Determination of Chlorogenic Acid in Coffee Products According to DIN 10767

Application Note

Food Testing & Agriculture – Food Authenticity

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Abstract

This Application Note demonstrates the determination of chlorogenic acid in roasted coffee according to DIN 10767, which is part of a series of quality control measurements for coffee and coffee products. The performance of the Agilent 1260 Infinity LC System demonstrates linearity, retention time, area precision, as well as accuracy. The performance will also be shown on solvent saver columns with reduced inner diameter.

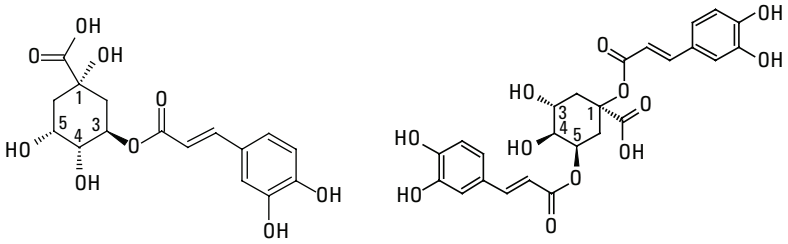
Verified for Agilent
1260 Infinity II LC

Agilent Technologies

Introduction

Chlorogenic acid is a natural product occurring in a large number of different plants or parts of the plant; for example, in green coffee beans. Chemically, it is the ester of caffeic acid and (–)-quinic acid, 3-O-caffeoylquinic acid (3-CQA). Other isomers are crypto-chlorogenic acid (4-CQA) and neo-chlorogenic acid (5-CQA). Additionally, there are other isomers, called iso-chlorogenic acids, with two caffeic acid moieties such as 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 1,5-dicaffeoylquinic acid (cynarine) (Figure 1). Chlorogenic acids are the ingredient of coffee considered to cause problems for coffee drinkers with a sensitive stomach. On the other hand, as a polyphenol compound, it is a highly potent antioxidant with some attributed positive health effects[1]. To control the content of chlorogenic acid in coffee, different roasting procedures are applied. More chlorogenic acid is degraded at a slow roasting process at lower temperature (20 minutes at 200 °C) compared to a faster roasting procedure at higher temperature (3–5 minutes at 400–600 °C). Regular coffee contains about 3.5 g chlorogenic acid per 100 g[2].

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



n-Chlorogenic acid
(3-O-caffeoylquinic acid, 3-CQA)

Cynarine
(1,5-dicaffeoylquinic acid)

Figure 1. Formula of chlorogenic acid as an example for the mono caffeic acid esters and cynarine as an example for the dicaffeic acid esters of (–)-quinic acid (other isomers can be draw according to the numbers and chemical names given in the text).

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 flow cell (G4212-60008)

Software

Agilent OpenLAB CDS ChemStation Edition for LC & LC/MS Systems, Rev. C.01.04

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. 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). Coffee was purchased from a local super market.

Standards

- Chlorogenic acid stock solution: 100 mg/L
- Dilution series for calibration (1:2): 20.0, 10.0, 5.0, 2.5, 1.25, 0.625, 0.3125 (312.5 µg/L), and 0.15625 (156.25 µg/L) mg/L

Sample preparation

A 2 g amount of the roasted coffee sample was extracted with 150 mL methanol/water (50/50, v/v) in a Soxhlet extractor. After four to five extraction cycles, the solution was allowed to cool down to room temperature. The extract was transferred quantitatively to a 200-mL volumetric flask and filled up to the 200 mL level with the methanol-water mixture. After mixing, the extract was filtered by a syringe filter (Agilent Captiva Premium Syringe Filter, Regenerated Cellulose, 0.45 µm, 25 mm, p/n 5190-5111) and the filtrate was used directly for injection.

Results and Discussion

For *n*-chlorogenic acid, a calibration curve was created from 0.156 mg/L up to 20 mg/L (Figure 2). As a standard the isomer *n*-Chlorogenic acid (3-CQA) was used for the calibration. Due to its similar extinction coefficient with neo- and crypto-chlorogenic acid, the calibration can also be used directly for these compounds. The values for the iso-chlorogenic acids can be converted to *n*-chlorogenic acid with an extinction relation (factor 0.77) published in the scientific literature[10].

