Quantitative Determination of Caffeine and Alcohol in Energy Drinks and the Potential to Produce Positive Transdermal Alcohol Concentrations in Human Subjects

Jessica Ayala, Kelsie Simons, and **Sarah Kerrigan***

Forensic Science Program, College of Criminal Justice, Sam Houston State University, Box 2525, 1003 Bowers Blvd., Huntsville, Texas 77341

Abstract

The purpose of this study was to determine whether non-alcoholic energy drinks could result in positive "alcohol alerts" based on transdermal alcohol concentration (TAC) using a commercially available electrochemical monitoring device. Eleven energy drinks were quantitatively assayed for both ethanol and caffeine. Ethanol concentrations for all of the non-alcoholic energy drinks ranged in concentration from 0.03 to 0.230% (w/v) and caffeine content per 8-oz serving ranged from 65 to 126 mg. A total of 15 human subjects participated in the study. Subjects consumed between 6 and 8 energy drinks over an 8-h period. The SCRAM® II monitoring device was used to determine TACs every 30 min before, during, and after the study. None of the subjects produced TAC readings that resulted in positive "alcohol alerts". TAC measurements for all subjects before, during and after the energy drink study period (16 h total) were < 0.02% (w/v). Subjects in the study consumed a quantity of non-alcoholic energy drink that greatly exceeds what would be considered typical. Based on these results, it appears that energy drink consumption is an unlikely explanation for elevated TACs that might be identified as potential drinking episodes or "alcohol alerts" using this device.

Introduction

The popularity of energy drinks has increased at a dramatic rate since the introduction of Red Bull in the United States in 1997. U.S. consumers spent $744 million on caffeinated energy drinks during the year ending June 2007 (1). Although part of a growing trend, energy drinks are niche products and comprise a small percentage of the non-alcoholic beverage market. A wide variety of these non-alcoholic beverages claim to improve performance and boost energy. These products are typically targeted to young adult consumers; a survey of energy drink consumption among college students in the United States indicated that 51% reported using energy drinks on a regular basis (more than one drink per month) (2). Of these, 67% consumed energy drinks to counteract the effects of insufficient sleep, 65% for increased energy, and 54% to drink with alcohol while partying. Students that used energy drinks to offset the effects of alcohol typically consumed three or more drinks while partying. The use of combined energy drinks and alcohol has been the focus of several studies. Co-ingestion of energy drinks and alcohol has been shown to reduce a subject's perception of effects, including impairment of motor coordination. However, it did not significantly reduce the actual deficits in performance caused by alcohol, including motor coordination and visual reaction time (3).

Although many energy drinks are promoted as being nutraceutical foods, boosting health, energy, or otherwise having sought-after benefits, there is some concern among health professionals that these beverages, and the drinking behaviors of the targeted consumers, may in fact have adverse health consequences. The most commonly reported adverse effects include insomnia, nervousness, headache, and tachycardia (4). In a recent study, heavy consumption of energy drinks was attributed to new onset seizures in four patients (5) and hospitalization of individuals with preexisting mental illness (6).

In addition to caffeine, energy drinks frequently contain mixtures of amino acids, herbals, dietary supplements and other substances such as taurine, guarana, ginko, ginseng, and others. There is still much debate surrounding the purported physiological benefit and the overall safety of these ingredients. A recent study concluded that although the amount of guarana, taurine, or ginseng in most energy drinks was below the amount expected to deliver therapeutic benefit or adverse effect, caffeine and sugar were present in amounts known to cause a variety of adverse effects (4). Energy drinks are reported to contain as much as 80–300 mg of caffeine and 35 g of processed sugar per 8-oz serving (4).

The purpose of the study was to investigate transdermal alcohol concentration (TAC) following nonconventional alcohol

* Author to whom correspondence should be addressed: Sarah Kerrigan, Ph.D., Sam Houston State University, Box 2525, 1003 Bowers Blvd., Huntsville, TX 77341. E-mail: sarah.kerrigan@shsu.edu.

exposure that may result from the consumption of energy drinks. Previous studies indicated that consumption of non-alcoholic beverages containing < 0.5% (w/v) alcohol had the potential to produce positive breath alcohol test results if the statutory 15-min deprivation period was not observed (7). In this study, the potential for similar results was investigated using a commercially available transdermal alcohol monitoring device. In order to determine the feasibility of a positive TAC following energy drink consumption, it was necessary to quantitatively determine caffeine and ethanol in a variety of popular energy drinks. The objective was to select an energy drink with the highest concentration of ethanol, but one that was not going to pose an unusual health risk to participants in terms of excessive caffeine intake. A total of 11 energy drinks were evaluated in the study.

