A Novel Automated Liquid/Liquid Extraction Technique for the Determination of Caffeine in Coffee

Application Note

Environmental

Author

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Abstract

Coffee is a requirement for many people to start their day. However, the reason people drink coffee may be more for the caffeine than for the taste. In order to determine the amount of caffeine in coffee, many coffee producers use liquid-liquid extraction. How much caffeine is in your cup of coffee? Assorted coffee blends will be extracted for caffeine using an automated liquid-liquid extraction technique with the intention of answering this question.

Introduction:

Caffeine is a natural component of coffee, acting as a stimulant. Thus, drinking coffee can wake you up and enhance your alertness. Many people feel that they cannot function without some coffee to start their day. Furthermore, the amount of caffeine a person is receiving from their coffee can vary with the type of bean and the brewing process.

This study will examine automated extraction of caffeine from assorted coffee blends. In order to distinguish caffeine amounts from the blends, brand A will be used for examination of light, medium, dark and flavored coffees. Brands A, B, and C will be used for assessing medium blend differences. Finally, brand C will be used to investigate brewing disparities.

Discussion:

Liquid-liquid extraction takes advantage of a compound's solubility in different solvents. Since caffeine is more soluble methylene chloride it diffuses readily from the coffee into the methylene chloride. Additionally, methylene chloride is denser than water, so the extracted caffeine separates into the bottom methylene chloride portion of the liquid system. Using the FLEX autosampler, an automated liquid-liquid extraction was performed. The FLEX added methylene chloride to a coffee sample, mixed the sample, let it settle and injected the extracted caffeine onto the GC.

Experimental:

The sampling system used for this analysis was the EST Analytical FLEX autosampler fitted with a 250µl liquid syringe. A Restek Rxi-5 Sil MS 30m x 250mm x 0.25μm column was installed in the GC. The Agilent 7890 GC and 5975 MS were employed for separation and analysis. Furthermore, the GC inlet was equipped with the Titan PTV LVI for sample injections. Refer to Tables 1 and 2 for the sampling and analysis parameters

Autosampler	Flex
General	
Method Type	Liquid
Sample Preparation (Run Twice)	
Rinse Volume	100% (250µl)
Rinse Fill Rate	100%
Rinse Cycles	2
Rinse Dispense Rate	100%
Solvent Pump Cycles	1
Solvent Pump Volume	100% (250µl)
Pump Dispense Rate	100%
Solvent Volume	100% (250µl)
Solvent Fill Rate	2%
Solvent Fill Delay	0 sec
Sample Vial Needle Depth	100%
Incubate/Agitate	
Incubation Temperature	25°C
Incubation Time	5.1 min
Agitate	Yes
Agitation Time	5.0 min
Agitation Delay	0.1 min
Agitation Speed	100%
Ambient Equilibration Time	5.0 min
Rinse	
Rinse Volume	8% (20µl)
Rinse Fill Rate	100%
Rinse Cycles	2
Sample	
Sample Volume	4% (10µl)
Sample Depth	100%
Sample Depth Speed	90%
Sample Fill Rate	1%
Sample Fill Delay	5 sec
Sample Rinse Volume	8% (20µl)
Sample Rinse Cycles	1%
Sample Pump Volume	8% (20µl)
Sample Pump Cycles	2
Dispense Rate	50%
Air Volume Gap	
Air Fill Volume	4% (10µl)
Single Injection Port	
Injection Rate	100%
Injection Volume	8% (20µl)
Pre-Injection Delay	1 sec
Post-Injection Delay	1 sec
Rinse	
Rinse Volume	8% (20µl)
Rinse Fill Rate	100%
Rinse Cycles	2