HPLC method

Parameter	Value
Solvents	A) water + 1 % phosphoric acid B) acetonitrile
Flow rate	1.0 mL/min with Column 1 0.43 mL/min with Column 2 0.43, 0.86, and 1.72 mL/min with Column 3
Gradient	0 minutes – 10 % B, 20 minutes – 20 % B, 25 minutes – 30 % B, 35 minutes – 40 % B, 40 minutes – 40 % B Stop time: 40 minutes Post time: 10 minutes This gradient was used for Column 1 at a flow rate of 1 mL/min. For the other columns, the gradient and flow rate were adjusted accordingly.
Injection volume	10 µL with Column 1 4.3 µL with Columns 2 and and 1.4 µL with Column 3
Needle wash	In vial with acetonitrile
Column temperature	25 °C
Detection	324 nm bandwidth: 8 nm Ref.: Off Data rate: 10 Hz

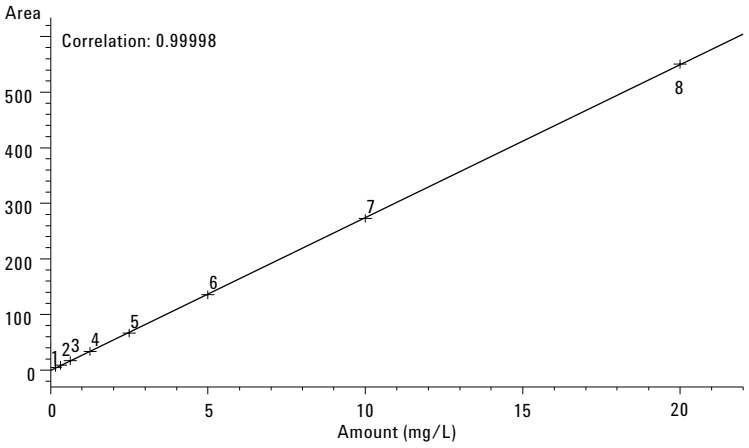


Figure 2. Calibration curve for *n*-chlorogenic acid for the concentration range 0.156–20 mg/L.

The calibration showed excellent linearity. The limit-of-quantification (LOQ) was 0.09 mg/L (signal-to-noise (S/N) = 10) and the limit-of-detection (LOD) at 0.03 mg/L (S/N = 3). Under the chosen HPLC conditions, *n*-chlorogenic acid eluted at 7.0 minutes and an overlay of the injected concentrations from the calibration shows good peak shapes for all concentrations and retention time conformance (Figure 3).

To demonstrate the performance, a statistical evaluation was done by multiple injections (n = 10) of the *n*-chlorogenic acid concentration level at 10 mg/L (Table 1A).

The retention time RSD and area RSD were at 0.07 % and 0.15 %, respectively. For the determination of carryover, the highest concentration used for the calibration was injected and followed by a blank injection. In this blank, no carryover from *n*-chlorogenic acid could be detected (Figure 4). The concentration precision and accuracy was measured for repeated injection (n = 10) of 12 mg/L *n*-chlorogenic acid. The precision was determined to be 0.12 % and concentration accuracy was found to be 98.1 % (Table 1A).

