# Determination of Caffeine in Coffee Products According to DIN 20481

Suitable for Agilent
1260 Infinity III LC

## Application Note

Food Testing & Agriculture – Food Authenticity


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### Abstract

This Application Note demonstrates the determination of caffeine in coffee products according to DIN 20481, which is part of a series of quality control measurements of coffee products. The performance of the system is shown for linearity, retention time, and area precision as well as accuracy. The performance is also shown on solvent saver columns with reduced id.


### Introduction

Today, coffee is the second most valuable product (besides crude oil) exported from developing countries, with a trading volume of approximately 22 billion US $. In 2007, the world coffee production was approximately 7,742,675 tons, with Brasilia as the largest producer of approximately 28 %. On the consumer side, the USA has the highest total consumption and Finland the highest consumption per person. One of the main ingredients of coffee is the alkaloid caffeine, with approximately 80–120 mg per cup. Caffeine is responsible for the stimulating effect of coffee. Decaffeinated coffee is produced in large amounts by extraction of the caffeine from green coffee beans with hot water, organic solvents, or supercritical carbon dioxide[1]. Decaffeinated coffee must contain less than 0.1 % caffeine[2].

In the European Union, only beverages that do not typically contain caffeine, for example energy drinks, must be labeled with the amount of caffeine the beverages contain.

The measurement of caffeine in coffee products is standardized in the DIN ISO regulations[3]. Besides caffeine, other important compounds inherent in coffee have to be controlled like chlorogenic acids[4,5], 16-O-methyl cafestol[6,7] and contaminants such as mycotoxins[8,9].

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**1260 Infinity II LC**

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## Experimental

### Equipment

Agilent 1260 Infinity LC System:

- Agilent 1260 Infinity Binary Pump (G1312B) with external degasser (G1322A)
- Agilent 1260 Infinity Standard Autosampler (G1329B) with Sample Thermostat (G1330B)
- Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)
- Agilent 1260 Infinity Diode Array Detector (G4212B) with a 10-mm flow cell (G4212-60008)

### Software

- Agilent OpenLAB CDS ChemStation Edition for LC & LC/MS Systems, Rev. C.01.05

### Columns

1. Agilent ZORBAX Eclipse Plus, 4.6 × 150 mm, 5 µm (p/n 959993-902)
2. Agilent Poroshell 120 EC-C18, 3.0 × 150 mm, 2.7 µm (p/n 693975-302)
3. Agilent Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm (p/n 699975-302)

### Chemicals

All Chemicals were purchased from Sigma/Aldrich, Germany. Methanol was purchased from Merck, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak). Regular and decaffeinated instant coffee was purchased from a local super market.

### HPLC method

| Parameter | Value |
|---|---|
| Solvents | A) Water<br>B) Methanol |
| Flow rate | 1.0 mL/min with Column 1,<br>0.43 mL/min with Column 2 and Column 3,<br>0.86 mL/min with Column 3 |
| Elution conditions | Isocratic, 25 % methanol |
| Stop time | 12 minutes |
| Injection volume | 10 µL with Column 1,<br>4.3 µL with Column 2,<br>1.4 µL with Column 3 |
| Needle wash | In vial with methanol |
| Column temperature | 25 °C |
| Detection | 272 nm<br>Bandwidth 4 nm; Reference: 360 nm<br>Bandwidth 16 nm<br>Data rate 10 Hz |

### Standards

Caffeine stock solution: 200 mg caffeine (water free) was dissolved in 500 mL warm water in a 1-L volumetric flask and filled with water to 1 L after cooling down to room temperature. A 1/10 and a 1/50 dilution was used as starting concentration for the calibrations.

### Sample preparation

Instant coffee (0.5 g) and 5 g MgO were combined in 200 mL water at 90 °C and stirred for 20 minutes at 90 °C in a water bath. After removal from the water bath and cooling down to room temperature, a part of the liquid was filtrated through a cellulose syringe filter (Agilent Captiva Premium Syringe Filter, Regenerated Cellulose, 0.45 µm, 25 mm, p/n 5190-5111). The filtered extract from the decaffeinated product was used directly for injection and the extract from regular coffee after a 1:10 dilution.