Transdermal alcohol measurement is a nontraditional means of determining alcohol exposure. Other nontraditional techniques include the use of ethanol biomarkers, sweat patches, and near-infrared spectroscopy (or diffuse reflectance from the skin). The concept of determining the presence of ethanol in sweat is not new, but the introduction of commercially available devices for this purpose is relatively recent, and the published literature on this topic is somewhat limited to date. Transdermal alcohol measuring devices that rely upon electrochemical detection are commercially available and are used in a variety of criminal justice settings (8). One such device is the Secure Continuous Remote Alcohol Monitor (SCRAM II) by Alcohol Monitoring Systems (Highlands Ranch, CO).

Less than 1% of ethanol is lost through the skin as vapor via passive diffusion through the skin or active excretion from sweat glands (9). Transdermal alcohol measurements determine a person's alcohol consumption through insensible perspiration which is the constant, unnoticeable excretion of sweat through the skin. The SCRAM II device consists of a 6-oz ankle bracelet and a portable modem. The bracelet detects alcohol using a fuel cell device similar to that which is used in portable breath test devices, in addition to infrared and temperature sensors that are used for tamper detection. At specific intervals, the device draws in ethanol vapor from the skin surface into the fuel cell. TACs are measured every 30 min after the bracelet has been initialized and a baseline established. Devices like this are used in a variety of criminal justice settings. Their principal benefit is the ability of the individual to be continuously monitored while they go about normal activities. Such "passive participation" is noninvasive and produces minimal discomfort to the user. The stored data are transmitted by radio frequency through the modem and sent to a remote server for data analysis. An "alcohol alert" occurs when the TAC exceeds 0.02% (w/v) on three or more consecutive readings and other criteria are met according to a proprietary algorithm. The process by which an alcohol drinking episode is identified is somewhat similar to the way in which breath testing instruments detect mouth alcohol, by looking at the exhalation profile and evaluating the change in concentration over time. In the case of TAC, an alcohol alert may be issued if there are three consecutive readings above 0.02% (w/v) and absorption and elimination rates meet certain criteria.

The average person emits approximately 1 L of insensible perspiration each day. Previous studies have indicated a negative shift in the peak TAC relative to the peak BAC (10). Delays in peak TAC of 30 min to 3 h have been reported (11–13). The relationship that exists between TAC and BAC and the subsequent partition ratio is a matter of ongoing investigation, but the devices appear to have the potential to qualitatively identify drinking episodes, and therefore many uses in criminal justice settings or situations where alcohol abstinence is required.

Methods

Reagents and materials

Energy drinks were purchased from retail outlets and convenience stores. Samples were stored at 4°C prior to analysis. Caffeine was purchased from Cerilliant (Round Rock, TX), and deuterated internal standard (caffeine-d_{10}) was purchased from Toronto Research Chemicals (North York, ON, Canada). Poly-Chrom Cerex Clin II solid-phase extraction cartridges were obtained from SPEWare (Baldwin Park, CA). Mono and dibasic sodium phosphate, glacial acetic acid, ammonium hydroxide, ethanol (100%), *n*-propanol, methanol, and ethyl acetate were purchased from Sigma-Aldrich (St. Louis, MO). A DB-5 capillary column (30 m × 0.25-mm i.d., 25 µm) and Restek BAC-2 capillary column (30 m × 0.32-mm i.d., 1.2 µm) were purchased from VWR (West Chester, PA). Secure Continuous Remote Alcohol Monitor (SCRAM II) devices and modems were requested from the manufacturer (Alcohol Monitoring Systems). Fifteen devices were provided to our institution at no charge, and they were returned to the manufacturer upon completion of the study.