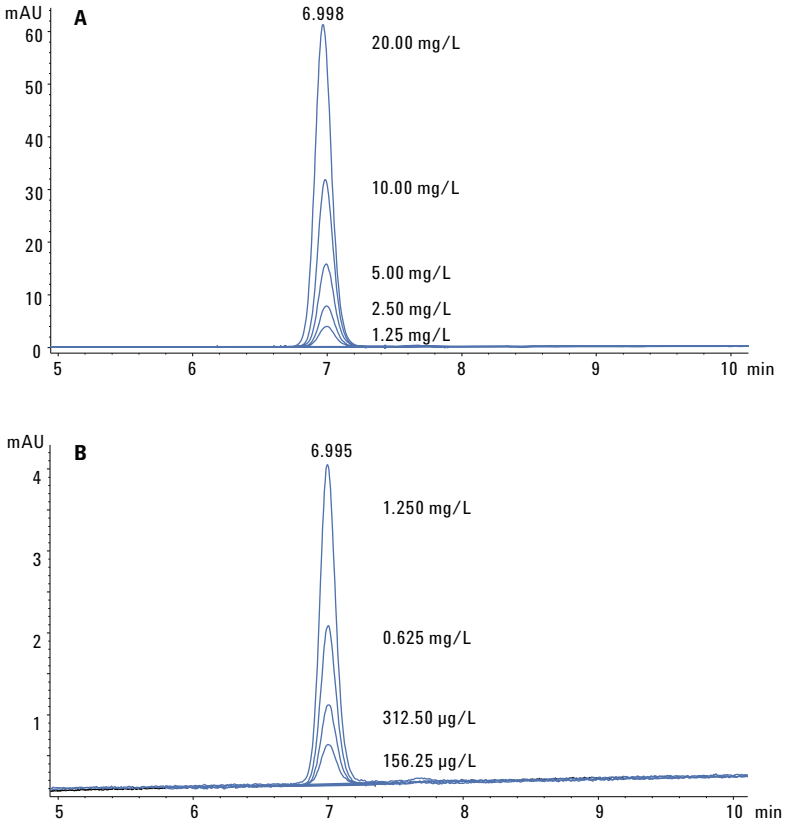


Figure 3. Overlay of chlorogenic acid peaks of different concentrations used as calibration levels. A) Concentrations 1.25–20 mg/L., B) Concentrations 0.1563–1.250 mg/L.

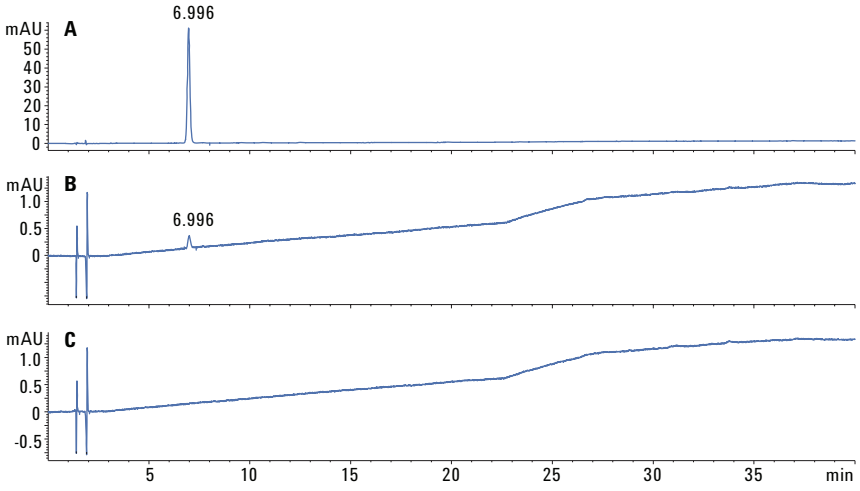


Figure 4. Determination of carryover of *n*-chlorogenic acid, for the maximum concentration used. A) Maximum concentration of *n*-chlorogenic acid at 20 mg/L. B) Lowest level of *n*-chlorogneic acid used for calibration at 156.25 µg/L (LOQ = 90 µg/L) as comparison. C) Blank injection following maximum *n*-chlorogenic acid concentration injection showing no carryover.

The analysis according to the description in the DIN Norm was done under conventional HPLC conditions at a flow rate at 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 1B). Retention time was found at 6.4 minutes due to the low delay volume configuration of the pump (by removing mixer and damper). The retention time and area RSD as well as linearity were in the same range as the 4.6 mm id column.

In contrast to the conventional columns, lower LOQ and LOD were, as found with the solvent saver column, at 0.05 mg/L and 0.015 mg/L, respectively. This effect was due to the higher separation power of this column with its 2.7-µm fused core shell particles and hence higher and sharper peaks delivering improved S/N performance.

Table 1A. Performance data measured for 10 mg/L chlorogenic acid with the Eclipse plus 4.6 × 150 mm, 5 µm column as well as concentration precision and accuracy.

	Column: 4.6 × 150 mm, Eclipse plus C18, 5 µm
	Chlorogenic acid 10 mg/L
r.t. (min)	7.021
r.t. RSD (%)	0.07
area RSD (%)	0.15
Calibration	156.25 µg/L–20.0 mg/L
Linearity, R^2	0.99998
LOD	0.03 mg/L
LOQ	0.09 mg/L
Carry over	from 20.0 mg/L - n.d.
Concentration precision	0.12 % at 12.0 mg/L
Concentration accuracy	98.1 % at 12.0 mg/L

Table 1B. Performance data measured for 10 mg/L chlorogenic acid with the Poroshell 3.0 × 150 mm, 2.7 µm column as well as concentration precision and accuracy.

