European Journal of Chemistry

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# Quick and simultaneous determination of caffeine and taurine in beverages using UPLC-ESI-MS

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RESEARCH ARTICLE

10.5155/eurjchem.12.1.18-22.2036

Received: 26 August 2020
Received in revised form: 11 October 2020
Accepted: 07 December 2020
Published online: 31 March 2021
Printed: 31 March 2021

KEYWORDS

Taurine
Caffeine
Soft drinks
UPLC-ESI-MS
Energy drinks
Simultaneous determination

ABSTRACT

A rapid UPLC-ESI-MS method was developed for simultaneous determination of caffeine and taurine in beverages (energy drinks and soft drinks). The molecular ions of caffeine and taurine were identified in single ion recording mode at *m/z* 194.98 and 125.86, respectively. The mass spectrometer parameters were optimized as: capillary voltage 3.0 kV, cone voltage 35 V, extractor 3 V, RF Lens 0.1 V, source temperature 150 °C, desolvation temperature 350 °C, nitrogen 600 L/h, LMR1 7.9, HMR1 15.2, IE1 0.30. The mobile phase comprising methanol (0.1% formic acid) (A) and water (5 mM ammonium acetate) (B) was used in gradient mode. The mobile phase components A and B were pumped in 80:20 (*v*:*v*) ratio from 0-0.44 min, and then 100% of component A was pumped between 0.45-0.68 min, and at 0.69 min the composition was returned to 80:20 (*v*:*v*) ratio of A and B till 2.0 min. Caffeine and taurine were eluted at 0.46 and 0.43 min, respectively. The samples of energy drinks and soft drinks were diluted in a solvent system comprising methanol and water in 80:20 (*v*:*v*) ratio. Our investigations showed that soft drinks SD1 and SD2 have 88.8±4.2% and 110.7±3.6% (*w*:*w*) caffeine of their labeled claim. The caffeine content in energy drink brands ED1, ED2, ED3, and ED4 was 76.9±2.5, 65.6±3.4, 88.1±12.6, and 89.1±2.8% (*w*:*w*) of labeled claims, respectively. While taurine content in ED1, ED2, ED3, and ED4 was 86.5±8.4, 81.3±27.5, 101.9±4.8, and 97.1±0.3% (*w*:*w*) of labeled claim, respectively.

**Cite this**: *Eur. J. Chem.* **2021**, *12(1)*, 18-22 **Journal website**: www.eurjchem.com

## 1. Introduction

Beverages, such as energy drinks and soft drinks, are globally consumed and are fashionable among athletes and youths. Energy drinks are also consumed as a stimulant to increase physical and mental performance [1-3]. Caffeine, taurine, and sugar are the major constituents of energy drinks. Caffeine (1,3,7-Trimethylpurine-2,6-dione) is a central nervous system stimulant and rapidly absorbed into the systemic circulation. Taurine (2-Aminoethanesulfonic acid) is generally recognized as safe by The United States Food and Drug Administration (US-FDA) under specific conditions of use as an ingredient in enhanced water beverages. It is used as a nutritive ingredient in energy drinks, dietary supplements, and hypo-allergenic soy-based infant formula. Caffeine has been considered as the main ingredient for performance enhancement. There are reports which suggest the excessive consumption of energy drinks together with alcohol or other drugs, or both, may lead to adverse effects like cardiac problems, such as arrhythmias and heart attacks, including death [4-6]. Gray *et al.* recommend caution for patients with familial long QT syndrome using energy drinks [7]. Giles *et al.* reported that caffeine reduces the feeling of fatigue and increases vigor, while taurine reversed the effects of caffeine on vigor and caffeine withdrawal symptoms [8]. The safety issues of energy drinks have been raised over time, therefore it is important to establish the large-scale safety of energy drinks and maintain their quality.

There are only a few methods which can simultaneously determine caffeine and taurine in energy drinks, soft drinks and other beverages. Aranda and Morlock developed a planar chromatography (High-performance thin-layer chromatography, HPTLC)-multiple detection method for simultaneous estimation of caffeine, taurine, riboflavin, pyridoxine, and nicotinamide [9]. The analytes were measured in different modes. Nicotinamide and caffeine were measured in Ultraviolet (UV) absorption mode, riboflavin and pyridoxine were measured in fluorescence mode, and taurine was measured in visible absorbance mode after derivatization with ninhydrin solution [9]. Racz *et al.* demonstrated the application of near-infrared spectroscopy for quantitative determination of caffeine and sugar in energy drinks [10].


ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online) 
https://dx.doi.org/10.5155/eurjchem.12.1.18-22.2036

**Table 1.** Gradient scheme for elution of caffeine and taurine.

| Time (min) | Flow rate (µL/min) | Gradient percent composition | |
|---|---|---|---|
| | | Methanol (0.1% formic acid) | Buffer (5 mM $NH_4OAc$) |
| 0.00 | 300 | 80 | 20 |
| 0.44 | 300 | 80 | 20 |
| 0.45 | 300 | 100 | 0 |
| 0.68 | 300 | 100 | 0 |
| 0.69 | 300 | 80 | 20 |
| 2.00 | 300 | 80 | 20 |

Russo *et al.* developed an online extraction technique coupled to high-performance liquid chromatography (HPLC) for the determination of caffeine in coffee, tea, and cocoa [11]. Triebel *et al.* also determined the taurine in energy drinks using Fourier transform infrared (FTIR) spectroscopy [12]. Vochyanova *et al.* developed a method for simultaneous and fast determination of caffeine and taurine in energy drinks by micellar electro kinetic chromatography (MEKC). Caffeine and taurine were detected using a dual contactless conductometry/ ultraviolet photometry detector [13]. After derivatization with ninhydrin, taurine can be analyzed using UV spectrophotometer [14]. Caffeine and taurine were simultaneously determined by Chirita *et al.* in energy drinks using hydrophilic interaction chromatography with UV and evaporative light scattering detection [15]. Ricciutelli *et al.* provided an ultraperformance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method for analyzing taurine in energy drinks without derivatization [16]. Al-Bratty *et al.* determined caffeine in commercial energy beverages by using Gas chromatography–mass spectrometry (GC-MS) [17].

The aim of the present investigation was to develop a simple and fast ultra-performance liquid chromatography tandem mass spectrometry (UPLC-ESI-MS) method for simultaneous estimation of caffeine and taurine in beverages (energy drink and soft drinks).

## 2. Experimental

### *2.1. Materials and instrumentation*

Caffeine (99.7% pure) was manufactured by Alfa Aesar, A Johnson Matthey Co., Ward Hill, MA. Taurine was obtained as a gift sample from Saudi Food and Drug Authority, Riyadh. Formic acid was purchased from Loba Chemie Pvt. Ltd. Mumbai, India. Ultrapure water was prepared using Milli-QR Gradient A10R (Millipore, Moscheim Cedex, France). Bath sonicator (ultrasonic cleaner) was from Kode Technical Research Co. Ltd. Vortex was from SciLogex. Weighing balance was from Sartorius AG Gottingen, Germany. HPLC gradient grade methanol was procured from Panreac Quimica (Barcelona, Espana). The pH meter was Seven Easy pH, Mettler Toledo AG, Switzerland.

The energy drinks and soft drinks were obtained from a local Saudi Market. The samples were analyzed using a Waters® Acquity H-Class UPLC®-tandem quadrupole mass spectrometer (TQD) (Waters, Milford, USA). Acquity UPLC® BEH C18 1.7 µm, 2.1×50 mm column was made in Ireland. The H-Class UPLC® system comprises an acquity quaternary solvent manager and an acquity sample manager coupled with a column heater. TQD was equipped with an electrospray ionization (ESI) probe. The system was controlled by MassLynx 4.1 Software. Data acquisition, processing, and reporting were carried out automatically using the application manager 'QuanLynx' included with MassLynx 4.1 Software (Version 4.1, SCN 714). The detector tuning was assisted with the help of IntelliStart®. The other instruments were: a rotary pump (Sogevac, France) for assisting vacuum and a nitrogen generator (Peak Scientific, Scotland) to supply desolvation gas.