Quantitative caffeine determination

Quantitative analysis of caffeine was performed by gas chromatography–mass spectrometry (GC–MS) using an Agilent 5975 MSD based upon a previously published procedure (14). Briefly, energy drinks were diluted 1:100 with 100 mM pH 6.0 phosphate buffer prior to analysis. Energy drinks, calibrators and controls (1 mL) were fortified with 50 µL 0.1 mg/mL caffeine-d_{10} in methanol. Because of the large dilution of energy drinks (1:100), calibrators and controls were prepared directly in phosphate buffer. A methanolic working standard was used to prepare caffeine calibrators in the range 1–10 mg/L (0.001–0.010 mg/mL). Samples were transferred to SPE columns and drawn through the column under vacuum. Columns were then successively rinsed using 1 mL deionized water, 1 mL acetic acid (1 M) and dried under full vacuum for 5 min. Ethyl acetate (1 mL) was added to the column and the eluate collected. Columns were rinsed once again using methanol (1 mL). A second eluent consisting of ethyl acetate with 2% concentrated ammonium hydroxide (1 mL) was added and the eluate collected. The two fractions were combined, evaporated to dryness under nitrogen at room temperature, and reconstituted in 25 µL of ethyl acetate.

Samples were analyzed by GC–MS using an Agilent 6890

GC with a 5975 MSD. The injector and interface were set at 250 and 280°C, respectively. Separation of components in each 2-µL injection was achieved using a 30-m DB-5 capillary column. Injections were made in split mode with a 10:1 split ratio. Following an initial oven temperature of 160°C and hold time of 0.5 min, the temperature was increased at 30°C/min to 290°C. The final hold time was 7.17 min, and the total run time was 12 min. Helium was used as the carrier gas at a flow rate of 1.3 mL/min. Caffeine-d_{10} (*m/z* **204**, 115, 70) was used as the internal standard for the quantitative determination of caffeine (*m/z* **194**, 109, 67). Acquisition was in selected ion monitoring mode, and quantitation ions are shown in bold. The limit of quantitation (LOQ), defined as the concentration of caffeine that produced a signal-to-noise ratio of at least 10:1 with a calculated concentration within 20% of the expected value, was < 1 mg/L. The linear range of the assay was 1–25 mg/L, accuracy was 102%, and intra-assay CV was < 3% (n = 2) at 1 mg/L.

Quantitative ethanol determination

Ethanol was quantitatively determined using GC with flame-ionization detection (FID) using an Agilent 7890 GC and a 30-m Restek BAC-2 capillary column. Minor modifications to a previously published procedure for blood alcohol analysis were used to determine ethanol in energy drinks (15). Briefly, blanks and ethanol calibrators ranging from 0.003 to 0.50% (w/v) were prepared in deionized water using 100% ethanol. In a 10-mL headspace vial, 2 mL of each calibrator, control or energy drink was mixed with 1 mL of internal standard solution consisting of 0.03% (w/v) n-propanol in deionized water. Samples were equilibrated at 50°C for 10 min, and a 5-µL headspace sample was injected onto the GC–FID in splitless mode. The injector and isothermal column temperature were maintained at 180 and 50°C, respectively. Limit of detection and LOQ were 0.001 and 0.005% (w/v), respectively. Accuracy was 96-102% (0.070–0.300% w/v) and intra-assay CVs were 1.1–4.4% (n = 8) in the range 0.039–0.300% (w/v).

Table I. Characteristics of Human Subjects

Participant Number	Sex	Age (years)	Ethnicity	Weight (lbs)	Height (ft, in.)
1	Female	35	Caucasian	293	5′ 10″
2	Male	25	Caucasian	155	5′ 8″
3	Male	25	Asian	218	5′ 10″
4	Female	29	Caucasian	174	5′ 9″
5	Male	23	Hispanic	256	5′ 9″
6	Male	24	Caucasian	271	5′ 11″
7	Female	22	Caucasian	168	5′ 8″
8	Male	25	Caucasian	224	5′ 8″
9	Male	25	Caucasian	207	6′ 3″
10	Female	23	Caucasian	130	5′ 2″
11	Female	24	Caucasian	131	5′ 6″
12	Female	25	Hispanic	152	5′ 2″
13	Female	24	Caucasian	197	5′ 6″
14	Female	26	Caucasian	141	5′ 4″
15	Female	23	Caucasian	167	5′ 10″

