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Rapid analysis of taurine in energy drinks using amino acid analyzer and Fourier transform infrared (FTIR) spectroscopy as basis for toxicological evaluation

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Summary. So-called energy drinks with very high amounts of taurine (up to 4000 mg/l are usually granted by certificates of exemption) are increasingly offered on the market. To control the currently valid maximum limits of taurine in energy drinks, a simple and rapid analytical method is required to use it routinely in food monitoring. In this article, we describe a fast and efficient analytical method (FTIR-spectroscopy) that is able to reliably characterize and quantify taurine in energy drinks. The determination of taurine in energy drinks by FTIR was compared with amino acid analyzer (ion chromatography with ninhydrin-postcolumn derivatization). During analysis of 80 energy drinks, a median concentration of 3180 mg/l was found in alcohol-free products, 314 mg/l in energy drinks with spirits, 151 mg/l in beer-containing drinks and 305 mg/l in beverages with wine. Risk analysis of these products is difficult due to the lack of valid toxicological information about taurine and its interferences with other ingredients of energy drinks (for example caffeine and alcohol). So far, the high taurine concentrations of energy drinks in comparison to the rest of the diet are scientifically doubtful, as the advertised physiological effects and the value of supplemented taurine are unproven.

Keywords: Taurine – Amino acid analyzer – FTIR-Spectroscopy – Energy drinks

Introduction

Drinks containing ingredients with presumed stimulant properties (so-called energy drinks) are increasingly offered on the market. In addition, alcohol containing energy drinks are highly consumed among young adults (BfR, 2003). The market for these drinks has increased in the past years, and, although they might be harmless, overdoses or combination of these with other drinks could be harmful to the health of some consumers in certain circumstances (Santa-Maria et al., 2002). Energy drinks often contain ingredients such as caffeine, guarana, glucuronolactone and, especially regarded in this study, taurine.

Taurine (2-aminoethyl sulphonic acid) is an amino sulphonic acid naturally present in the diet in foods such as meat, seafood and milk. Taurine is thought to play an extensive role in numerous physiological processes, including the formation of bile salts, modulation of calcium flux and neuronal excitability (Huxtable, 1992). It was proposed to act as an antioxidant, an intracellular osmolyte, a membrane stabilizer, and a neurotransmitter (Brosnan and Brosnan, 2006).

A review of the literature shows that taurine may cause physiologically beneficial effects (e.g. like decreased blood pressure in hypertensives) (Ikeda, 1977; Kendler, 1989). However, there is very little research published about the toxicity and synergistic or antagonistic effects of taurine as ingredient of energy drinks, especially in combination with alcohol, to suggest that consumption of taurine is safe to human health (Finnegan, 2003). Therefore, it is necessary to control the maximum limits of taurine in food that many European countries have established.

To measure the amount of taurine in food, different analytical methods have been developed over the past years. Most of these methods use high-performance liquid chromatography (HPLC) with different detection systems. Taurine as a sulphur amino acid is a compound lacking in a chromophore group. Therefore, the majority of the chromatographic methods involves pre- or postcolumn-derivatization to allow ultraviolet (UV), visible or fluorometric detection (Chaimbault et al., 2004). A few alternative methods use other detection modes, such as electrochemical or refractive index detection (Chaimbault et al., 2004). Recently, planar chromatography with VIS-absorbance measurement and confirmation by electrospray mass spectrometry was used to detect taurine in energy drinks (Aranda and Morlock, 2006). In general, most of the employed detection methods are of low specificity and a time-consuming (40–60 min) chromatographic method is required to separate taurine from other interfering compounds. Thus, there is still a need for a simple (avoiding a derivatization step or other tedious sample treatments) and rapid (<10 min) analytical method that is able to reliably characterize and quantify taurine.

In this article, we describe a fast and efficient analytical method (FTIR-spectroscopy), making it possible to determine the amounts of taurine in energy drinks without any derivatization step or chromatographic separation. The determination of taurine in energy drinks by FTIR is compared with amino acid analyzer (ion chromatography with ninhydrin-postcolumn-derivatization). The results of a large sample collective are presented and evaluated.

