Determination of Methylcafestol in Roasted Coffee Products According to DIN 10779

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 16-O-methyl cafestol in roasted coffee products according to DIN 10779, which is part of a series of quality control measurements of coffee products. The performance of the system shows linearity, retention time, area precision, and accuracy. The performance is also shown on solvent saver columns with reduced inner diameter. The sample preparation procedure is described and the analysis of a real sample is shown.

Verified for Agilent
1260 Infinity II LC

Agilent Technologies

Introduction

Chemically, cafestol belongs to the group of diterpenes, which naturally occur in coffee beans. Typically, it is present in coffee beans up to 0.06 % and bound as an ester of fatty acids. These compounds and cafestol itself are degraded during the roasting process, and the content of cafestol in the final roasted coffee product depends on the roasting process. Cafestol is soluble in water only in minor amounts and is present in highest quantities in French press coffee or Turkish/Greek coffee. In filtered coffee, it is present in only negligible amounts[1].

Biologically, cafestol has an increasing effect on the serum cholesterol level. Conversely, there are studies, showing anti-carcinogenic, anti-inflammatory and anti-genotoxic effects of cafestol[2].

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

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 10-mm path length flow cell	
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)	
HPLC method	
Solvents	A) Water B) Acetonitrile
Flow rate	1.0 mL/min with Column 1 0.43 mL/min with Column 2 and Column 3 0.86 mL/min and 1.72 mL/min with Column 3
Elution conditions	Isocratic, 50 % acetonitrile
Stop time	30 minutes
Injection volume	20 µL with Column 1 8.6 µL with Column 2 2.8 µL with column 3
Sample temperature	8 °C
Needle wash	In vial with acetonitrile.
Column temperature	25 °C
Detection	220 nm, bandwidth 4 nm, Ref. 360 nm, bandwidth 80 nm, 10 Hz

Chemicals

All chemicals were purchased from Sigma-Aldrich, Germany. Acetonitrile 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 roasted coffee was purchased from a local supermarket.

Standards

Cafestol stock solution: 2 mg/10 mL acetonitrile (200 ppm). A 100 ppm dissolution in acetonitrile/water (50/50, v/v) was used as stock solution for the calibration. A 1:2 dilution pattern was used to generate the concentration levels for the calibration.

Sample preparation

Extraction

A 5-g amount of roasted coffee was mixed with 20 g of sodium sulfate and extracted for 5 hours in a Soxhlet extractor with *tert*-butyl methyl ether (tBME) at 4–5 cycles per hour. After the extraction, the tBME was removed in vacuum and the residue was dissolved in 50 mL of tBME in a volumetric flask.

Saponification of coffee lipids

A 20-mL amount of the obtained solution was transferred to a round bottom flask and the tBME was removed. The residue was dissolved in 40 mL KOH/ethanol (10 g KOH, 100 mL water, and 900 mL EtOH). After adding approximately 25 mg sodium ascorbate and boiling chips, the solution was refluxed for 2 hours. After the saponification, the solvent was removed in vacuum and the residue was dissolved in approximately 80 mL of water at 70 °C and transferred to a separation funnel. The round bottom flask was cleaned with 25 mL MeOH and transferred into the separation funnel together with 20 mL 10 % NaCl. This solution was extracted twice with 100 mL tBME for 3 minutes each time. After separation of the phases, the tBME phases were collected. The combined tBME was washed with 100 mL of 2 % NaCl and dried with sodium sulfate. The tBME was removed in vacuum to dryness. The residue was dissolved in 20 mL methylene chloride in a volumetric flask. A 2-mL amount of this solution was evaporated under a nitrogen gas stream and the residue was dissolved in 2 mL water/acetonitrile 50/50. After membrane filtration, it was used for injection.