Energy drink consumption and transdermal alcohol detection

A total of 15 volunteers (6 males, 9 females, aged 22–35 years, weighing 130–293 lbs, and in reported good health) were selected for the study (Table I). Subjects were required to wear the SCRAM II ankle bracelet for 11 days during which time they participated in a number of investigational activities. Participants were required to maintain food and beverage logs, refrain from alcohol use unless directed, and facilitate data downloads via modem at least once every 24 h. The study was approved by the Institutional Review Board (Sam Houston State University Protection of Human Subjects Committee) to ensure the safety, protection and privacy of all participants. Subjects gave informed consent and received an honorarium ($200) for participation in the study. Neither the institution nor the investigators received any compensation. The experimental design and execution of the study were solely the work of the investigators. Participation by the ankle bracelet manufacturer was limited to the loaning of the devices during the study period and attaching and initializing the devices to the participants. Devices were returned to the manufacturer upon completion of the study.

Subjects were instructed to consume no more than 8 energy drinks over an 8-h period during the regular course of their daily activities. They recorded their food and beverage intake and documented any discomfort or unusual effects during this time. Subjects were specifically instructed to stop drinking the energy beverage if they felt uncomfortable or experienced any unpleasant effects. They were also instructed not to consume caffeinated products during the study period.

To ensure that the devices were able to detect actual drinking episodes, volunteers also participated in a controlled drinking experiment in which they consumed a quantity of alcohol sufficient to produce an estimated peak blood alcohol concentra-

Table II. Number of Servings of Alcohol (1.5 oz, 40%) and Energy Drink (8 oz, 0.23%) Consumed During Each Study Period

	Alcohol Consumption		Energy Drink Consumption	
Participant Number	Number of servings	Duration (h)	Number of servings	Duration (h)
1	5	0.4	8	7.0
2	3	0.3	8	4.8
3	4	0.3	8	7.5
4	3	0.2	7	3.8
5	5	0.5	8	5.3
6	5	0.5	8	6.0
7	3	0.2	8	7.3
8	4	0.3	8	6.5
9	4	0.3	8	7.8
10	2	0.1	8	5.8
11	2	0.2	6	5.8
12	2	0.1	7	7.8
13	3	0.3	8	6.5
14	2	0.1	6	7.9
15	3	0.3	8	7.1

tion (BAC) of 0.08% (w/v). Subjects consumed 1.5-oz servings of 80 proof liquor (vodka) diluted with fruit juice over a period of approximately 30 min. The estimated dose was determined using the Widmark equation and assuming a volume of distribution of 0.7 L/kg for men and 0.55 L/kg for women. Table II summarizes the approximate number of servings of alcohol and energy drinks that were consumed by each subject over a specified period.

Results and Discussion

Quantitative caffeine determination

Linear regression analysis of calibrators in the range 0–10 mg/L yielded an R^2 value of 1.000 and the control sample fortified with 1.0 mg/L caffeine produced a calculated concentration of 1.02 mg/L (102%). Quantitative caffeine determinations in diluted samples yielded concentrations ranging from 2.74 to 5.31 mg/L. These correspond with caffeine doses of 65–126 mg per 8-oz serving. Nutritional labeling information and actual caffeine concentrations are summarized in Table III, and Figure 1 depicts actual SIM data for the No Fear Super Cherry beverage, which was representative of all of the samples. In another study where caffeine content of energy drinks was quantitatively determined, doses of 33–77 mg were reported per 8-oz serving (16). Although caffeine content in the beverages tested in this study were considerably higher (65–126 mg) for equivalent serving sizes, results for the one energy drink (Red Bull) that was included in both studies were in excellent agreement: 67 mg and 69 mg, respectively.

Caffeine (1,3,7-trimethylxanthine, guaranine) is a plant-derived alkaloid and psychostimulant that is present in tea leaves, coffee, cocoa beans, and kola nuts. Individuals may be exposed to caffeine via beverages, food, over-the-counter drugs, prescription drugs, dietary supplements, and cosmetic treatments. An average cup of coffee is reported to contain 100 mg caffeine, although much higher doses have been reported, particularly among specialty coffees (17). Caffeine is also available in numerous dietary supplements, over-the-counter drugs, and in prescription drug mixtures at doses ranging from 32 to 200 mg (18). A dose of 50–200 mg is generally consistent with mild stimulation.

