SHIMADZU
Excellence in Science

Application News

High Performance Liquid Chromatograph Nexera™ lite

Simultaneous Quantitative Analysis of Coumarin and Cinnamaldehyde in Cinnamon Produced in Different Regions

N. Iwata

User Benefits

- Quickly achieves separation between coumarin, cinnamaldehyde, and contaminants.
- Pretreatment can be accomplished easily using a protocol based on solid phase extraction without conditioning.
- Enables quantitative analysis at optimal detection wavelengths with one analysis.

■ Introduction

Cinnamon, which is ground from the dried bark of an evergreen tree in the Lauraceae family, has been used from ancient times as a food spice and herbal remedy. As a spice, it is commonly used in confections, teas, curries, and meat dishes. It can promote digestion, stomach health, intestinal regulation, detoxification, pain relief, and other benefits, and has even been reported to be effective in preventing or improving diabetes.[1),2)]

The main types of cinnamon are *Cinnamomum verum* (Ceylon cinnamon) and *Cinnamomum cassia*. Both types contain compounds such as coumarin and cinnamaldehyde, but their concentrations vary significantly depending on the region where it is produced. Due to the hepatotoxicity of coumarin, the German Federal Institute for Risk Assessment (BfR) specified a maximum recommended tolerable daily intake of 0.1 mg of coumarin per kg of body weight.[3)] Meanwhile, animal studies of cinnamaldehyde have shown detrimental fetal effects.[3)] Consequently, determining the concentration of those compounds in cinnamon is considered important.

This article describes a method for using high-performance liquid chromatography to simultaneously quantify the content of coumarin and cinnamaldehyde in cinnamon samples from different regions.

■ Analysis of Mixed Standard Solution

Fig. 1 shows chromatograms of mixed standard solution of coumarin and cinnamaldehyde (500 µg/L each, prepared with acetonitrile) acquired using the analytical conditions indicated in Table 1. Coumarin was detected at 280 nm, near the maximum absorption wavelength. Though the maximum absorption wavelength for cinnamaldehyde is 287 nm, taking into consideration sensitivity and separation from contaminants in the cinnamon sample, cinnamaldehyde was detected at 320 nm.

Table 1 Analytical Conditions

System:	Nexera lite
Column:	Shim-pack™ GIST-HP C18*1 (150 mm × 3.0 mm I.D., 3 µm)
Flowrate:	0.8 mL/min
Mobile Phase:	A) Water B) Acetonitrile
Time Program:	50 %B (0.00-2.00 min)→60 %B (4.00 min) →100 %B (4.01-5.00 min)→50 %B (5.01-10.00 min)
Mixer:	180 µL
Column Temp.:	25 °C
Injection Volume:	5 µL
Vial:	SHIMADZU LabTotal™ for LC 1.5 mL, Glass*2
Detection (PDA):	Ch1 (Coumarin): 280 nm, Ch2 (Cinnamaldehyde): 320 nm (SPD-M40)