Materials and methods

Reference procedure (amino acid analyzer)

The chromatographic analysis of taurine was carried out on an LC 3000 Amino Acid Analyzer (Eppendorf, Hamburg, Germany). The system comprises an cooled compartment for autosampler, 5 buffer bottles, ninhydrin and washing solution, helium-inertgas manifold for the solutions, heating compartment for analytical column and derivatization-reactor, as well as two-channel photometric detection. The device is controlled by WinLC combined with EZChrom data software v 6.7 (Scientific Software, Pleasanton, CA).

The chromatographic separation was achieved with a cation exchange column BTC 2410 – 4 μm, 125 × 4 mm and lithium citrate buffer system (Onken, Gründau-Breitenborn, Germany). Taurine elution is accomplished by using a 5 buffer system eluting with increasing pH, starting at pH 2.2 followed by on-line post column reaction with ninhydrin. Identification is performed by detection at 570 nm (Channel A) and in case of fault of detection or higher taurine concentration detection on a second channel (Channel B) at 470 nm is possible. Quantification is carried out with glucosaminic acid as internal standard. For validation of the reference procedure, authentic samples were analyzed under repeatability conditions within a day (intraday) and under laboratory conditions on different days (interday) for determination of the precision. The accuracy was assessed by repeated analysis of spiked samples.

Fourier transform infrared (FTIR) spectroscopy

The WineScan FT 120 instrument (Foss Deutschland, Hamburg, Germany) was used to generate the FTIR spectra. The Wine Scan is a task-specific Fourier Transform Infrared Interferometer for beverages. It scans the full infrared spectrum. The instrument has been approved for wine analysis since 1996 with ready-to-use must and wine calibrations provided by the manufacturer (Patz et al., 2004) and it was recently introduced to the analysis of beer and spirit drinks (Lachenmeier et al., 2005; Lachenmeier, 2005, 2007). The conventional and part of the near-infrared range is scanned between 10.8 and 2 μm, which corresponds to the wave numbers of 926–5011 cm^{-1}. It acquires 1060 data points for data analysis. The spectral regions of water absorption between 1447–1887 and 2971–3696 cm^{-1} were eliminated to prevent noise being included in the calculation.

No prior preparation of the samples is required. For sampling, the injection nozzle of the spectrometer is plunged directly into the sample. The sample is then thermostated at 40 °C in the analyzer, so that no external thermostating is necessary. After measurement in the sample cuvette, the whole system and tubes are automatically cleaned by a built-in cleaning system.

Multivariate data analysis

As usual, the sample interferogram is Fourier transformed in the first step. Next, the water spectrum is divided from the sample spectrum to eliminate the background absorbance of water. In the third step, the sample is standardized using an equalizer sample, so that a transfer of calibrations between instruments is possible (e.g. the calibrations may be used in other laboratories that don't have the capabilities for reference analytics). The absorbance is calculated and the multivariate data analysis is performed.

For quantitative determination from the FTIR spectra (applying PLS regression), the standard software FT 120 v 2.2.2 was used (Foss Deutschland, Hamburg, Germany). Prior to PLS regression, the following appropriate wavelength ranges for the analyte were selected using the automatic filter selection tool of the FT 120 software that applies multivariate data analysis: 1196–1211, 995–999, 1389–1400, 1543–1562, 1084–1107, 1725–1736, 1026–1030, 1736–1759, 1775–1782, 1173, 1130–1134, 2967, 1292–1316, 1802–1813, and 1061 cm^{-1}. The ranges were selected based on the correlation between the reference results for the component in question and the sample variation in each wave number in the spectra by a non-disclosed algorithm of Foss.

Results and discussion

Performance of the analytical procedures

A typical chromatogram of an authentic energy drink sample is presented in Fig. 1. Table 1 summarises validation results of the amino acid analyzer. The precision of the method never exceeded 0.5% (intraday) and 1.0% (interday). The accuracy was between 0.2 and 2.1%. Regarding the validation data, the procedure is selective, and reproducible and can be treated as reference procedure. The values determined with the amino acid analyzer were then used to calibrate the FTIR instrument.