Quantitative ethanol determination

Linear regression analysis of calibrators in the range 0.031–0.50% (w/v) yielded an R^2 value of 1.000 and the control sample fortified with 0.30% produced a calculated concentrations of 0.313 (104%). Quantitative analysis of ethanol in the energy drinks indicated concentrations ranging from 0.030 (180 Blue Energy) to 0.230% (w/v) (180 Red Energy). Ethanol concentrations for all 11 energy drinks are summarized in Table IV.

Table III. Nutritional Labeling Information and Actual Caffeine Content of Energy Drinks

	Nutritional Label Information		Quantitative Analysis	
Energy Drink	Serving size (oz)	Caffeine content per serving	Concentration in undiluted beverage (mg/L)	Estimated total mg caffeine per 8-oz serving
Rockstar Black (Original)	8	80 mg	274	65
Red Bull	8.3	Not specific; "contains caffeine"	293	69
Full Throttle	8	Not specific; "709.7 mg energy blend"	294	70
Amp Energy (Green)	8	143 mg	326	77
Vitamin Energy Dragonfruit	8	150 mg	341	81
Monster (Green)	8	Not specific; "2500 mg energy blend"	357	85
180 Energy (Red)	8.2	Not specific; "contains caffeine"	360	85
180 Energy (Orange)	8.2	Not specific; "contains caffeine"	364	86
180 Energy (Blue)	8.2	Not specific; "contains caffeine"	369	87
No Fear Super Cherry	8	87 mg	420	99
NOS High Performance (Blue)	8	125 mg	531	126

Energy drink selection

Of the 11 energy drinks tested, 180 Red Energy had the highest ethanol concentration, estimated to be 0.230% (w/v). In earlier studies, ethanol concentrations in soft drinks and flavored beverages were reported in the range 0–0.096% (w/v) (19) and 0–0.084% (w/v) (7). Even at the highest concentration tested, the ethanol concentration was less than one-tenth of the concentration typically found in domestic beer. One would have to consume more than 30 8-oz servings of the energy drink (180 Energy Red) to achieve the equivalent of a standard 12-oz serving of beer containing 5% alcohol (w/v).

The United States Food and Drug Administration (FDA) does not consider the terms "non-alcoholic" and "alcohol-free" to be equivalent (20). The term "alcohol-free" may be used only when the product contains no detectable alcohol. Beverages such as soft drinks, fruit juices, and certain other flavored beverages that are traditionally perceived by consumers to be "non-alcoholic" may contain traces of alcohol (less than 0.5% alcohol by volume) derived from the use of flavoring extracts or other sources. The FDA considers beverages containing less than 0.5% alcohol to be "non-alcoholic", and these do not need to contain the government warning statement. Although these very low concentrations of

ethanol in "non-alcoholic" beverages are too low to have a physiological effect, they may produce a positive breath alcohol test result if the deprivation period to allow mouth alcohol to dissipate is not observed (7). The purpose of the study was to investigate whether the consumption of these "non-alcoholic" beverages could produce transdermal alcohol measurements that were sufficiently high enough to produce a "positive result" or "alcohol alert" that might indicate alcohol consumption. The energy drink 180 Energy Red (Anheuser-Busch) was selected for the study because it contained the highest concentration of ethanol but a moderate concentration of caffeine (85 mg), which is less than an average dose resulting from a typical serving of coffee (17,21).

The FDA considers caffeine to be both a drug and a food additive. They report an average daily intake of 200 mg among adults and recommend that daily intake of caffeine should not exceed 600 mg (22). Although the adverse health consequences of caffeine have been widely studied, fatal caffeine overdoses in adults are quite rare and typically require the ingestion of very large quantities of the drug, typically in excess of 5 g. For the purposes of the study, subjects were instructed not to consume more than 8 energy drinks during the course of the day and to refrain from all other caffeinated products, including coffee, tea, sodas, or supplements.