Results and Discussion

Method performance

Starting with the 100 ppm standard solution, a calibration curve was created over eight concentration levels using a 1:2 dilution pattern down to 781.25 ppb on the standard 4.6 × 150 mm column under standard HPLC conditions at a 1 mL/min flow rate and 20-µL injection. Cafestol eluted at 18.00 minutes (Figure 1). The calibration showed excellent linearity with a coefficient of 0.99993 (Figure 2). The limit-of-quantification (LOQ) was calculated for a signal-to-noise ratio (S/N) of 10 to be 580 ppb and the limit of detection (LOD) was calculated for a S/N of 3 to be 170 ppb.

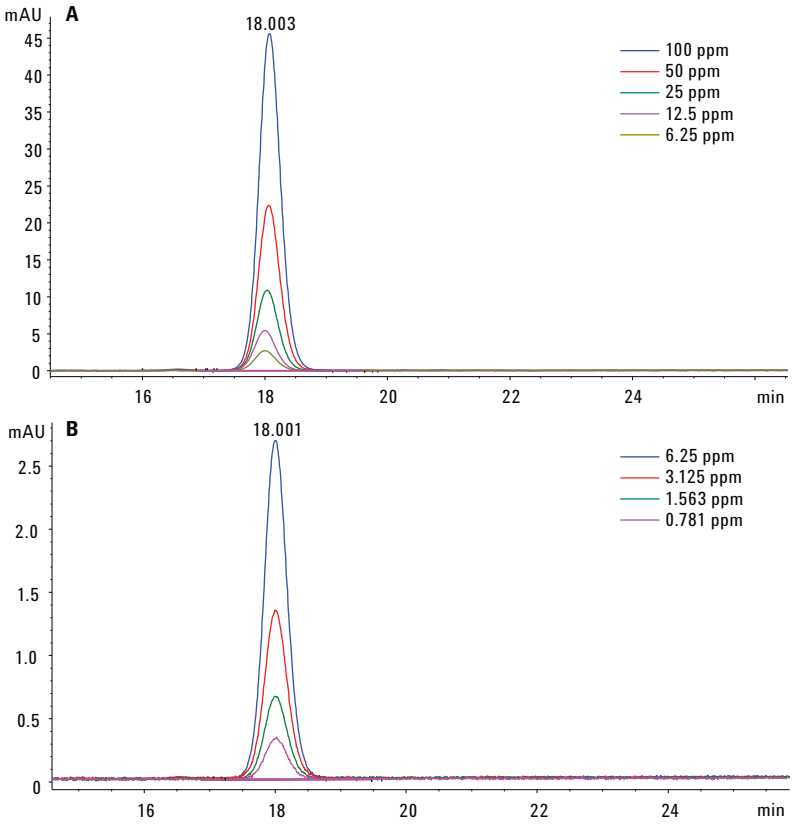

Figure 1. Overlay of 16-O-methyl cafestol peaks of different concentrations used as calibration levels. A) Concentrations 6.25–100 mg/L. B) Concentrations 0.7813–6.25 mg/L.

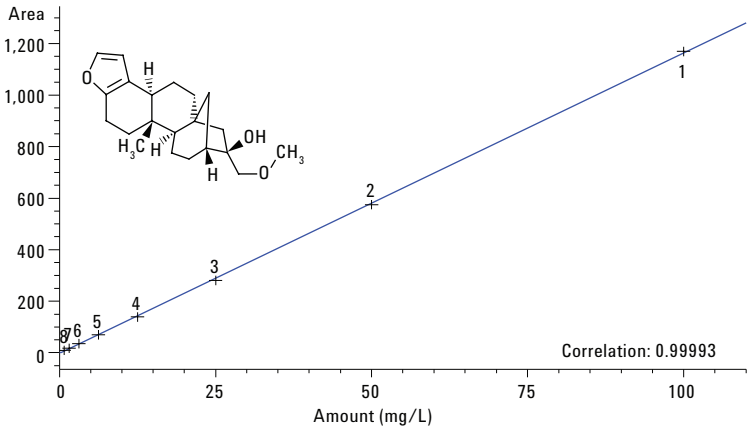

Figure 2. Calibration curve for 16-O-methyl cafestol for the concentration range 0.781–100 mg/L.