In the infrared spectra, water, ethanol and many other compounds of beverages contain absorptions of various functional groups. However, the constituents are chemically very similar and therefore display similar and overlapped absorptions, which cannot be assigned to individual compounds (Fig. 2). Therefore, chemometric techniques have to be used to calibrate the instrument against the chemical reference method, which makes FTIR a secondary analytical technique. However, it is notable that the wavelength ranges selected by multivariate data analysis predominantly are positioned in the fingerprint region between 900 and 1600 that shows the strongest absorptions in pure taurine FTIR spectra. The bands between 1000 and 1100 cm^{-1} may be explained by the characteristic strong

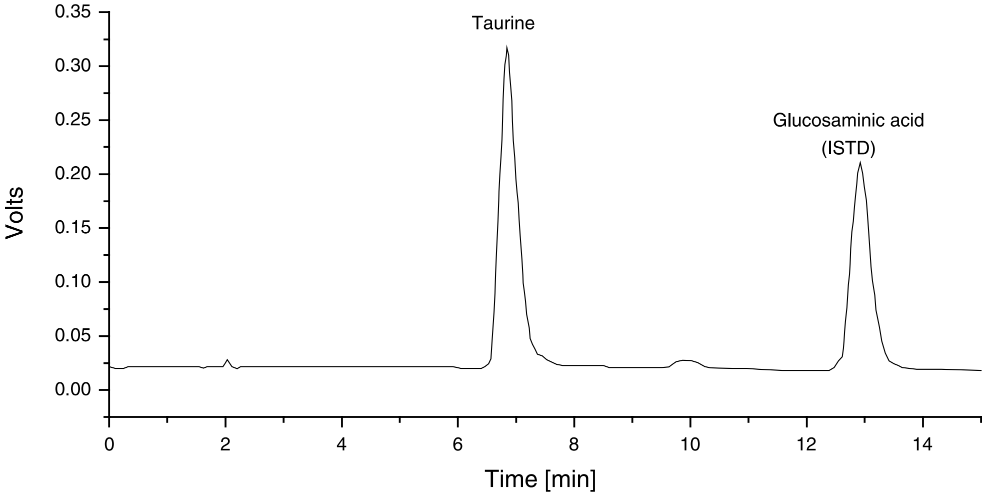

Fig. 1. Chromatogram of the taurine analysis with the amino acid analyzer

Table 1. Validation results of the chromatographic reference method (amino acid analyzer) for the determination of taurine in energy drinks

Sample	Precision (Intraday) [%]	Precision (Interday) [%]	Accuracy [%]
Energy drink 1	0.30	0.34	2.1
Energy drink 2	0.46	0.98	1.3
Energy drink 3	0.33	0.43	0.2
Energy drink 4	0.44	0.47	2.4

absorption of $=SO_2$. The bands around $1600\,cm^{-1}$ may have resulted from $-NH_2$ bending vibrations.

The analysis results of energy drink samples showed an excellent correlation ($R = 0.99$) between amino acid analyzer and FTIR. The PLS calibration for taurine is usable for a quantitative screening analysis. Table 2 illustrates information concerning the results obtained through FTIR-PLS regression.

The fact that calibration methods will never perform better than the reference method must also be considered. As expected, in the case of taurine, the precision and accuracy results of FTIR show significantly higher values than those of the reference method. FTIR should be treated as a fast, reliable screening method. For the first time, the FTIR method allows to check the taurine concentrations in energy drinks in less than two minutes. The validation results show that the method is able to quickly check the taurine maximum limits. Due to the calibration sets and not to the FTIR technique itself, the quantitative results have not enough confidence for official complaints against manufacturers. With the information gained by FTIR screening, decisions can be made whether additional analyses (with more time-consuming and expensive but more accurate standard procedures, like the amino acid analyzer) are required.

FTIR/PLS offers considerable advantages when measured against conventional methods of analysis and will acquire increasing importance as an efficient high-throughput tool for screening procedures (30 samples/hour). This leads to an remarkable reduction of the analysis time compared with chromatographic methods for taurine analysis. It supplies simple and cost-effective control of parameters like maximum limits of taurine in food.

Taurine concentrations in energy drinks

There is currently no specific legislation governing energy drinks within the EU, nor is there a general consensus with regard to the permissible concentrations of the individual ingredients.

Furthermore there is no agreed definition in the regulatory framework for the products referred to as "energy" or "stimulant" drinks.

The results regarding the taurine concentrations of energy drinks obtained by the analysis with the amino acid analyzer are summarized in Table 3. The median concentration was 3180 mg/l for the analysed alcohol-free energy drinks, 314 mg/l for energy drinks with spirits, 151 mg/l for beer-containing drinks and 305 mg/l for beverages with wine.