Table 1: FLEX Autosampler Experimental Parameters

GC/MS	Agilent 7890/5975
Inlet	Titan LVI PTV
Inlet Temp.	45°C for 0.15 min, 500°C/min to 325°C for 14min
Inlet Head Pressure	14.956 psi
Split	250:1
Split Flow	350ml/min
Mode	Pulsed Split
Injection Pulse Pressure	25psi until 0.05 min
Cryo	On
Liner	TITAN XL SB Deactivated Baffled Liner
Column	Rxi-5Sil MS 30m x 0.25mm I.D. x 0.25μm film thickness
Oven Temp. Program	40°C hold for 1.5 min, ramp 25°C/min to 310°C hold for 1.7 min, 14 min run time
Column Flow Rate	1.4ml/min.
Gas	Helium
Total Flow	354.4ml/min
Source Temp.	230°C
Quad Temp.	150°C
MS Transfer Line Temp.	280°C
Solvent Delay	3.0 min
Acquisition Mode	Scan
Scan Range	m/z 40-500
Sampling Rate	3.12 scans/sec

Table 2: GC/MS Experimental Parameters

Pure caffeine was acquired from Sigma Aldrich and diluted in water in order to make a 5000ppm standard. Next a calibration curve was prepared in order to calibrate the extraction of caffeine from a water matrix. Single cup coffee servings in assorted blends, flavors and brands were purchased. The assorted coffees were prepared and two milliliters of each coffee was measured and placed in mini reaction vials. The vials of coffee were then positioned in the FLEX sample tray for automated extraction. Coffee samples were run in triplicate in order to ensure reproducibility. Extraction results are listed in Table 3; Figures 1 through 3 displays the results in bar graph format while Figures 4 through 6 shows a comparison of the coffee chromatograms.

Coffee	Caffeine Amount (mg/Cup)
Decaf	27.41
Morning Roast	96.53
Medium Roast A	110.57
Dark Roast	110.84
Vanilla Flavored	116.54
Mocha Flavored	119.84
Medium Roast B	123.54
Medium Roast C	102.63
Brewed Cup C	181.44