## Results and Discussion

The content of caffeine in coffee initially depends on the biological coffee plant species. For instance, the species *Coffea arabica* contains about half of the species *Coffea robusta*, the first is approximately 60 % of the world production and the second 36 %. For the measurement of the content of caffeine in regular coffee and decaffeinated coffee products, two calibrations in the typical range were created. For regular coffee, the calibration was done from 1.25 mg/L to 20 mg/L and for decaffeinated and caffeine-reduced coffee products, the calibration was done from 0.125 mg/L to 4 mg/L (Figure 1).

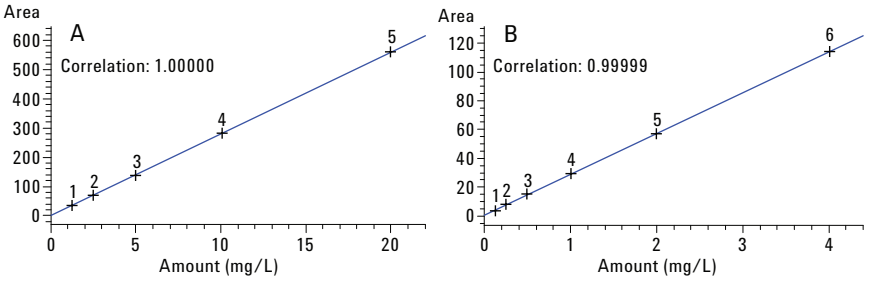


Figure 1. Calibration curves for caffeine, A) calibration for the concentration range 1.25 –20 mg/L. B) Calibration for the concentration range 0.125–4 mg/L.

Both calibrations show excellent linearity. The limit of quantification (LOQ) was found at 0.113 mg/L and the limit of detection (LOD) at 0.034 mg/L. Under the chosen HPLC conditions, caffeine eluted at 6.7 minutes and an overlay of the injected concentrations from each calibration shows good peak shapes for all concentrations and retention time conformance (Figure 2).

To demonstrate the performance, a statistical evaluation was done by multiple injection (n = 10) of the caffeine concentration level at 10 mg/L for the higher concentration calibration and at 1 mg/L for the lower level caffeine concentration calibration (Table 1).