TAC

In the controlled drinking experiment, subjects consumed the approximate number of 1.5-oz servings of 80 proof liquor to achieve an estimated BAC of 0.08% (w/v). This ranged from 2 to 5 drinks for all participants, depending on sex and weight (Table II). TACs for all participants following actual drinking are shown in Figure 2. There was a great deal of intersubject variability with respect to maximum TAC and time to reach maximum TAC. The average peak TAC for all subjects was 0.064% (w/v), and this was achieved, on average, 2 h after the last drink. The purpose of including the controlled drinking study was for comparison purposes and a full discussion of the TACs is beyond the scope of this publication and will be presented elsewhere. TACs for all participants following con-

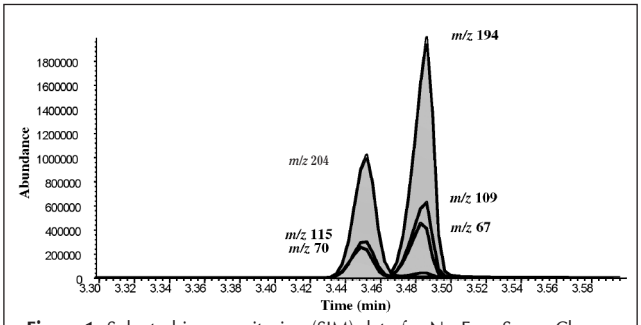

Figure 1. Selected ion monitoring (SIM) data for No Fear Super Cherry (4.2 mg/L). Quantitation ions for caffeine (*m/z* **194**, 109, 67) and caffeine-d_{10} (*m/z* **204**, 115, 70) are shown in bold.

Table IV. Ethanol Content of Energy Drinks

Energy Drink	Ethanol Concentration (%, w/v)
180 Energy (Blue)	0.030
AMP Energy (Green)	0.035
NOS High Performance (Blue)	0.038
No Fear Super Cherry	0.053
Red Bull	0.067
Vitamin Energy Dragonfruit	0.110
Full Throttle	0.119
180 Energy (Orange)	0.127
Monster (Green)	0.151
Rockstar Black (Original)	0.162
180 Energy (Red)	0.230

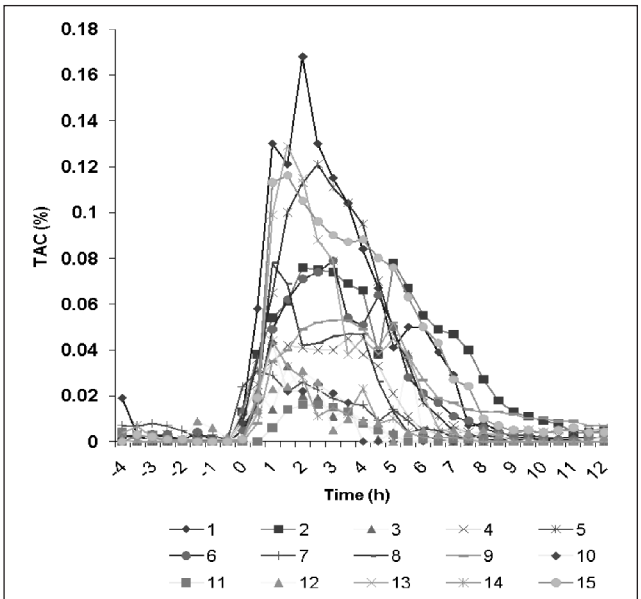

Figure 2. Transdermal alcohol concentration (%, w/v) following actual ethanol consumption. Sufficient alcohol to achieve an estimated BAC of 0.08% (w/v) was consumed over a period of approximately 30 min beginning at time "0". TAC is also depicted for the 4 h prior to alcohol consumption (–4 to 0 h or baseline) and a total of 12 h after the first drink.

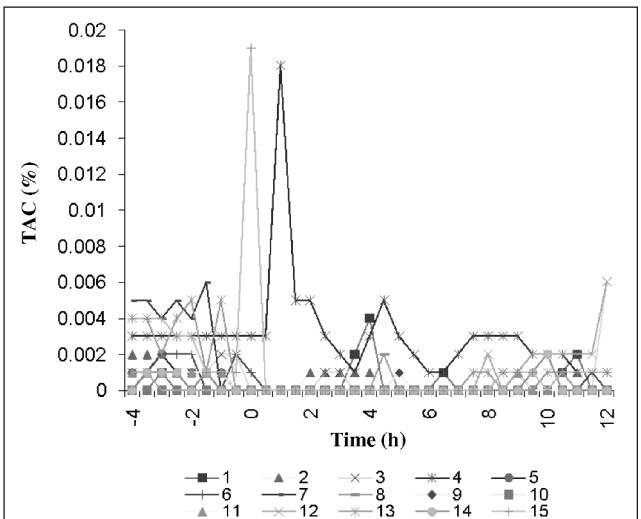

Figure 3. Transdermal alcohol concentration (%, w/v) following consumption of energy drinks. Energy drink consumption took place over 8 h, starting at time "0". TAC during the 4 h preceding consumption of the energy drinks (–4 to 0 h, "baseline") and the 4 h following the last energy drink (8–12 h) are also depicted.

