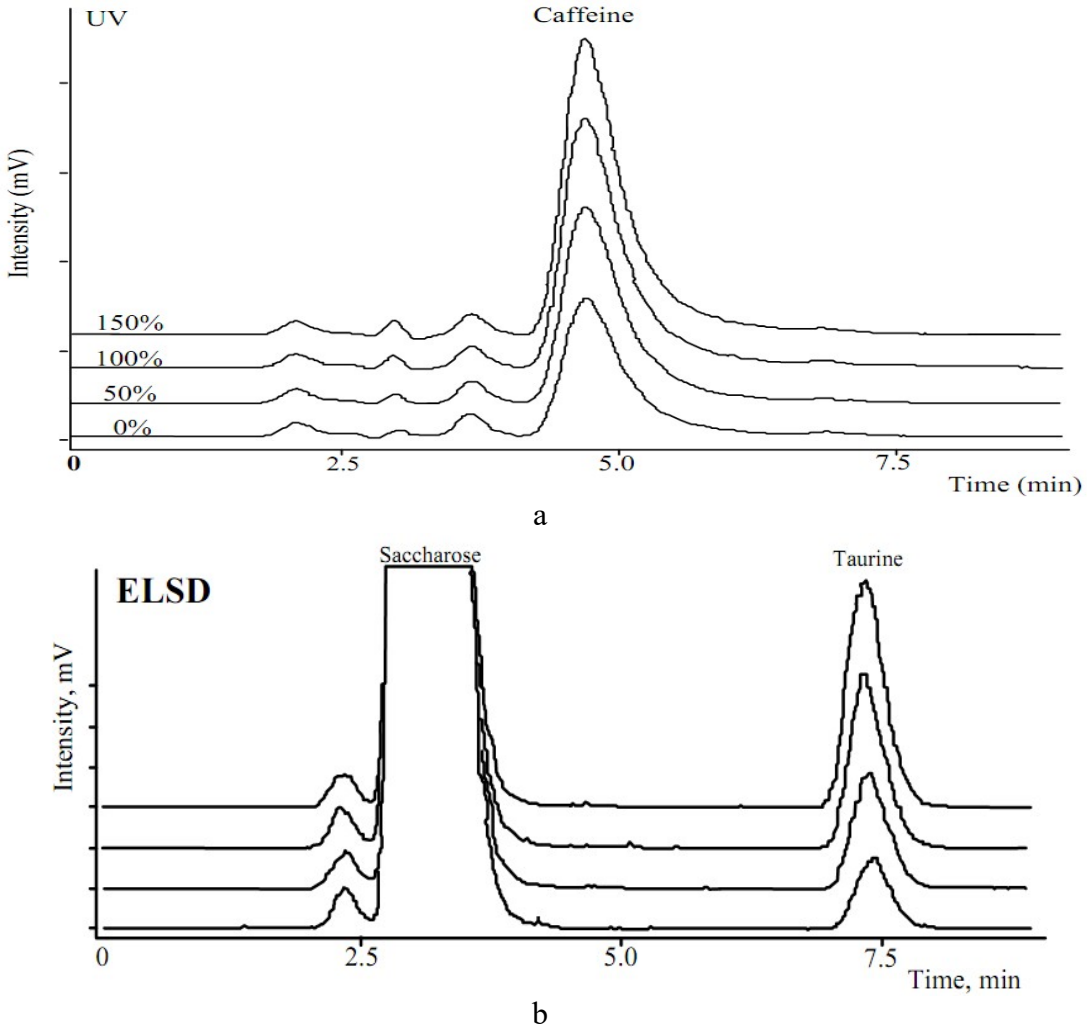

Figure 3. *LC Analysis of a diluted (1/50) energy drink with standard additions (0 %, 50 %, 100 % and 150 % of the claimed label concentration for taurine and caffeine) (a): UV detection at 272 nm; (b): ELS detection*

CONCLUSION

We developed a HILIC/UV/ELSD method for the simultaneous determination of caffeine and taurine in energy drink. The presented method presents the following advantages:

i) The method is time-efficient as it doesn't require any sample preparation (beside sample dilution) and the analysis time is about 8 minutes, resulting in the possibility of analyzing a large amount of samples in a short time.
ii) The procedure is simple and inexpensive as only basic LC instrumentation is necessary.

The developed method was validated in terms of linearity, accuracy and precision as presented in a previous paper [2]. Thus the proposed method can be used to inspect the final product quality as well as for the step by step product control during the fabrication process. We also believe that this method could be used to analyze the target compounds in other matrices.

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