### *2.2. Chromatographic conditions*

Caffeine and taurine were eluted on an Acquity UPLC®BEH C18 (1.7 µm, 2.1×50 mm) column. Analytical column was supported with Acquity UPLC®BEH 1.7 µm, VanGuardTM Precolumn 2.1×5 mm. The column temperature was maintained at 40±5 °C. The mobile phase comprising methanol (0.1% formic acid) (A) and water (5 mM ammonium acetate) (B) was used in gradient mode. The mobile phase components A and B was pumped in 80:20 (*v*:*v*) ratio from 0-0.44 min, and then 100% of component A was pumped between 0.45-0.68 min, and at 0.69 min the composition was returned to 80:20 ratio of A and B till 2.0 min (Table 1). In gradient mode, the flow rate of the mobile phase was maintained at 300 µL/min. The composition of the strong wash and purge solvent was methanol: water in 80:20 (*v*:*v*) ratio. The sample run time was 2 min. The sample injection volume was 10 µL. The temperature of auto-sampler was maintained at 15±3 °C.

### *2.3. Mass spectrometer conditions*

The protonated ions $[M+H]^+$ of caffeine and taurine were determined in single ion recording mode. The instrument was operated in positive electrospray ionization (ESI+) mode. The preliminary tuning of mass spectrometer parameters was performed with the help of IntelliStart®. The IntelliStart® is a feature within MassLynx® software to aid automatic detector tuning and optimization of mass parameters. The final tuning was performed manually through fluidics to improve selectivity and signal intensity. The molecular ions of caffeine and taurine were identified in single ion recording mode at *m/z* 194.98 and 125.86, respectively. Optimized conditions of tune page (mass parameters) were set as: capillary voltage 3.0 kV, cone voltage 35 V, extractor 3 V, RF Lens 0.1 V, source temperature 150 °C, desolvation temperature 350 °C, nitrogen gas 600 L/h, LMR1 7.9, HMR1 15.2, IE1 0.30. Nitrogen was used as the desolvation gas.

### *2.4. Calibration curve*

Standard stock solutions of caffeine (1.0 mg/mL) and taurine (1.0 mg/mL) were separately prepared in water and stored in refrigerator. The predetermined amounts of stock solutions were mixed and diluted to give a working solution. From the working solution, a series of calibration standards for caffeine and taurine were prepared in methanol: water (80:20, *v*:*v*) by serial dilution. At every serial dilution step, the diluted standards were vortexed for 20 sec to mix the solution properly. The calibration standards were filled in the glass inserts for loading in an autosampler. Six replicates of calibration curves were prepared for caffeine and taurine.

### *2.5. Sample preparation*

Four brands of energy drinks and two brands of soft drinks were purchased from a local market in Riyadh, Saudi Arabia. The brands of energy drinks were coded as ED1, ED2, ED3, and ED4; while the brands of soft drinks were coded as SD1 and SD2.

**Table 2.** Caffeine and taurine content in energy drinks and soft drinks.

| Brand | pH | Volume (mL) | Labelled content (mg/100 mL) | | Measured content (mg/100 mL) | |
|---|---|---|---|---|---|---|
| | | | Caffeine | Taurine | Caffeine | Taurine |
| ED1 | 3.33 | 250 | 30 | 30 | 23.0 | 25.9 |
| ED2 | 3.20 | 250 | 30 | 30 | 19.6 | 24.4 |
| ED3 | 2.60 | 250 | 32 | 400 | 28.2 | 368.5 |
| ED4 | 3.45 | 250 | 30 | 400 | 30.2 | 339.5 |
| SD1 | 3.10 | 330 | 14 | 0 | 11.4 | 0.0 |
| SD2 | 2.56 | 330 | 10 | 0 | 11.0 | 0.0 |