sumption of the energy drinks are shown in Figure 3. TAC estimates never exceeded 0.02% (w/v) during or following energy drink consumption in any of the subjects. In order for an "alcohol alert" to be triggered, the device must record three consecutive readings of 0.02% (w/v) or more, in addition to other criteria. Figure 4 shows the average TAC readings for all 15 subjects for the 4 h before, during, and after consuming alcohol and energy drinks. The averaged TAC readings for all subjects show that at no point did TAC even approach what might be considered to be an elevated TAC. Instead, average TAC readings remained at "baseline" or "negative", which was on average for all subjects < 0.001% (w/v). Figure 4 also shows the 95% confidence intervals for actual drinking, compared with energy drink consumption. The confidence intervals are large due to the significant intersubject variability in TAC described earlier. Despite the expected variability, at no point during the drinking experiments do the confidence intervals overlap above TACs above 0.02% (w/v).

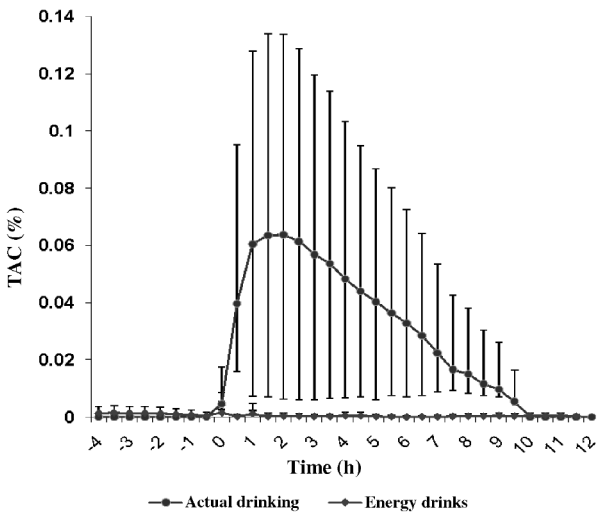

Figure 4. Mean transdermal alcohol concentrations (%, w/v) for all subjects (n = 15) following the controlled drinking study (actual drinking) and the consumption of "non-alcoholic" energy drinks. TAC is also depicted for the 4 h prior to the study period (–4 to 0 h or baseline) and a total of 12 h following the start of the study. Error bars indicate 95% confidence intervals.

Table V. Self-Reported Effects in 15 Human Subjects Following Ingestion of 6–8 Energy Drinks Over Approximately 8 h

Observation	Number of subjects
Energized/wide awake	8
Jittery or jerky movements	8
Cold and/or numbness	4
Light-headed	3
Dizziness	2
Tired	2
No unusual effects	1
Focused	1
Irritable	1

Conclusions

Transdermal alcohol measurement has been proposed as a valuable tool for the qualitative identification of drinking episodes. However, scientific studies to date are still in their infancy and the investigation of interferences or nonconventional alcohol exposure deserves additional study. "Non-alcoholic" beverages, which may contain as much as 0.5% ethanol by volume, have been shown to produce positive breath alcohol test results if the statutory deprivation period is not observed. Transdermal alcohol measurement devices such as the SCRAM II device have a variety of uses in criminal justice settings, particularly where remote, long-term monitoring is desirable such as probation or parole.

The purpose of the study was to determine whether the consumption of energy drinks could produce TAC measurements that were suggestive of an actual drinking episode. In testing whether energy drinks could produce what might be considered a false positive, subjects were required to consume a large number of drinks that greatly exceeded what might be considered typical. Upon completion of the energy drink study, more than 50% of subjects reported some sort of "unpleasant" effect, most commonly "jittery" or "jerky movements" (Table V). Eight of the 15 subjects reported feeling "wide awake" and only one subject reported no effect. Even after consuming as many as eight energy drinks, to the extent that most subjects experienced some sort of unpleasant effect, TAC readings were not elevated (< 0.02%) and no "alcohol alerts" were generated for any of the subjects. Based on these results, it seems unlikely that non-alcoholic beverage consumption alone is a viable defense for elevated TAC.

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