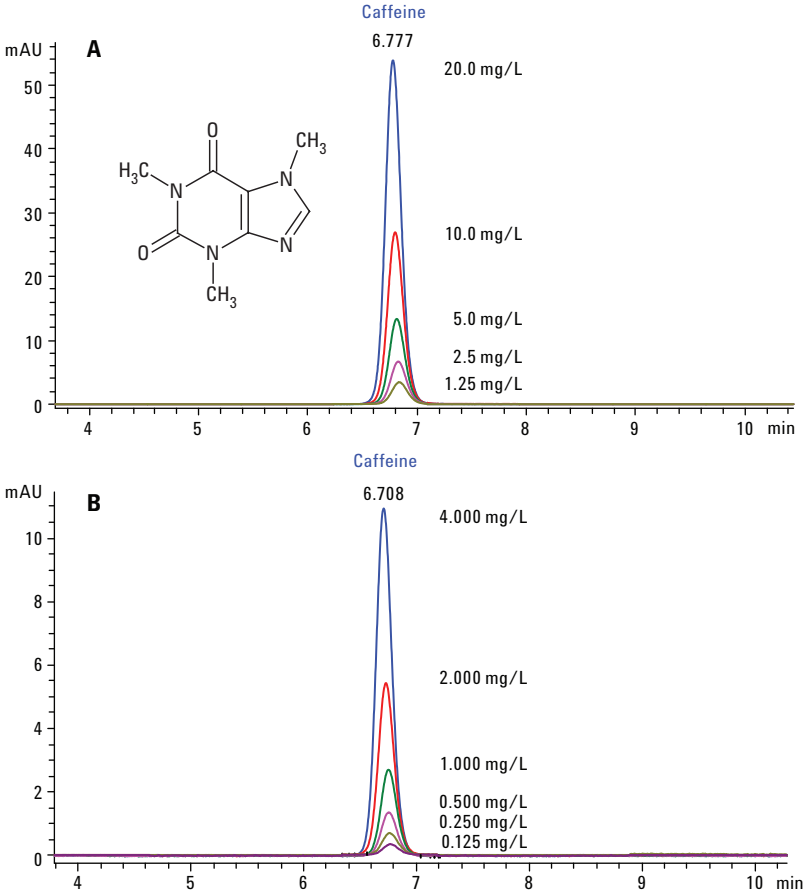


Figure 2. Overlay of caffeine peaks of different concentrations used as calibration levels. A) Caffeine concentrations, 1.25–20 mg/L. B) Caffeine concentrations 0.125–4 mg/L.

Table 1. Performance data measured for 10 mg/L and 1mg/L of caffeine with the Agilent ZORBAX Eclipse Plus C18, 4.5 × 150 mm column as well as concentration precision and accuracy.

| Parameter | Value | |
|---|---|---|
| Column | Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm | |
| | Caffeine 10 mg/L | Caffeine 1 mg/L |
| r.t. (min) | 6.805 | 6.755 |
| r.t. RSD (%) | 0.06 | 0.10 |
| area RSD (%) | 0.20 | 0.40 |
| Calibration | 1.25–20.0 mg/L | 0.125–4.0 mg/L |
| $R^2$ | 1.0000 | 0.9999 |
| LOD | 0.034 mg/L | |
| LOQ | 0.113 mg/L | |
| Carryover | from 20.0 mg/L - n.d. | |
| Concentration precision | 0.14 % at 12.0 mg/L | |
| Concentration accuracy | 101.03 % at 12.0 mg/L | |

The retention time RSD and area RSD were at 0.1 % and 0.4 % for the lower concentration, respectively. For the determination of carryover, the highest concentration used from the calibration was injected and followed by a blank injection. In this blank, no caffeine carryover could be detected (Figure 3). The concentration precision and accuracy were measured for repeated injection (n = 10) of 12 mg/L caffeine. The precision was determined to be 0.14 % and concentration accuracy was found to be 101.4 %.

The analysis according to the description in the DIN ISO Norm was done under conventional HPLC conditions at a flow rate of 1 mL/min with a 4.6-mm id column. To save solvent and costs, the described calibration and statistical evaluation was repeated with a solvent saver column of the same length but with a 3.0-mm id at a flow rate of 0.43 mL/min (Table 2). Retention time and area RSD as well as linearity were in the same range as found for the 4.6-mm id column. In contrast to the conventional columns, a lower LOQ and LOD were found with the solvent saver column at 0.051 mg/L and 0.015 mg/L, respectively. This effect was due to the higher separation power of this type of column with its 2.7-µm fused core shell particles delivering higher and sharper peaks yielding improved signal-to-noise performance.

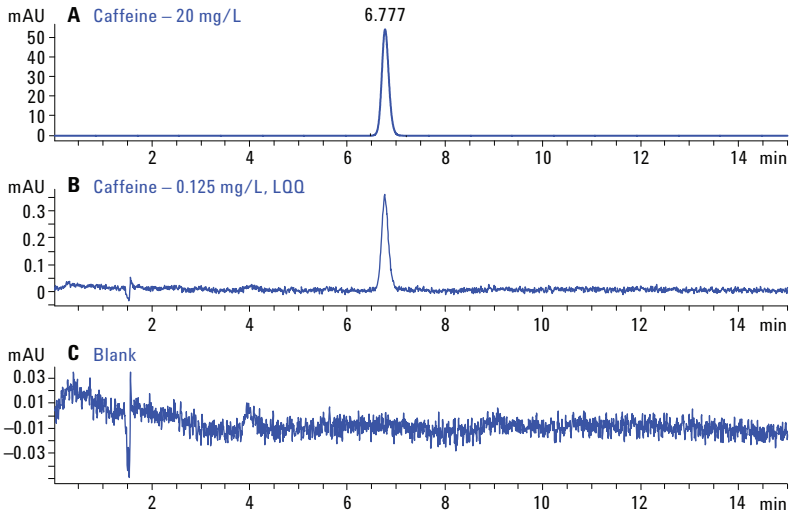


Figure 3. Determination of carryover of caffeine for the maximum concentration used. A) Maximum concentration of caffeine at 20 mg/L. B) Lowest level of caffeine used for calibration at 0.125 mg/L (LOQ = 0.051 mg/L), as comparison. C) Blank injection following maximum caffeine concentration injection showing no carry over.