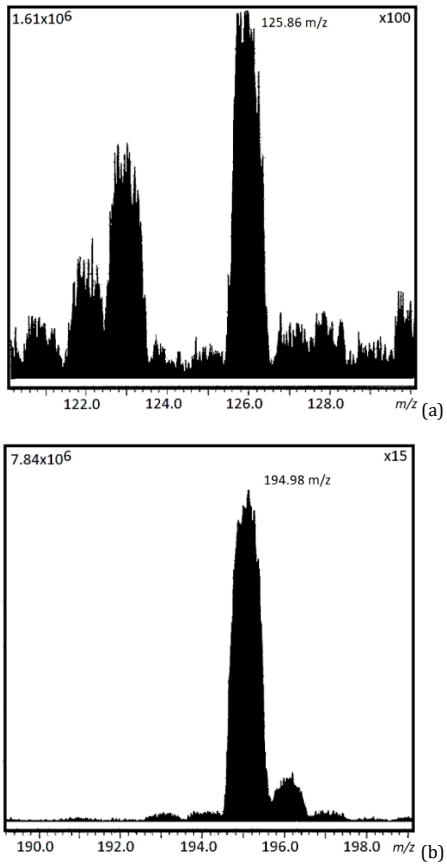


**Figure 1.** Representing tuning peaks of taurine (a) and caffeine (b).

The amounts of caffeine and taurine in individual energy drinks and soft drinks are presented with the corresponding code in Table 2. Cans of energy drinks and soft drinks were stored in refrigerator. Before analysis, the cans of energy drinks and soft drinks were removed from the refrigerator and held at laboratory temperature (23±2 °C), until the equilibrium temperature was reached. The five mL sample was withdrawn from each can and transferred into correspondingly labeled test tubes. These samples containing test tubes were pulse sonicated in a bath sonicator to remove entrapped air. The pulse sonication was preferred over continuous sonication to prevent the flow of the sample from the tubes (due to gas release). After sonication, samples were withdrawn from these tubes and diluted in a solvent system comprising methanol: water in 80:20 (*v*:*v*) ratio. The diluted samples were mixed for 20 sec using vortex. The 160-170 µL of finally diluted samples were transferred into glass inserts and analyzed. The 10 µL injection volume was withdrawn from each insert and injected for analysis automatically.

### *2.6. pH measurement*

The pH values of energy drinks and soft drinks were measured at laboratory temperature (23±2 °C). Before pH measurement, the samples were transferred into 50 mL tubes and allowed to equilibrate at laboratory temperature and sonicated in a sonication bath to remove the gas from the samples.

## 3. Results and discussions

An UPLC-ESI-MS method was developed for simultaneous determination of caffeine and taurine in energy drinks and soft drinks. Triple-quadrupole mass spectrometer (TQD) was tuned for determination of caffeine and taurine. Tuning of the mass spectrometer was performed to increase the signal intensity of the analytes in question. The representative tuning peaks of caffeine and taurine are presented in Figure 1. The protonated parent ions $[M+H]^+$ of caffeine and taurine were monitored in single ion recording mode. The mass/charge ratio of protonated ions of caffeine and taurine was *m/z* 194.98 and 125.86, respectively. A common optimum cone voltage 35 V was selected for both analytes. At this cone voltage, sufficient analyte (caffeine and taurine) signals were obtained, without compromising the sensitivity of any analytes.

After injection, both analytes were quickly eluted. The developed method is fast, since the sample run time is short (2.0 min) and the retention time for caffeine and taurine was 0.46 and 0.43 min., respectively.