*1 P/N: 227-30040-05 *2 P/N: 227-34001-01

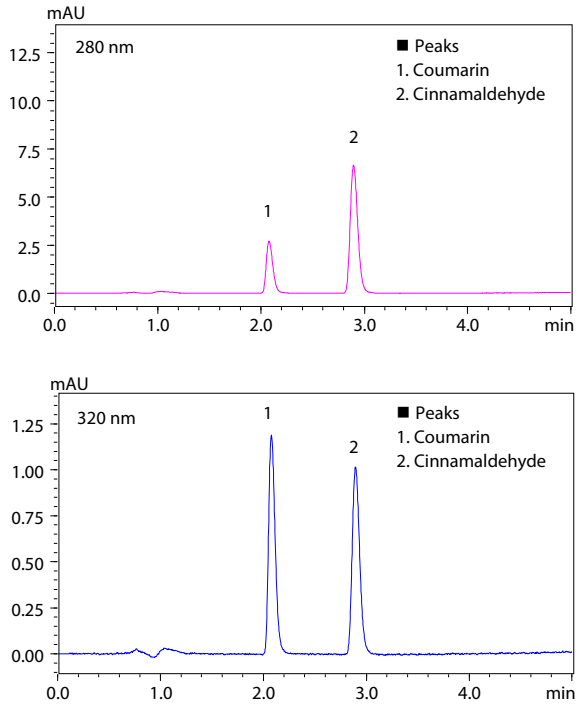

Fig. 1 Chromatograms of Mixed Standard Solution of Coumarin and Cinnamaldehyde (500 µg/L each)

■ Reproducibility

Table 2 shows the reproducibilities (%RSD) of the retention time and the peak area of a 500 µg/L mixed standard solution in six repeated analyses.

Table 2 Reproducibility (%RSD) in Six Repeated Analyses

Compound	Retention time	Peak area
Coumarin	0.00	0.22
Cinnamaldehyde	0.07	0.49

■ Calibration Curve

Calibration curves for coumarin and cinnamaldehyde are shown in Fig. 2. The concentration range was from 12.5 to 1,000 µg/L for coumarin and from 500 to 40,000 µg/L for cinnamaldehyde. Both curves indicated good linearity, with an r^2 contribution of 0.9999 or greater.

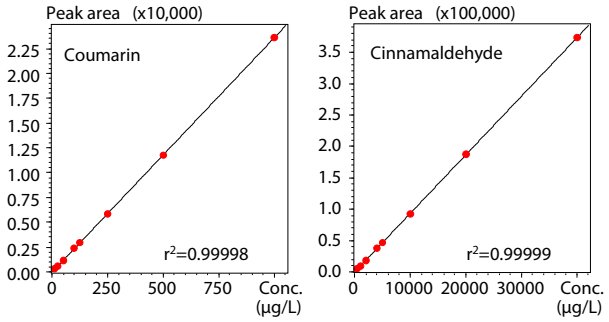

Fig. 2 Calibration Curves

■ Analysis of Cinnamon Samples from Different Region

Samples of commercial *Cinnamomum cassia* produced in Vietnam and *Cinnamomum verum* produced in Sri Lanka were used. The pretreatment protocol is shown in Fig. 3. Acetonitrile was used as the extraction solvent. Lipids were removed using a dispersive solid phase extraction (dSPE) cartridge (Merck Supel™ QuE Z-Sep+). The cartridge eliminates the need to carry out conditioning before loading samples, which simplifies operations. Supernatant was filtered through a 0.2 µm membrane filter and diluted with acetonitrile before analysis by HPLC.

Chromatograms of *Cinnamomum cassia* and *Cinnamomum verum* are shown in Figs. 4 and 5. For cinnamaldehyde, the detection wavelength was optimized to improve its separation from contaminants. Analysis results and concentration are indicated in Tables 3 and 4.