Table 2. Performance data measured for 10 mg/L and 1 mg/L of caffeine with the Agilent Poroshell 120 EC-C18, 3.0 × 150 mm column as well as concentration precision and accuracy.

| Parameter | Value | |
|---|---|---|
| Column | Agilent Poroshell 120 EC-C18, 3.0 × 150 mm, | |
| | Caffeine 10 mg/L | Caffeine 1 mg/L |
| r.t. (min) | 6.005 | 5.810 |
| r.t. RSD (%) | 0.10 | 0.12 |
| area RSD (%) | 0.11 | 0.31 |
| Calibration | 1.25–20.0 mg/L | 0.125–4.0 mg/L |
| $R^2$ | 1.0000 | 0.9999 |
| LOD | 0.015 mg/L | |
| LOQ | 0.051 mg/L | |
| Carryover | from 20.0 mg/L - n.d. | |
| Concentration precision | 0.17 % at 12.0 mg/L | |
| Concentration accuracy | 100.26 % at 12.0 mg/L | |

Finally, a sample of regular instant coffee and decaffeinated instant coffee were analyzed (Figures 4 and 5). A content based on used instant coffee granules of less than 0.1 % caffeine was found for the decaffeinated product (Figure 4). This is in accordance with the regulation for decaffeinated coffee products to be below 0.1 % caffeine. The regular coffee product contained approximately 2 % of caffeine based on the used instant coffee granules (Figure 5).

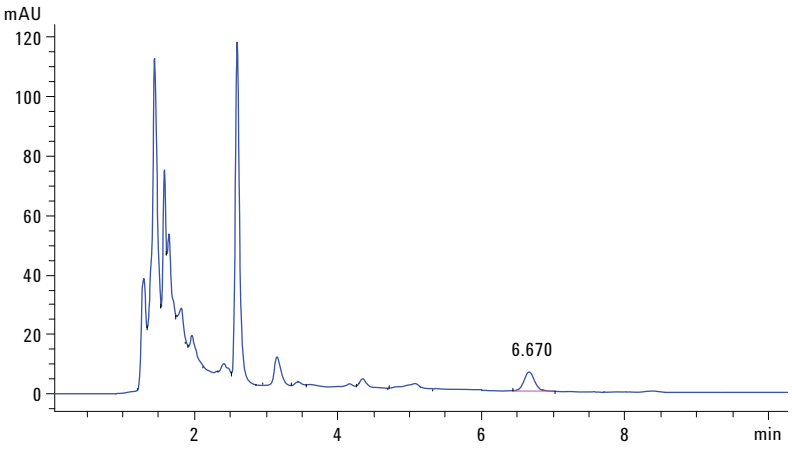


Figure 4. Determination of caffeine in decaffeinated instant coffee. 2.3 mg/L from 0.5 g in 200 mL yields 0.92 mg/g instant coffee granules, < 0.1 %.

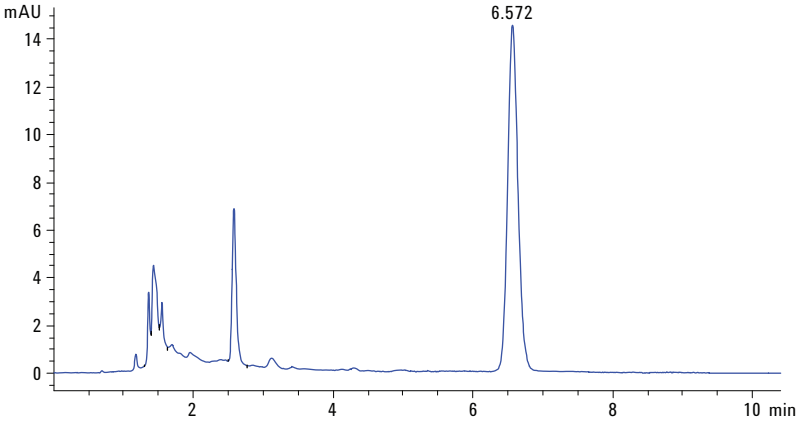


Figure 5. Determination of caffeine in regular instant coffee. 5.34 mg/L from 0.5 g in 200 mL (1:10 dilution) yields 21.36 mg/g instant coffee powder, approximately 2 %.