A statistical evaluation of the analytical method was done by multiple injections of the 25 ppm concentration level. Table 1A shows that a retention time RSD of 0.05 %, and an area RDS of 0.15 % were found.

To determine the method accuracy, a dilution of 20 mg/mL was used and injected multiple times. For the measured concentrations, a precision RSD of 0.29 % and for the concentration accuracy 90.6 % were found.

Table 1A. Performance data measured for 25 mg/L cafestol with the Agilent ZORBAX Eclipse Plus 4.6 × 150 mm, 5 µm column as well as concentration precision and accuracy for 20 mg/mL.

Parameter	Value
Column	Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm
Sample	Cafestol 25 mg/L
RT (min)	18.00
RT RSD (%)	0.05
Area RSD (%)	0.15
Calibration	0.781–100.0 mg/L
Linearity, R^2	0.99993
LOD	0.17 mg/L
LOQ	0.58 mg/L
Carryover	from 100.0 mg/L–n.d.
Concentration precision	0.29 % at 20.0 mg/L
Concentration accuracy	90.6 % at 20.0 mg/L

Table 1B. Performance data measured for 25 mg/L cafestol with the Agilent Poroshell 120 EC-C18, 3.0 × 150 mm, 2.7 µm column as well as concentration precision and accuracy for 20 mg/mL.

Parameter	Value
Column	Agilent Poroshell 120 EC-C18, 3.0 × 150 mm, 2.7 µm
Sample	Cafestol 25 mg/L
RT (min)	16.65
RT RSD (%)	0.20
area RSD (%)	0.34
Calibration	0.781–100 mg/L
Linearity, R^2	0.99995
LOD	0.12 mg/L
LOQ	0.38 mg/L
Carryover	from 100.0 mg/L–n.d.
Concentration precision	0.31 % at 20.0 mg/L
Concentration accuracy	92.0 % at 20.0 mg/L

To determine carryover, the 100-ppm solution was injected followed by a blank solvent injection. No carryover was detected from the highest concentration level of the calibration to the following blank (Figure 3).

Analysis of an actual live sample

To show an actual example with enriched content of cafestol, a commercially available roasted coffee was treated as described in the sample preparation section. This sample was measured on both Columns 1 and 2 as described in the method section. The content of cafestol in the roasted coffee in Column 1 was determined using the previously created calibration. The roasted coffee sample contained approximately 50 mg/kg cafestol (Figure 4A). The measurement with the solvent saver Column 2, containing a comparable stationary phase with the 2.7-µm fused core shell particles, delivered better separation performance with higher and narrower peaks at less than half of the solvent consumption (Figure 4B).

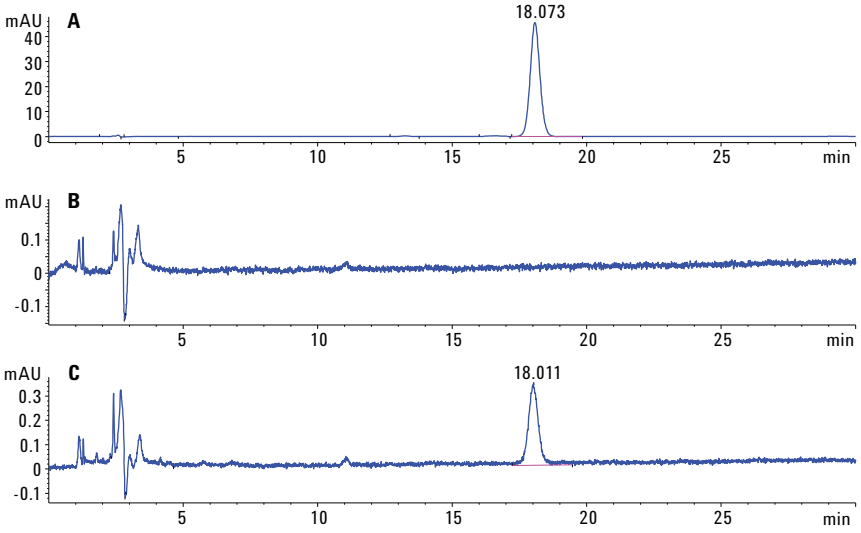

Figure 3. Determination of carryover of 16-O-methyl cafestol for the maximum concentration used. A) Maximum concentration of cafestol at 100 mg/L. B) Blank injection following maximum cafestol concentration injection showing no carry over. C) Lowest calibration concentration of cafestol (LOQ = 0.58 mg/mL) at 0.781 mg/L, as comparison.