As shown in Fig. 3, the widest variation of taurine concentrations was found in alcohol-free energy drinks, that is also the group with the highest market share. The concentrations in alcohol-free drinks were usually lower than 4000 mg/l. Only in 8 samples (16%) concentrations above

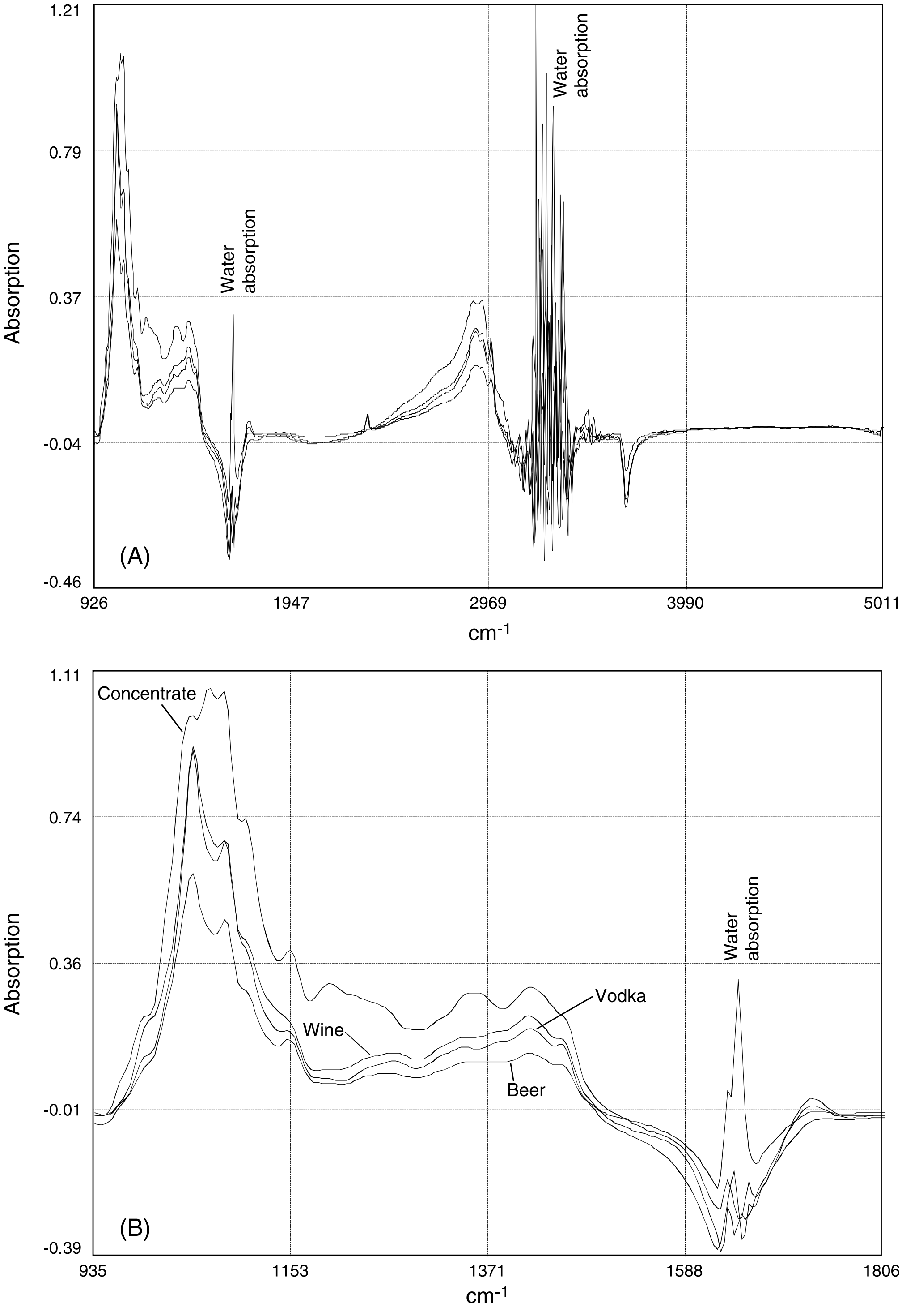

Fig. 2. FTIR spectra of four energy drinks with taurine showing the total spectal range between 926 and 5011 cm^{-1} (**A**) and a strong vertical expansion of the characteristic region between 935 and 1806 cm^{-1} (**B**). The "concentrate" contained 8515 (8594) mg/l, the energy drink with wine contained 4838 (4868) mg/l, the energy drink with vodka contained 291 (273) mg/l, and the energy drink with beer contained 141 (151) mg/l of taurine (reference values of amino acid analyzer in brackets)