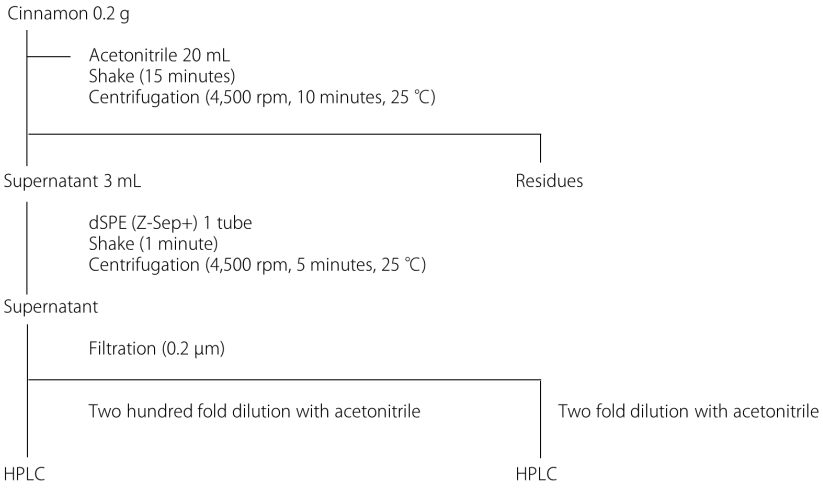

Fig. 3 Pretreatment Protocol

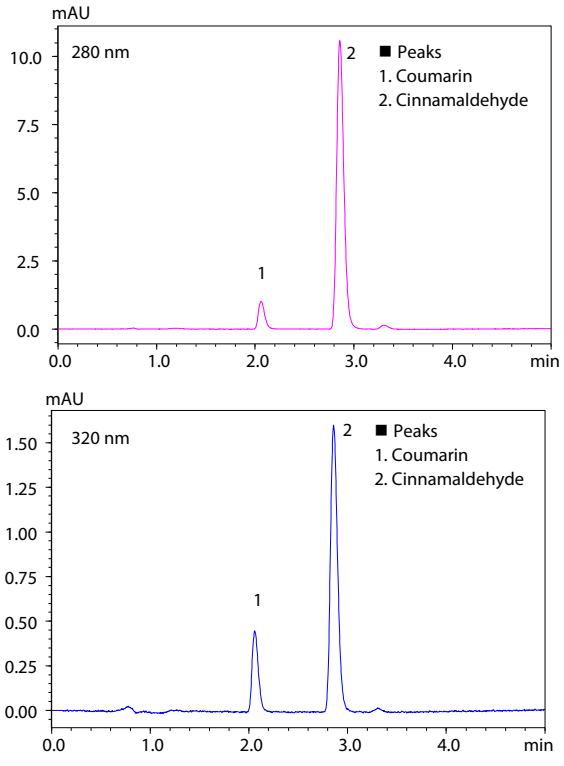

Fig. 4 Chromatograms of *Cinnamomum Cassia*

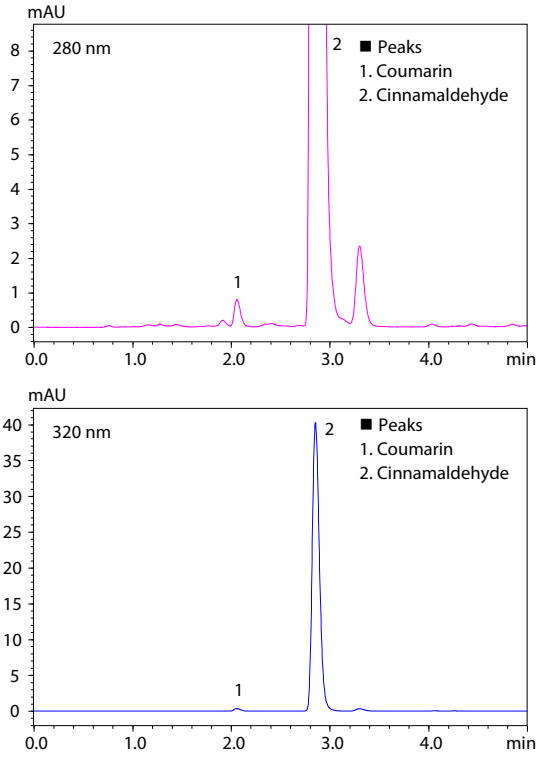

Fig. 5 Chromatograms of *Cinnamomum Verum*

Table 3 Analysis Results

	Coumarin	Cinnamaldehyde
	µg/L	µg/L
Cinnamomum Cassia	190	871
Cinnamomum Verum	148	21,821

Table 4 Concentration

	Coumarin	Cinnamaldehyde
	µg/g	µg/g
Cinnamomum Cassia	3,791	17,428
Cinnamomum Verum	30	4,364

■ Recovery Rates

Cinnamomum cassia was used for recovery rates testing. 0.2 g of *Cinnamomum cassia* was spiked with coumarin and cinnamaldehyde, prepared to concentrations of 2 mg/g and 20 mg/g, respectively. Then six samples were pretreated simultaneously according to the pretreatment protocol in Fig. 3. At this time, sample solutions containing coumarin and cinnamaldehyde at concentrations of 100 µg/L and 1000 µg/L were injected into the HPLC system, respectively. Table 5 shows the average recovery rates obtained from the results of six samples.