To improve productivity, the 150 mm Poroshell column was replaced by a 3.0 × 50 mm Poroshell column, which allowed finishing the separation in one third of the time, 4 minutes, improving sample throughput three times (Figure 6). A further improvement of the throughput could be achieved by a doubling of the solvent flow, leading to a total run time of 2 minutes.

## Conclusion

This Application Note demonstrates the use of a standard HPLC to determine caffeine in coffee products according to the DIN ISO 20481. The linearity of the calibration curves in the concentration ranges for the determination of caffeine in regular coffee and decaffeinated coffee products is excellent, as well as the RSD values for retention time and area. It was shown that comparable results with even lower LOD and LOQ can be achieved by means of solvent a saver column on the same instrument with 57 % less solvent consumed.

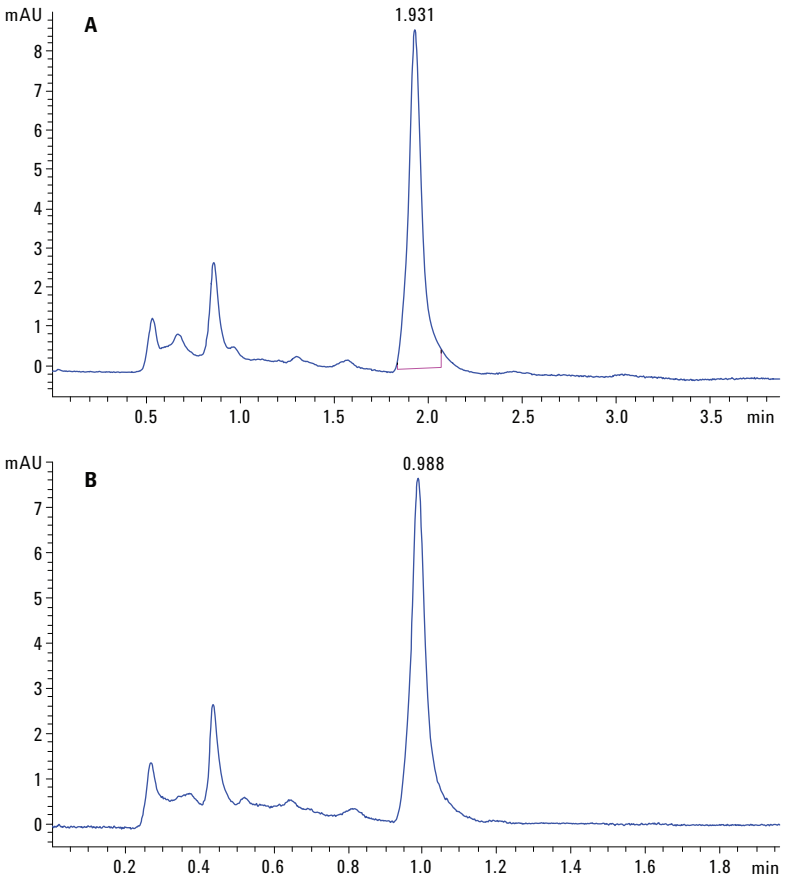


Figure 6. Improved efficiency by means of a shorter column (3.0 × 50 mm, 2.7 µm at 0.43 mL/min) and higher flow rates. A) Reduction of column length to one third reduces the elution time of caffeine to 1.93 minutes, run time to 4 minutes, and increases sample throughput three times. B) Doubling the flow rate to 0.86 mL/min reduces the run time to 2 minutes and the elution time of caffeine to 0.988 minutes.

## References


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2. Guidelines of the European Commission about labeling of quinine and caffeine containing beverages, 18. July **2002**.
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DE84992766





Published in the USA, October 15, 2024
5991-2851EN


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