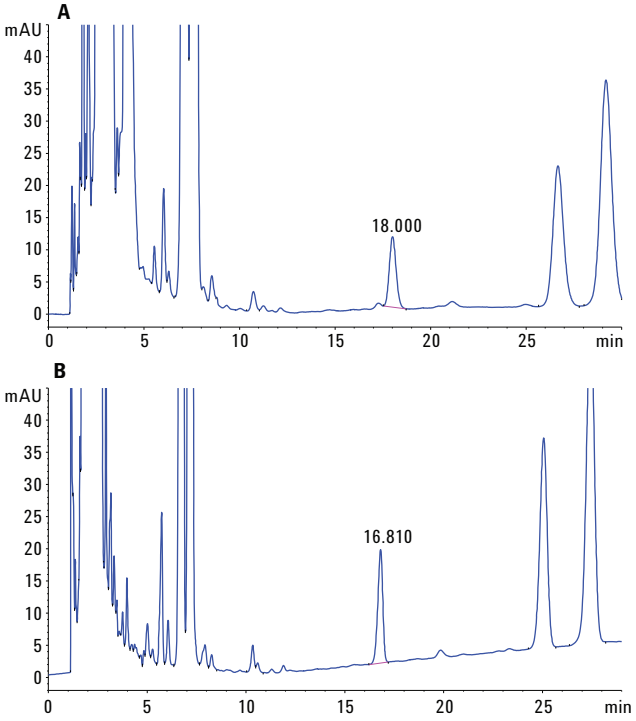

Figure 4. Determination of 16-O-methyl cafestol in roasted coffee according to the described sample preparation. According to the calibration the sample solution had a concentration of 24.2 ppm of caftestol. Calculating the volume and extracted amount the roasted coffee sample contains about 50 mg/kg of cafestol. A) Column 1: 1 mL/min, 20 µL injection. B) Column 2: 0.43 mL/min, 8.6 µL injection.

Optimizing sample throughput

The above described experiments were repeated with a Poroshell 120 EC-C18, 3.0 × 150 mm, 2.7 µm solvent saver column. The flow rate and the injection volume were adjusted according to the narrower id of this column to 0.43 mL/min and 8.6 µL, respectively.

For the calibration, similar linearity was found but the LOQ and LOD were lower on the 2.7-µm solvent saver column. This was due to the better separation performance, showing narrower and sharper peaks with improved S/N enabled by the 2.7-µm fused core shell particles used in this column (Figure 4B). Other statistical performance parameters like retention time and area RSDs as well as concentration precision and accuracy were in the same order for both columns. The advantage of Column 2 with the lower id is the solvent consumption, which was 57 % lower than Column 1.

To improve the analysis efficiency, the 150-mm column was exchanged with a 3.0 × 50 mm column with the identical stationary phase. This immediately allowed a reduction of the run time to approximately one third and improved the throughput by a factor of three (Figure 5A). Further improvement was achieved by doubling the flow rate to 0.86 mL/min, which reduced the run time to 5 minutes and the elution time of cafestol to 2.888 minutes (Figure 5B). With a flow rate of 1.72 mL/min, the total run time was reduced to 2.7 minutes and the elution time of cafestol to 1.559 minutes (Figure 5C).

