### **Technical Note**

# **Caffeine Content of Specialty Coffees**

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#### Abstract

Caffeine is the world's most widely consumed drug with its main source found in coffee. We evaluated the caffeine content of caffeinated and decaffeinated specialty coffee samples obtained from coffee shops. Caffeine was isolated from the coffee by liquid–liquid extraction and analyzed by gas chromatography with nitrogen-phosphorus detection. In this study, the coffees sold as decaffeinated were found to have caffeine concentrations less than 17.7 mg/dose. There was a wide range in caffeine content present in caffeinated coffees ranging from 58 to 259 mg/dose. The mean (SD) caffeine content of the brewed specialty coffees was 188 (36) mg for a 16-oz cup. Another notable find is the wide range of caffeine concentrations (259–564 mg/dose) in the same coffee beverage obtained from the same outlet on six consecutive days.

#### Introduction

Caffeine (1,3,7-trimethylxanthine) is the world's most widely consumed drug with its main source found in coffee (1). Estimates of daily caffeine consumption in the United States in 1978 indicated that approximately 200 mg was consumed daily by adults over the age of 18 with coffee accounting for about 75% of the total caffeine consumed (2). An average cup of coffee contained approximately 85 mg of caffeine; hence average consumption was slightly over two cups of coffee per day. Individual consumption varied considerably, with those in the 99th percentile consuming 563 mg of caffeine (2). This is equivalent to approximately seven cups of coffee per day.

Caffeine is rapidly absorbed in the stomach and small intestine and metabolized primarily in the liver. It is excreted in 24-h urine as dimethylxanthines, uric acid derivatives, and 2.4% caffeine (3). The plasma half-life ranges from 2.3 to 12 h (3) with caffeine plasma concentrations reaching peak levels at 45 min to 2 h after ingestion (1). The half-life of caffeine is increased for those with liver disease, in newborns, and during pregnancy (1).

Caffeine produces central nervous system stimulation and has been found to positively influence human performance. In two separate studies, in which caffeine doses of 60 mg and 100 mg were administered, caffeine affected human performance by speeding reaction time and increasing alertness (4,5). The effects of low dosage have been investigated with as little as 32 mg improving auditory vigilance and visual reaction time (6). Further, it has been suggested that caffeine might improve road safety since driving simulation tests have found that caffeine ingestion increases alertness and driving performance (7).

Although there are beneficial effects of caffeine ingestion, there may also be potentially harmful effects. There has been considerable study of the effects of caffeine on the cardiovascular system. In one study in which doses of 45–360 mg of caffeine were administered, both systolic and diastolic pressures increased, with a significant heart rate increase after the 360-mg caffeine dose (8). In another study, it was concluded that anxiety may be increased with doses of 300 mg or higher (9), and a separate study found increased anxiety with as little as 125 mg of caffeine (10). Care should be exercised when consuming caffeine with medications such as bronchodilators (both stimulate the central nervous system), quinolones (increases caffeine levels leading to excitability and nervousness), and anti-anxiety drugs (lessens effects of drug) (11).

Popularity of espresso and other specialty coffees has risen considerably over the last decade, especially for younger adults. The current study arose out of the concern that there is little awareness regarding the amount of caffeine present in these specialty coffees. Substantial variations in caffeine content arise from the variety of coffee drinks, their preparation (such as percolation, drip, or espresso) and from the geographical source of the coffee bean (12). Previous studies have been conducted to determine the mean value of caffeine content in regularly brewed coffee. Barone and Roberts (13) arrived at the standard value of 85 mg in a cup of ground-roasted coffee (150 mL, 5 oz). However, there is a paucity of information on the caffeine content of specialty coffees.

#### **Materials and Methods**

In this study (phase-one), 20 caffeinated and 7 decaffeinated specialty coffee samples were obtained from coffee shops in Severna Park, MD and Bethesda, MD. In addition (phase-two),

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samples of Starbucks<sup>®</sup> Breakfast Blend were purchased over the course of six consecutive days from a Starbucks coffee shop in Gainesville, FL. These samples were then evaluated for their caffeine content. The caffeine was extracted from the coffee using a liquid–liquid extraction procedure previously published, with some modifications (14). To 0.1 mL of coffee, 10  $\mu$ L of mepivacaine and 10  $\mu$ L of 10M NaOH was added. All samples were vortex mixed for 5 s, and 4.0 mL of chloroform (Fisher, certified ACS) was added. All samples were placed on a rotator for 15 min and then centrifuged for 10 min at 3000 rpm. The organic layer was then transferred and evaporated under a stream of nitrogen at 40°C. The residue was then reconstituted in 100  $\mu$ L of methanol, transferred to autosampler vials, and analyzed by gas chromatography (GC).

A 1.0-mg/mL caffeine (Alltech-Applied Science Labs) stock standard solution was prepared in methanol (Fisher Scientific, certified ACS). Caffeine stock control solutions were prepared in methanol from two separate sources (Alltech-Applied Science Labs and Sigma-Aldrich Company), both at concentrations of 1.0 mg/mL. A 1.0-mg/mL solution of mepivacaine (Alltech-Applied Science Labs) was prepared in methanol for use as an internal standard.

Quantitation of caffeine was based on a calibration curve prepared in a concentration range of 50–500 mg/L with mepivacaine as the internal standard. Control samples, prepared at concentrations of 75 and 250 mg/L (three each), were included in each batch.

