

# 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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## Introduction

Drinks containing ingredients with presumed stimulant properties (so-called energy drinks) are increasingly offered on the market. Energy drinks often contain ingredients such as caffeine, guarana, glucuronolactone and especially regarded in this study taurine (2-aminoethyl sulphonic acid). 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 (up to 4000 mg/l are usually granted by certificates of exemption) of energy drinks in comparison to the rest of the diet (mean daily intake of taurine in humans has been estimated between 40 and 400 mg) are scientifically doubtful as the advertised physiological effects and the value of supplemented taurine are unproven. To control the currently valid maximum limits of taurine in energy drinks that many European countries have established, a simple and rapid analytical method is required to use it routinely in food monitoring. Therefore, we describe a fast and efficient analytical method (FTIR-spectroscopy) that is able to reliably characterize and quantify taurine in energy drinks without any derivatization step or chromatographic separation. The determination of taurine in energy drinks by FTIR was compared with amino acid analyzer (ion chromatography with ninhydrin-postcolumn derivatization). The results of a large sample collective are presented and evaluated.

## Material 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 chromatographic separation was achieved with a cation exchange column BTC 2410 - 4  $\mu\text{m}$ , 125 x 4 mm and lithium citrate buffer system. 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 two-channel photometric 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. The device is controlled by WinLC combined with EZChrom data software. 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, while no prior preparation of the samples is required. The conventional and part of the near-infrared range is scanned between 10.8 and 2  $\mu\text{m}$ , which corresponds to the wave numbers of 926 to 5012  $\text{cm}^{-1}$ . It acquires 1060 data points for data analysis. The spectral regions of water absorption between 1447-1887  $\text{cm}^{-1}$  and 2971-3696  $\text{cm}^{-1}$  were eliminated to prevent noise being included in the calculation.

### 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. 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 v2.2.2 was used. Prior to PLS regression, the appropriate wavelength ranges for the analytes were selected using the automatic filter selection tool of the FT 120 software that applies multivariate data analysis. 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

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. The energy drink samples analysed by FTIR-Spectroscopy showed an excellent correlation ( $R = 0.9959$ ). The PLS calibration for taurine is usable for a quantitative screening analysis. Table 2 illustrates information concerning the results obtained through FTIR-PLS regression. 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.

Tab. 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 [%]
Sample 1	0.30	0.34	2.1
Sample 2	0.46	0.98	1.3
Sample 3	0.33	0.43	0.2
Sample 4	0.44	0.47	2.4

Tab. 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

### Taurine concentrations in energy drinks

The results regarding the taurine concentrations of energy drinks obtained by the analysis with the amino acid analyzer are shown in figure 1.

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 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 taurine concentrations up to 300 mg/l are generally permitted as flavouring (AromenV).

Amounts up to 4000 mg/l are permitted only if the producer has got a certificate of exemption. As 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%).

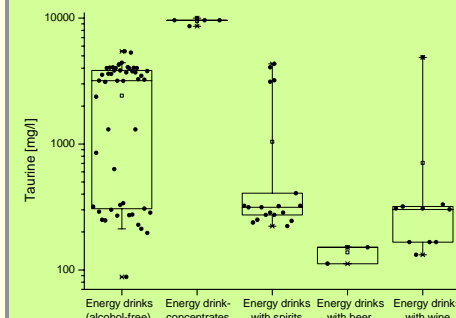


Fig. 1: Box chart of the taurine concentrations in energy drinks

### 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 to 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 achieve only the objective to sidestep the regular limits. In fact, 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). The products were subsequently withdrawn from the market.

## Conclusion

The lack of scientific research into some of the ingredients found in stimulant drinks in high concentrations, and the unrecorded health effects of the combined intake, has made safety evaluation very difficult. In light of the limited toxicological information and in order to protect public health it is necessary to control the currently valid maximum limits for the ingredients of energy drinks, as for example taurine. Therefore, FTIR/PLS-spectroscopy 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 a 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.

## References

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