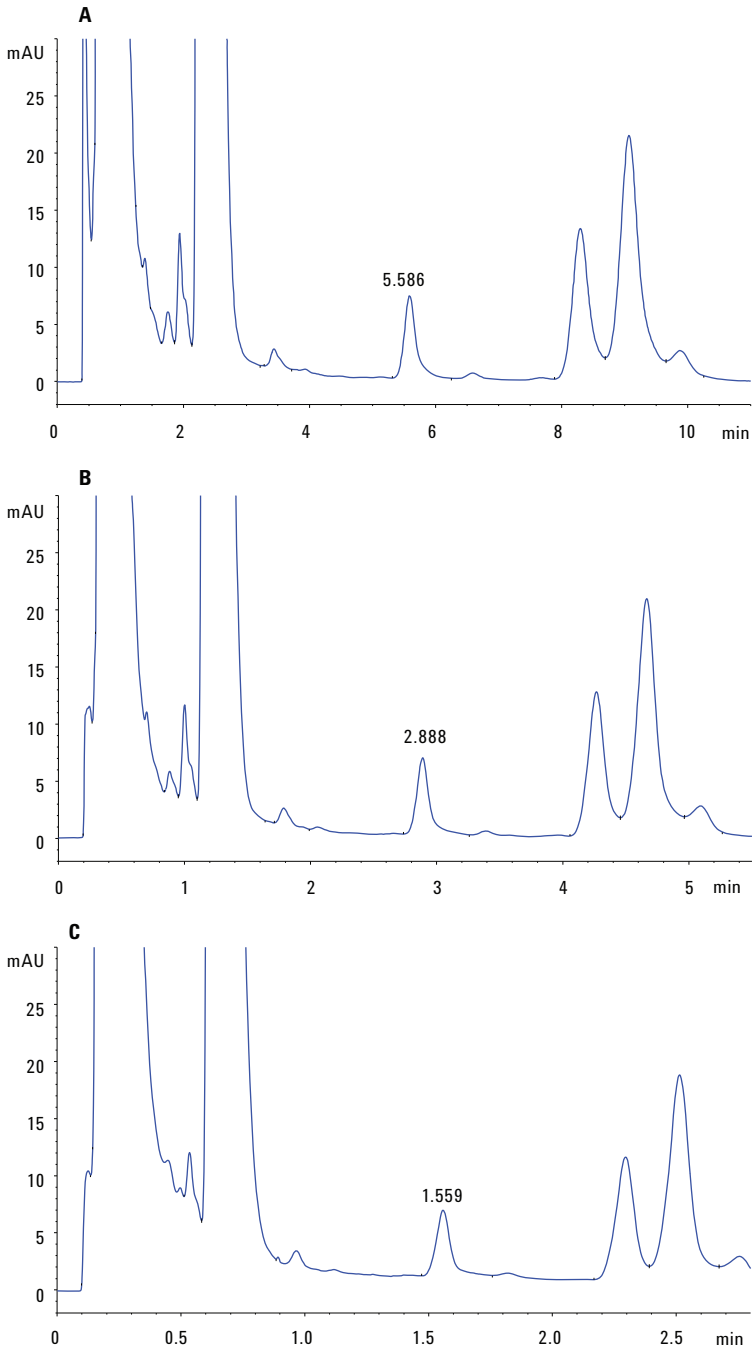

Figure 5. Improved efficiency by means of a shorter column (3.0 × 50 mm, 2.7 µm) and higher flow rates. Reduction of column length to one third reduced the elution time of cafestol to 5.586 minutes, total run time to 10 minutes and increased sample throughput three times. B) Doubling the flow rate to 0.86 mL/min reduced the run time to 5 minutes and the elution time of cafestol to 2.888 minutes. C) Four times higher flow rate of 1.72 mL/min reduced the run time to 2.7 minutes and the elution time of cafestol to 1.559 minutes.

Conclusion

This Application Note demonstrates the use of a standard HPLC method to determine 16-O-methyl cafestol in roasted coffee according to the DIN 10779. The linearity of the calibration curve is excellent as well as the RSD values for retention time and area. It shows that comparable results with even lower LOD and LOQ can be achieved by using solvent saver columns on the same instrument resulting in 57 % less solvent consumed.

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