	Column: 3.0 × 150 mm, Poroshell EC 120, 2.7 µm
	Chlorogenic acid 10 mg/L
r.t. (min)	6.425
r.t. RSD (%)	0.22
area RSD (%)	0.21
Calibration	156.25 µg/L–20 mg/L
Linearity, R^2	0.99999
LOD	0.015 mg/L
LOQ	0.05 mg/L
Carryover	from 20.0 mg/L - n.d.
Concentration precision	0.21 % at 12.0 mg/L
Concentration accuracy	96.0 % at 12.0 mg/L

The chromatogram of a real sample from regularly roasted coffee is shown in Figure 5. The peak for *n*-chlorogenic acid elutes at 6.95 minutes. Neo-chlorogenic acid eluted a little earlier at 4.35 minutes, and crypto-chlorogenic acid little later at 7.63 minutes. The three iso-chlorogenic acids eluted between 21.9 and 24.8 minutes. The concentration of *n*-chlorogenic acid was 1.19 g/100 g coffee. The combined content of all chlorogenic acids was 2.61 g/100 g.

The same sample was also measured on Column 2 under identical gradient conditions but at a flow rate of 0.43 mL/min to save about 57 % of solvent. The chromatogram showed all chlorogenic acid peaks with improved separation of especially the first three eluting chlorogenic acid isomers (Figure 6).

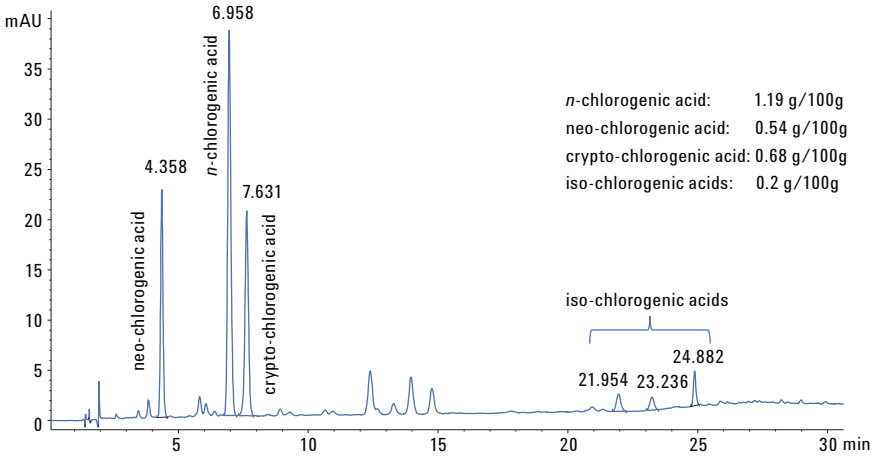


Figure 5. Determination of chlorogenic acids in regular coffee on column 1. *n*-Chlorogenic acid: 1.19 g/100 g. All chlorogenic acids: 2.61 g/100 g. (extraction of 2 g coffee, final extraction volume 200 mL, dilution of the extract was 1:10).

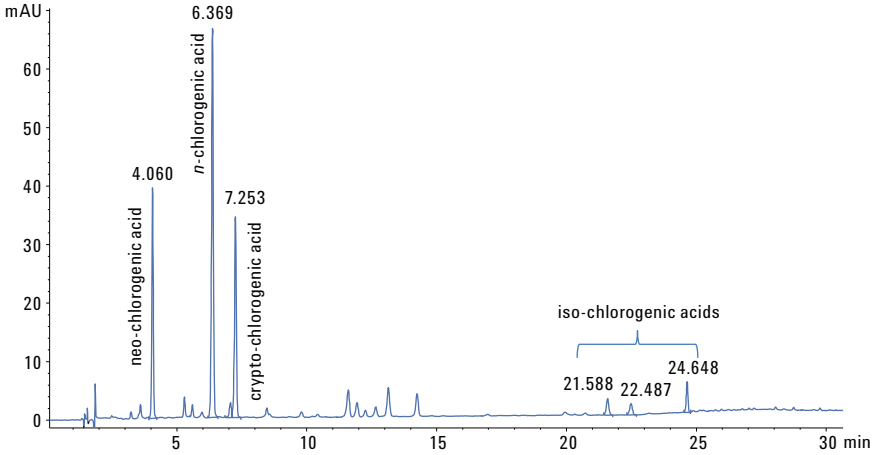


Figure 6. Determination of chlorogenic acids in regular coffee on Column 2 at a flow rate of 0.43 mL/min.

For a further improvement of the productivity, the 3.0 × 150 mm column was exchanged with a 3.0 × 50 mm column of the same stationary phase. The time steps of the gradient were reduced to one third and the flow rate was kept constant, which allowed finishing the separation in one third of the time, 13 minutes, improving sample throughput three times (Figure 7A). A further improvement of the throughput could be achieved by doubling the flow rate to 0.86 mL/min, which reduces the run time to 6.5 minutes (Figure 7B). A four times higher flow rate of 1.72 mL/min reduces the run time to 3.25 minutes (Figure 7C).