An Agilent 6890 series GC system with a nitrogen-phosphorous detector was utilized. The GC was fitted with a cross-linked methyl siloxane capillary column (HP-5, 30 m  $\times$  0.32-mm i.d., 0.25-mm film thickness) with ultra-high-purity helium as the carrier gas (constant flow rate, 1.0 mL/min). Injections (0.5 mL) were made in the splitless mode. The GC temperature settings were as follows: injection port, 250°C; detector, 300°C; initial column temperature, 90°C; hold time, 0.50 min; and temperature ramp, 25°C/min to 320°C for 2.00 min. The total run time was 11.70 min.

#### **Results and Discussion**

The identification of caffeine was based on retention time. The concentration of caffeine was determined by linear regression with the dependent variable being the ratio of caffeine to in-

Table I. Validation Data for the Caffeine Assay						
	Concentration (mg/L)	Day	N	Mean	%CV	%Accuracy
Intra-assay	75	1	3	72.57	2.18	96.76
		2	3	71.87	5.03	95.82
		3	3	75.97	1.90	101.29
	250	1	3	242.00	1.86	96.80
		2	3	236.87	1.21	94.75
		3	3	251.37	2.47	100.55
Interassay	75	-	9	73.47	3.85	97.96
	250	-	9	243.41	3.11	97.36

ternal standard peak areas and the independent variable being the ratio of caffeine to internal standard concentrations. All batches demonstrated excellent linearity with correlation coefficients of > 0.997.

A total of three batches were extracted on three consecutive days. A total of 18 control samples were assayed at concentrations of 75 and 250 mg/L (three at each concentration) with each batch. The intra-assay and interassay mean, %CV, and %accuracy values are listed in Table I.

The results of the caffeine analyses of the caffeinated espresso and brewed coffees appear in Tables II and III. The stores where the coffees were purchased are listed, as well as the

## Table II. Caffeine Content of Espresso and BrewedSpecialty Coffees

Coffee and Origin	Amount	Caffeine Dose (mg)
Espresso coffees		
Big Bean Espresso, 1-shot	1 shot	75.8
Big Bean Espresso, 2 short shots	2 short shots	140.4
Big Bean Espresso, 2 tall shots	2 tall shots	165.3
Starbucks Espresso, regular, small	1 shot	58.1
Hampden Café Espresso	2 shots	133.5
Einstein Bros® Espresso, double	2 shots	185.0
Brewed specialty coffees		
Big Bean, regular	16 oz	164.7
Big Bean Boat Builders Blend, regular	16 oz	147.6
Big Bean Organic Peru Andes Gold, regular, country origin, Peru	16 oz	186.0
Big Bean French Roast, regular	16 oz	179.8
Big Bean Ethiopian Harrar, regular, country origin, Ethiopia	16 oz	157.1
Big Bean Italian Roast, regular, country origin, Brazil	16 oz	171.8
Big Bean Costa Rican French Roast, regular, country origin, Costa Rica	16 oz	245.1
Big Bean Kenya AA, regular, country origin, Kenya	16 oz	204.9
Big Bean Sumatra Mandheling, regular, country origin, Indonesia	16 oz	168.5
Hampden Café Guatemala Antigua	16 oz	172.7
Starbucks regular	16 oz	259.3
Royal Farms regular	16 oz	225.7
Dunkin' Donuts regular	16 oz	143.4
Einstein Bros regular	16 oz	206.3

### Table III. Caffeine Content of Starbucks Breakfast Blend (Blend of Latin American Coffees)

Day	Caffeine Dose (mg) in 16 oz	
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1	564.4	
2	498.2	
3	259.2	
4	303.3	
5	299.5	
6	307.2	

brand and the country of origin of coffee, if known. The caffeine dose (mg) was determined by measurement of the volumes of coffee sold as one shot (42 mL), two short shots (40 mL), two tall shots (57 mL), espresso double (170 mL), espresso (83 mL), espresso small (30 mL), and medium regular coffee (473 mL). The caffeine dose of brewed specialty coffees is shown for a "medium" size coffee, which is the size reported to be the most popular size purchased at the coffee shops.

In phase-one of this study, the seven coffees sold as decaffeinated were found to have caffeine concentrations less than the low point on the curve (< 17.7-mg dose). There was a wide range in caffeine content present in caffeinated coffees (58-259 mg). Generally, caffeinated espresso coffees were lower in caffeine content than caffeinated brewed coffees. The mean (SD) caffeine content of the brewed specialty coffees was 188 (36) mg for a 16-oz cup. This would equate to a mean of approximately 59 mg of caffeine in a 5-oz cup of coffee as compared to earlier reports of 85 mg of caffeine in a standard 5-oz cup of coffee. Although the amount of caffeine appears to be lower in this comparison, it appears that larger volumes of coffee, and hence, increased amounts of caffeine, may be consumed at one time. Another notable finding is the variation between brands of specialty coffees with a 16-oz cup of Big Bean<sup>™</sup> coffee containing caffeine in the range of 148 mg to 245 mg and a 16-oz cup from Starbucks containing 259 mg of caffeine. Finally, data from phase-two indicate a wide range of caffeine concentrations (259–564 mg) in the same coffee beverage (Starbucks Breakfast Blend) obtained from the same outlet on six consecutive days. The variability in the caffeine content may be due to many factors, including the variety of coffee bean, roasting method, particle size (coffee "grind"), the proportion of coffee to water used in preparation, and the length of brewing time. Clearly, even under highly standardized conditions as presumably occurred in the preparation of the Starbucks Breakfast Blend coffee, the amount of caffeine may vary day-to-day by approximately twofold.

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