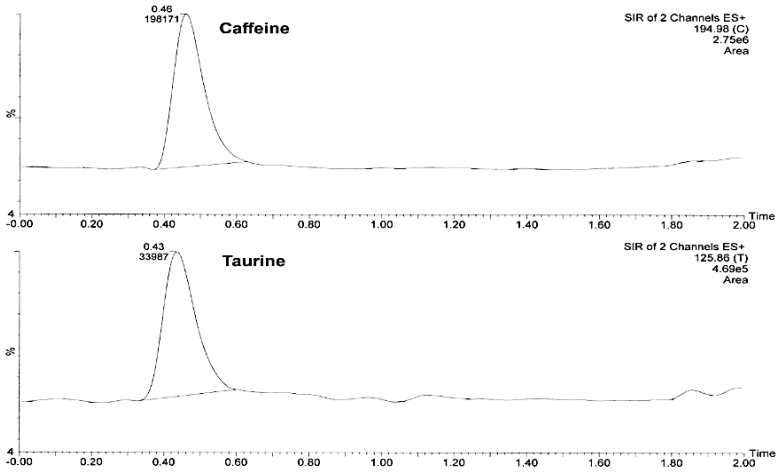


**Figure 2.** Representing chromatograms of caffeine and taurine.

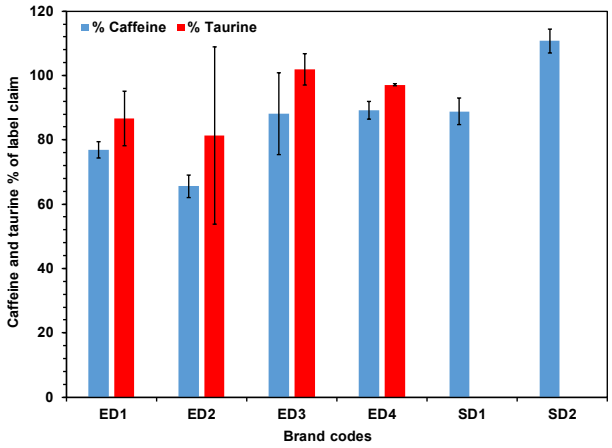


**Figure 3.** Percentage of caffeine and taurine found in energy drinks and soft drinks of their label claim.

The current method is simple also, since it does not require any tedious sample preparation or drug extraction steps. The samples were directly diluted in a methanol:water solvent system and thereafter directly injected for analysis. Furthermore, there was no need to do any derivatization of taurine before analysis, as done by Aranda and Morlock, and Draganov *et al*., where taurine was measured in visible absorbance mode after derivatization with ninhydrin solution [9,14]. The eluted peaks of caffeine and taurine are sharp and symmetrical, and there was no significant noise signal at the elution time of these drugs. Representative chromatograms of caffeine and taurine are presented in Figure 2. Six replicates of calibration curves of caffeine and taurine were run. The regression equations for caffeine and taurine were $y = 6273.7x + 33184$ and $y = 400.99x + 1238$, respectively. The regression coefficients ($r^2$) for caffeine and taurine calibration curves were 0.9993 and 0.9992, respectively. Linearity range for the analysis of caffeine was 12-400 ng/mL and for taurine the linearity range was 25-400 ng/mL.

Four different brands of energy drinks (coded as ED1, ED2, ED3, and ED4) and two brands of soft drinks (coded as SD1 and SD2) were sampled from the market and analyzed. The results of caffeine and taurine measurements are illustrated in Figure 3 as the percent of label claim on *y*-axis and product code on *x*-axis. Our investigations showed that soft drinks SD1 and SD2 have 88.8±4.2 and 110.78±3.6% (*w*:*w*) caffeine of their labeled claim. The caffeine content in energy drink brands coded ED1, ED2, ED3, and ED4 was 76.9±2.5, 65.6±3.4, 88.1±12.6, and 89.1±2.8% (*w*:*w*) of labeled claims, respectively. The caffeine content in ED1 was well below 20% and in ED2 it was below about 30% of their label claim. While taurine content in ED1, ED2, ED3, and ED4 was 86.5±8.4, 81.3±27.5, 101.9±4.8, and 97.1±0.3% (*w*:*w*) of labeled claim, respectively. The taurine content in ED1-ED4 was within ±20% of their label claim. From a safety point of view, the concentration of none of the analytes (caffeine or taurine) was in an unacceptable range in these tested products. The pH of SD1, SD2, ED1, ED2, ED3, and ED4 was in the acidic range and found to be 3.10, 2.56, 3.33, 3.20, 2.60 and 3.45, respectively. Because of their acidic pH, energy drinks and soft drinks have been associated with dental erosion [18-20]. Therefore, the precaution should be taken while consuming these products, especially the individuals feeling sensitivity in teeth.

## 4. Conclusions

An UPLC-ESI-MS analytical method has been developed for simultaneous determination of caffeine and taurine in energy drinks and soft drinks. The method is fast and selective for the routine analysis of caffeine and taurine in energy drinks and soft drinks. Four energy drinks and two soft drinks were successfully tested for the contents of caffeine and taurine, using the developed method. In one of the energy drinks, the caffeine content was found significantly low (65.6%) in the labeled claim. The developed method can be used for routine quality control of beverages comprising caffeine and or taurine.

**Acknowledgement**

The authors are thankful to the Research Center of College of Pharmacy and Deanship of Scientific Research, King Saud University.

**Disclosure statement**

Conflict of interest: The authors declare that they have no conflict of interest.
Author contributions: All authors contributed equally to this work.
Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

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