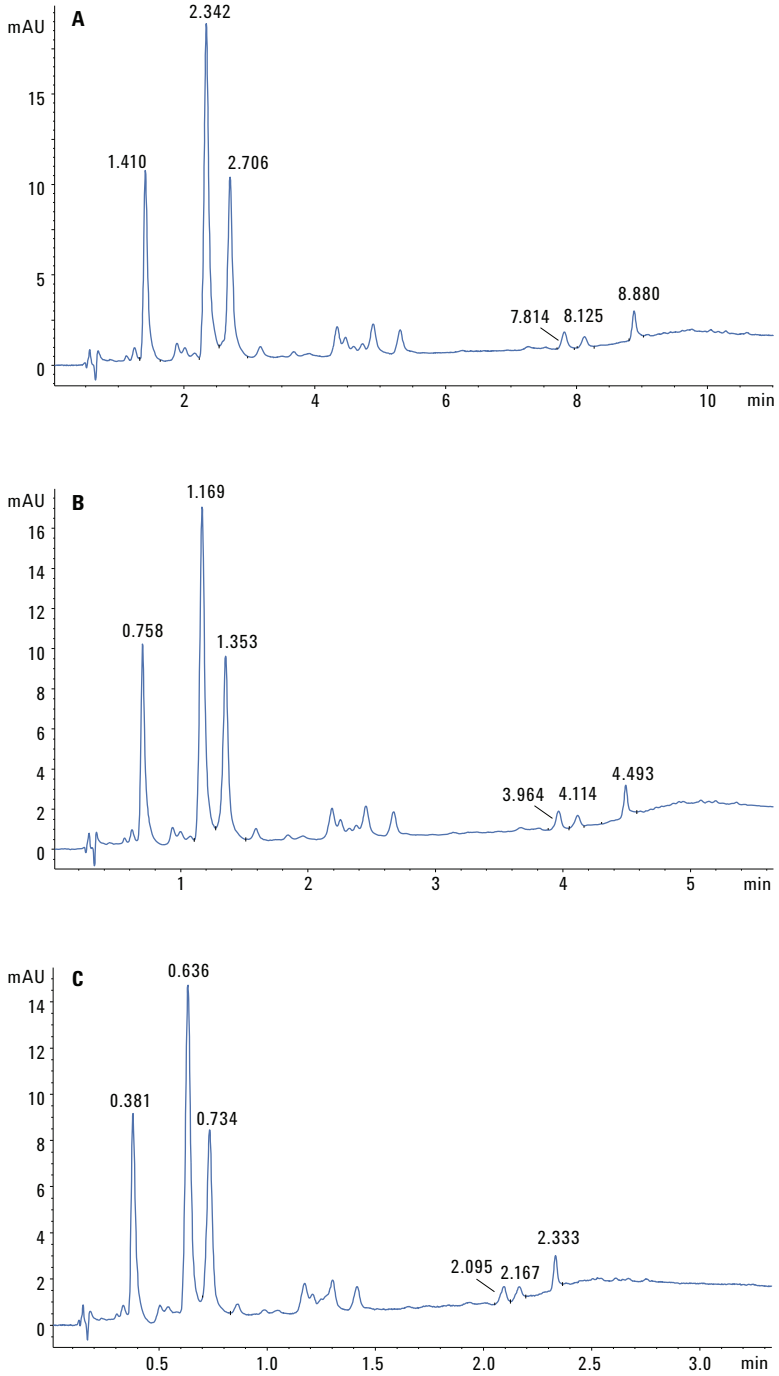


Figure 7. Improved efficiency by means of a shorter column (3.0 × 50 mm, 2.7 µm) and higher flow rates. A) Reduction of column length and gradient step times to one third reduces the elution time of *n*-chlorogenic acid to 2.342 minutes, total run time to 13 minutes and increases sample throughput three times. B) Doubling the flow rate to 0.86 mL/min reduces the run time to 6.5 minutes and the elution time of n-chlorogenic acid to 1.169 minutes. C) Four times higher flow rate of 1.72 mL/min reduces the run time to 3.25 minutes and the elution time of *n*-chlorogenic acid to 0.636 minutes.

Conclusion

This Application Note demonstrates the use of a standard HPLC to determine chlorogenic acids in roasted coffee according to the DIN 10767. The linearity of the calibration curve 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 whereas 57 % less solvent is consumed.

References

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