this limit could be detected with the highest value of 5435 mg/l. It is interesting to note, that 2 sub-groups can be seen in alcohol-free beverages. The first group comprises drinks with taurine concentrations around 300 mg/l, the second group has concentrations around 4000 mg/l. This grouping derives from the fact, that in Germany taur-

Table 2. Validation results of the FTIR PLS method for the determination of taurine in energy drinks

Parameter	Result
PLS factors	8
Precision [%]	6.6
Accuracy* [%]	7.3
R*	0.9959

* In comparison to the reference procedure (amino acid analyzer), $n = 85$

Table 3. Taurine concentrations in energy drinks (results of the reference procedure)

[mg/l]	n	Mean ± SD	Min–max	Median
Energy drinks (alcohol free)	49	2413 ± 1754	88–5435	3180
Drinkable Energy drink "concentrates"	5	9475 ± 523	8594–10000	9595
Energy drinks with spirits	18	1042 ± 1469	223–4325	314
Energy drinks with beer	3	138 ± 23	112–151	151
Energy drinks with wine	10	706 ± 1464	132–4868	305

ine concentrations up to 300 mg/l are generally permitted as flavouring (AromenV, 2006). Amounts up to 4000 mg/l are permitted only if the producer has got a certificate of exemption. As our results show, the producers generally exhaust the permitted limits. The significantly lower concentrations in the alcoholic energy drinks can be explained by the fact that no certificates of exemption are granted for such alcopops. Therefore regardless of the kind of alcohol (spirits, beer, wine), the drinks can only contain 300 mg/l in Germany. In the group of energy drinks with spirits, there were 5 samples (28%) with illegitimate high concentrations around 4000 mg/l and one illegitimate sample in the wine-group (10%).

Evaluation of so-called "energy drink concentrates"

In the last years a questionable tendency of marketing so-called "energy drink concentrates" was detected. Such products are labelled as energy drink concentrate or raw material to produce energy drinks, and a dilution of only 1:2.5 is prescribed. However, the organoleptical properties of the products do not correspond to those of conventional beverage concentrates that normally require a dilution of 1:50–1:70. The concentrates in question have the same aroma and taste as conventional energy drinks and are packed in normal beverage cans with single portions. Whereas a normal concentrate cannot be consumed directly without dilution due to the high concentrations of organic acids and flavours. On the internet the so-called "concentrates" were advertised that "the freak drinks it pure". The labelling of the energy drinks as "concentrate" achieves only the objective to sidestep the regular limits. In fact,