Table 3: Extraction Results

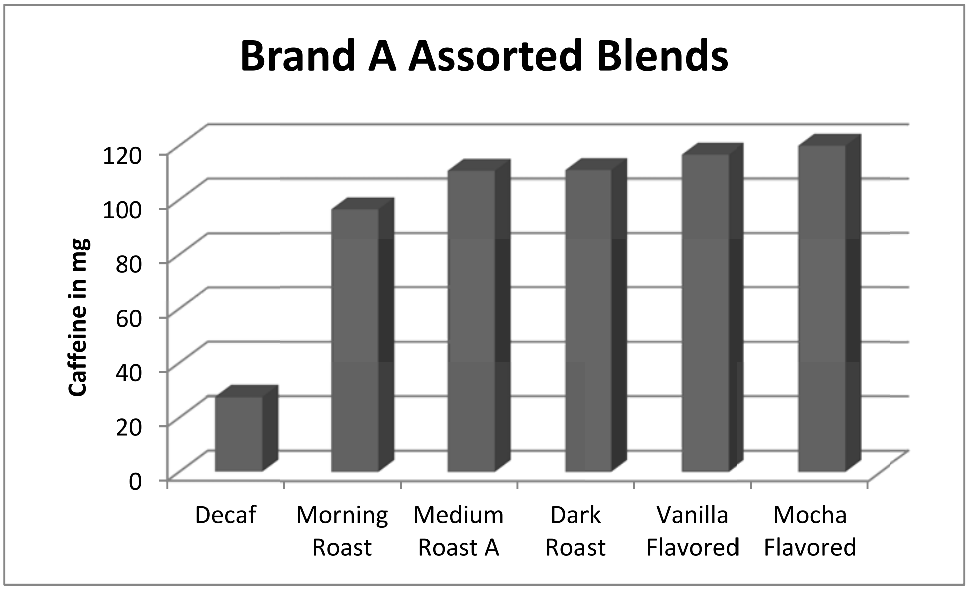

Figure 1: Caffeine Comparison of Different Blends

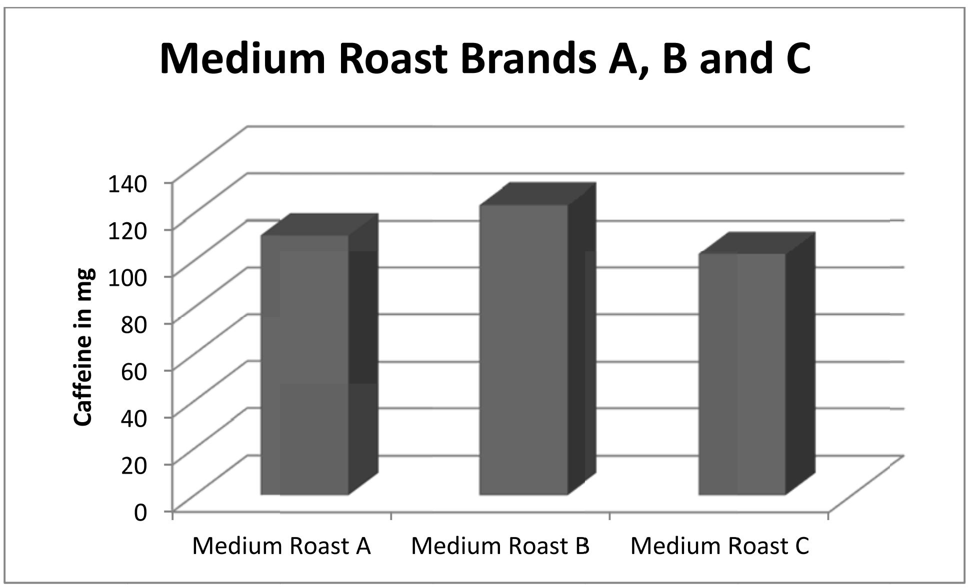

Figure 2: Caffeine Comparison of Different Brands

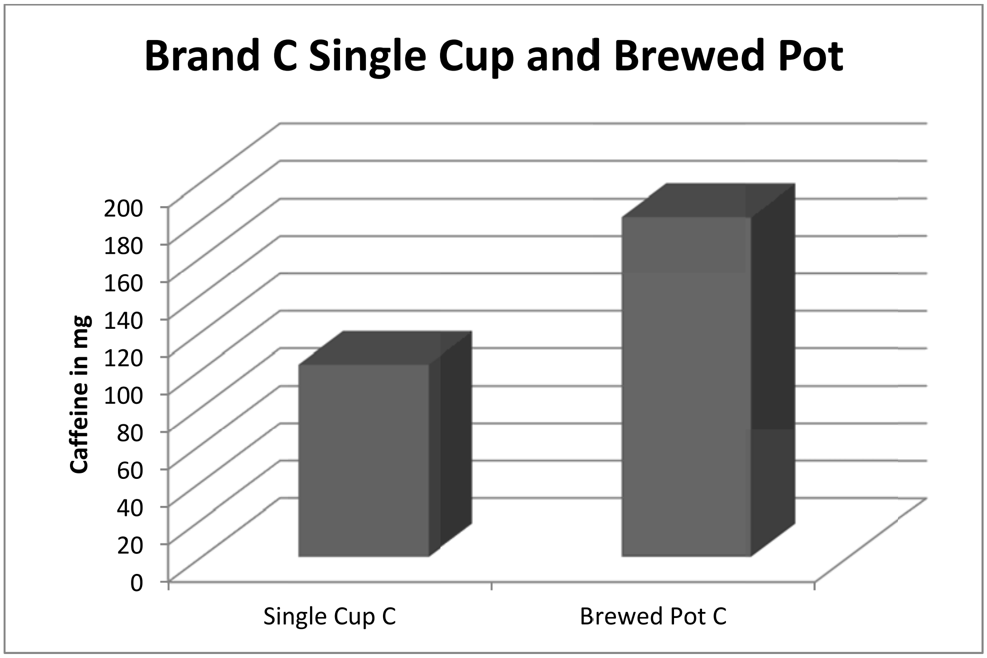

Figure 3: Caffeine Comparison of a Single Cup Brew versus a Brewed Pot of Coffee

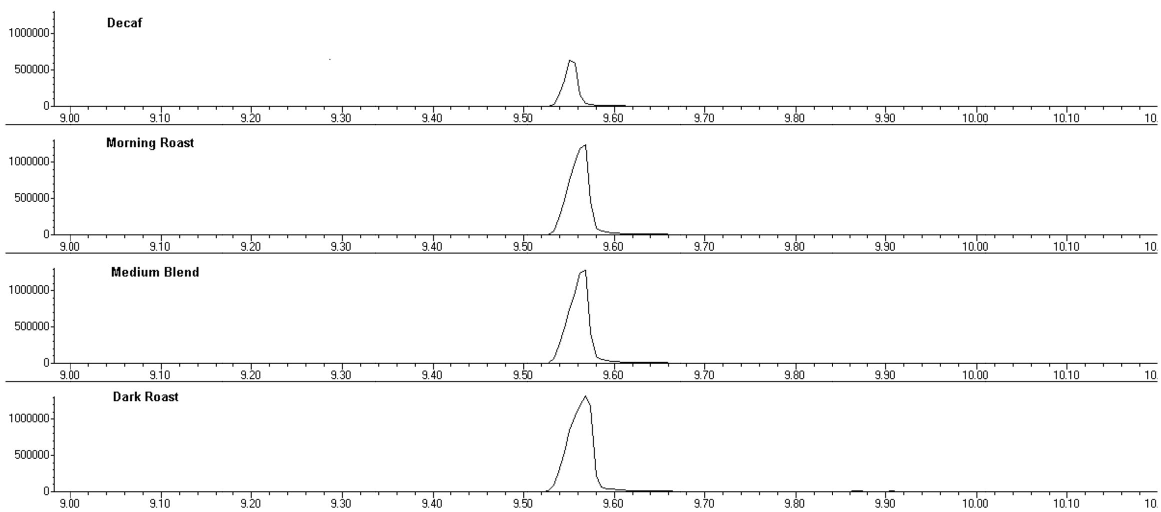

Figure 4: Chromatograms of Different Roasts of Brand A

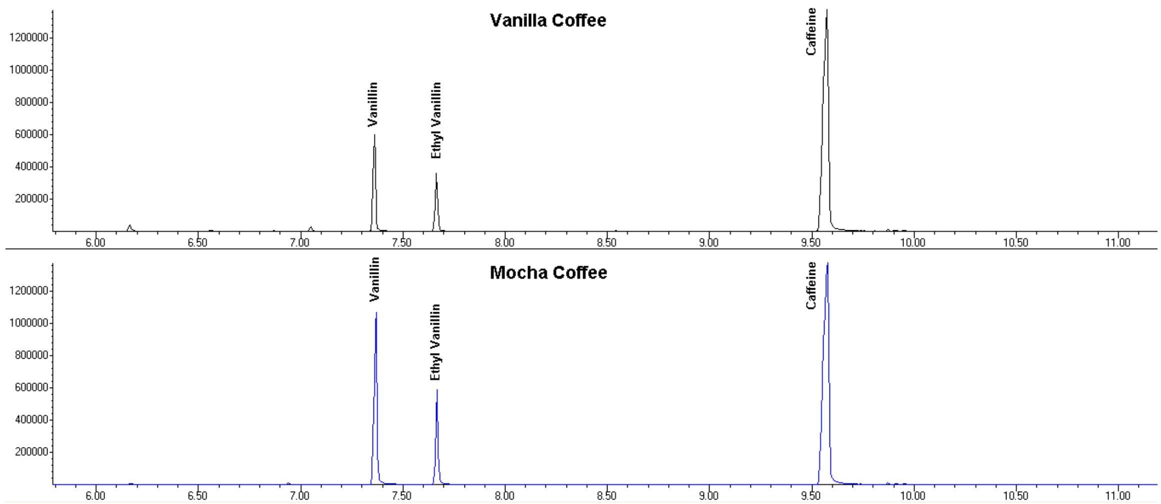

Figure 5: Chromatograms of Mocha Coffee versus Vanilla Coffee

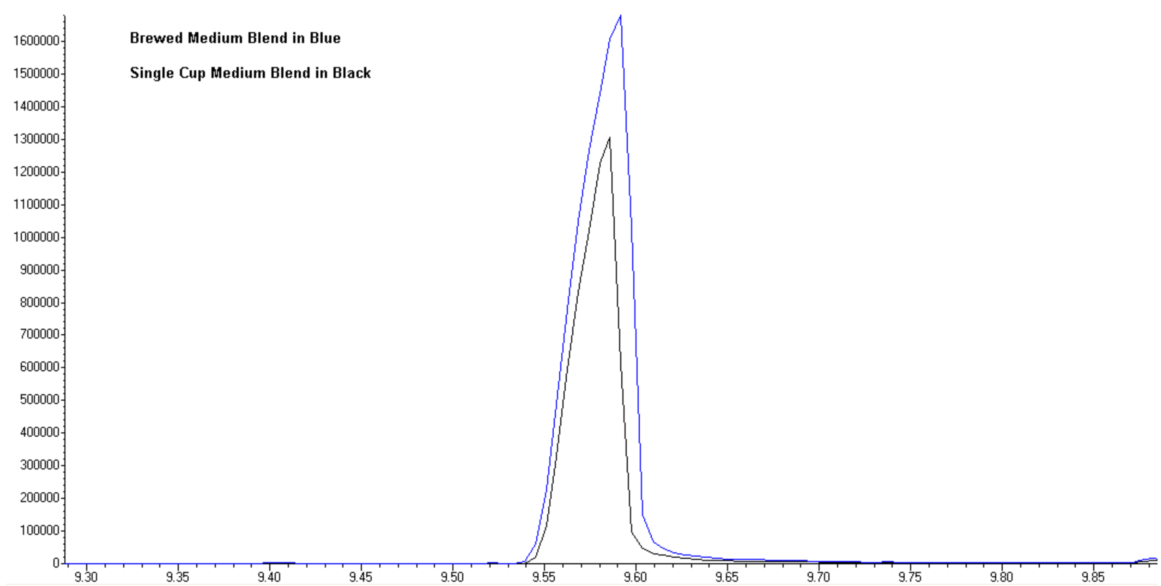

Figure 6: Overlay of Caffeine Extracted from a Single Cup versus a Pot of Coffee

Conclusions:

When analyzing the amount of caffeine in the same brand, the amounts were quite similar. Surprisingly, the dark coffee blend had the same amount of caffeine as the medium blend. The mocha flavor had the most caffeine of Brand A which was expected with the added caffeine in chocolate. The amount of caffeine in the medium roast did not differ much from brand to brand with Brand C having the least and Brand B having the most. The most marked difference between the coffees was found when comparing a single cup brew versus a pot of brewed coffee. The pot of brewed coffee had substantially more caffeine than the single cup. This was probably due to the amount of coffee used to brew the pot as compared to the controlled amount of coffee in a single cup. Finally, the extraction also proved to be quite efficient in removing vanillin and ethyl vanillin from the vanilla and mocha flavored coffees. These results displayed the mocha coffee to have more of the vanilla flavoring than the vanilla coffee.

Using the FLEX system, automated extraction proved to be an easy and accurate process. The caffeine extraction was efficient with the parameters established and the results were reproducible throughout the study.

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