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

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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, LMR1 15.2, IPI 1.0.3. 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 0.44 min, and then 0% 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% of labeled claims, respectively. The mobile phase components A and B were pumped in 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% of labeled claims, respectively. The mobile phase components A and B were pumped in 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% of labeled claims, respectively. The mobile phase components A and B were pumped in 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% of labeled claims, respectively. The mobile phase components A and B were pumped in 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% of labeled claims, respectively. The mobile phase components A and B were pumped in 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% of labeled claims, respectively. The mobile phase components A and B were pumped in 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% of labeled claims, respectively. The mobile phase components A and B were pumped in 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% of labeled claims.
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].

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 C18 (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 22.2036, 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, LMRI 7,9, HMRI 15,2, EI1 0.30. Nitrogen was used as the desolation 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 was 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.
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 a 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.

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

![Figure 1. Representing tuning peaks of taurine (a) and caffeine (b).](image-url)
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. 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.
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Disclosure statement

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Sample availability: Samples of the compounds are available from the author.

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