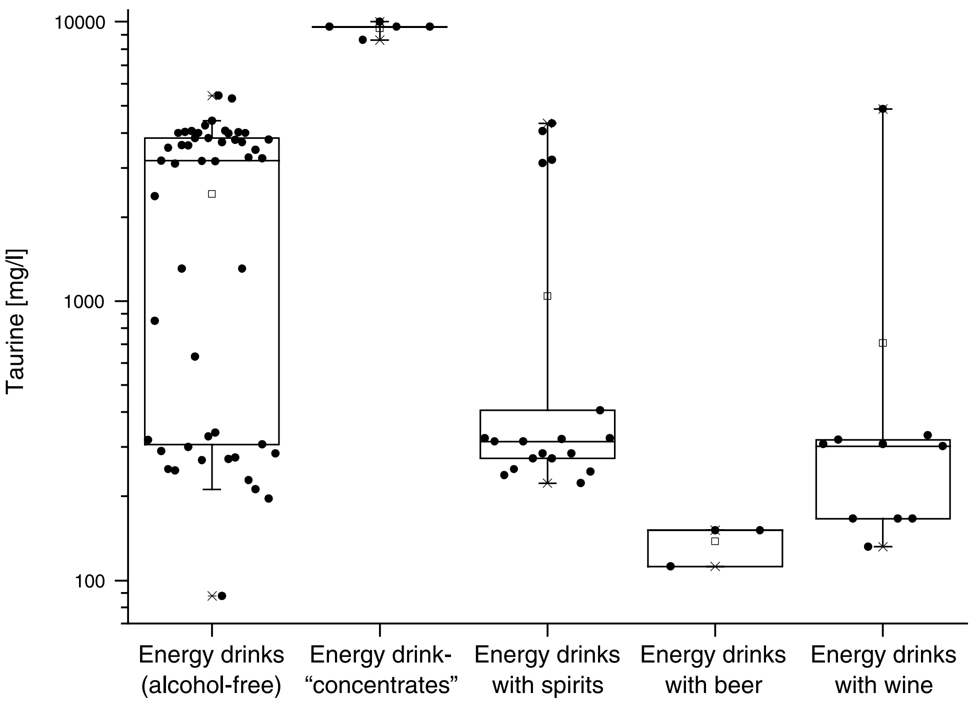

Fig. 3. Boxplot of the taurine concentrations in energy drinks

the taurine concentrations of the "concentrates" were higher than in every other analyzed sample with median value of 9595 mg/l. The labelling and advertising of the products misleads consumers and is a direct offence against European food law (article 16 of regulation (EC) No. 178/2002). These high concentrations led also to warnings in the European Unions rapid alert system for food and feed (RASFF, 2005). The products were subsequently withdrawn from the market.

Risk assessment of taurine in beverages

Over the last decade, energy drinks have developed a considerable share of the global soft drinks market. Legislation controlling their sale and marketing and scientific research into their ingredients lags behind the development of these "functional" foods. The lack of scientific research into some of the ingredients found in stimulant drinks such as taurine, and the unrecorded health effects of the combined ingredients, has made this task a difficult one.

In particular, during the combined intake of ethyl alcohol, caffeine, glucuronolacton and taurine in high concentrations, different interactions have to be taken into consideration. Because of identical targets (for example central nervous system (CNS), or cardiovascular system of ethyl alcohol, caffeine and taurine) or similar toxic effects (for example reproduction toxicity of ethyl alcohol and caffeine) of the individual components, amplified toxic effects could appear in comparison to the use of the isolated substances (Oborne and Rogers, 1983; BgVV, 2002). This problem is enforced by the fact that the intake of taurine from regular consumption of some energy drinks is several times higher than that from the rest of the diet. The mean daily intake of taurine in humans has been estimated between 40 and 400 mg (ANZFA, 2001).

Due to missing scientific risk evaluation and possible interactions with other ingredients this high content of taurine in energy drinks is irresponsible. A recent case about a possible taurine induced toxic encephalopathy underlined the dangers of uncontrolled abuse of nutritional supplements (Obermann et al., 2006).

In the following the main aspects and dangers of alcohol containing energy drinks are summarized:

- Lack of valid scientific information about interferences between the ingredients of energy drinks (for example taurine, caffeine) and alcohol.
- Scientifically not acceptable and misleading advertising-statements like "stimulants" (unproven physiological and health related effects are labelled).
- Possibility of fatal misjudgements by consumers that energy drinks could prevent or abate relevant alcoholic effects (for example in the traffic).

In reviewing the adverse health effects of energy drinks there is very little amount of comprehensive information, risk assessment data and peer reviewed scientific research in this area. Contrary to the recommendations on the labels of the products, the metabolic value of supplemental taurine in nondepleted adult subjects remains doubtful so far, because supplemented taurine is either excreted unchanged in the urine or degraded by enteral bacteriae (van de Poll et al., 2006).

In light of this limited information and in order to protect public health, it is necessary to invest in further scientific research and to control the currently valid maximum limits for the ingredients of energy drinks, as for example taurine. In the 5th amino acid assessment workshop, it was recently demanded that priority should focus on establishing safe upper levels for taurine because it is so widely available to the general population and because of concerns of the high intake that can arise from some beverages (Munro and Renwick, 2006). Whether or not there is a scientific basis for imposing upper limits on the key ingredients of energy drinks continues to be a question at both national and EU level. The lack of convincing evidence to date that energy drinks pose a real risk to public health has meant both that they have so far escaped product specific regulation and that national barriers which have attempted to restrict marketing of energy drinks and their key ingredients have been found to infringe Community law.

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