Table 5 Recovery Rates (n = 6)

N	Recovery rates (%)	
	Coumarin	Cinnamaldehyde
1	103.0	91.7
2	100.2	91.5
3	106.9	95.5
4	110.4	95.7
5	110.6	95.6
6	105.1	91.4
Average (%RSD)	106.0 (3.9%)	93.5 (2.4%)

■ Verification by UV Spectra

In addition to the peak retention time identification, qualitative analysis is also possible based on the similarity accordance of UV spectrum to that of a standard solution by a photodiode array (PDA) detector.

Fig. 6 shows three UV spectra of the peaks at about 2 minutes in Figs. 4 and 5, and the coumarin reference standard. All the UV spectra are shown normalized for comparison purposes. These peaks were identified as coumarin because the maximum absorption wavelength was determined at 275 nm for both peaks. Similarly, the UV spectra of the peaks at about 3 minutes in Figs. 4 and 5 were identified as cinnamaldehyde because the maximum absorption wavelength was determined at 287 nm for both peaks (Fig. 7).

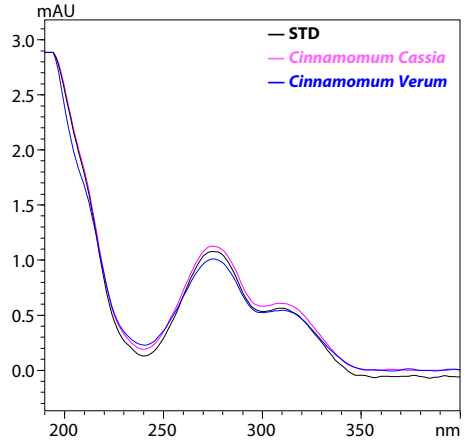

Fig. 6 Comparison of UV Spectra (Coumarin)

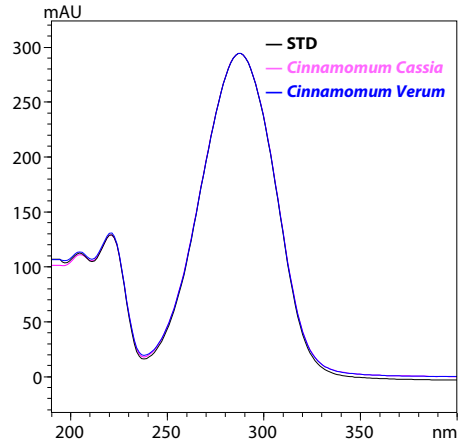

Fig. 7 Comparison of UV Spectra (Cinnamaldehyde)

■ Conclusion

A Method for pretreatment and analysis of coumarin and cinnamaldehyde in cinnamon produced in different regions was developed. Appropriate sensitivity can be ensured by optimizing the detection wavelength to achieve good separation from contaminants. The method in this article can be used to perform qualitative-quantitative analysis more quickly.

References
1) S. Kim, S. Hyun, S. Choung; *J. Ethnopharmacol.*, 104(1-2), 119-123 (2006).
2) R. Akilen, A. Tsiami, D. Devendra, N. Robinson; *Diabet. Med.*, 27(10), 1159-1167 (2010).
3) Hohe tagliche Aufnahmemengen von Zimt: Gesundheitsrisiko kann nicht ausgeschlossen werden (27.09.2006)

Shimadzu Corporation
Analytical & Measuring Instruments Division
Global Application Development Center

www.shimadzu.com/an/

01-00233-EN First Edition: Dec. 2021