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Application News

High Performance Liquid Chromatograph Nexera™ lite

Simultaneous Determination of Functional Components in Coffee

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User Benefits

- Simultaneous analysis of highly polar compounds from acidic to basic is possible.
- The peaks of the compounds with basic group that have generally poor retention in reversed phase columns can be retained, and stable analysis can be achieved.

■ Introduction

Recently, coffee drinking has been reported to be effective in preventing or improving dementia and lifestyle-related diseases such as diabetes and cancer.[1),2)] It has also been found that coffee components such as pyrocatechol and trigonelline contribute to these effects.[3),4)]

However, trigonelline, a compound with basic group, is difficult to analyze due to poor retention on a C18 column. Therefore, a PFPP column that has pentafluorophenylpropyl as the stationary phase was used for the analyses in this study. This column is expected to provide a unique separation selectivity as a result of π–π interaction and dipole-dipole interaction in addition to hydrophobic interaction.

This article describes simultaneous determination of functional components in coffee using a Shim-pack Scepter™ PFPP.

■ Analysis of Mixed Standard Solution

Fig. 1 shows the structural formula of trigonelline, pyrocatechol, chlorogenic acid, and caffeic acid, which were the targets of the analysis.

This article compared two analytical columns, a Shim-pack Scepter C18 and a Shim-pack Scepter PFPP. Fig. 2 shows chromatograms of the mixed standard solution (10 mg/L each) acquired by each column using the analytical conditions indicated in Table 1. Trigonelline was retained by the Shim-pack Scepter PFPP but was only poorly retained by the Shim-pack Scepter C18. Generally, longer retention provides improved separation, whereas shorter retention leads to inadequate separation with co-existing interferences.

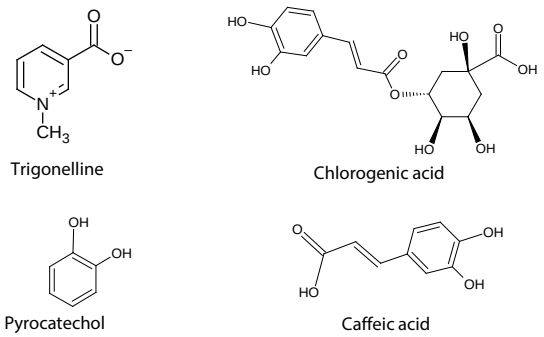
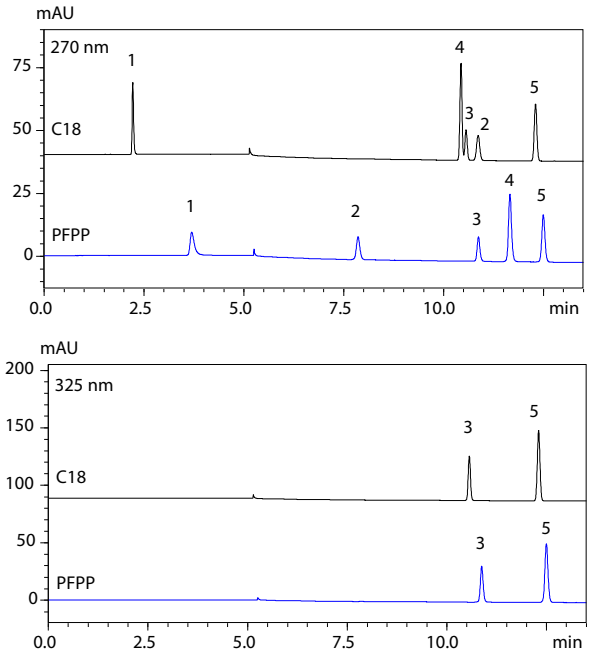


Fig. 1 Structure of Four Target Compounds



■Peaks 1. Trigonelline, 2. Pyrocatechol, 3. Chlorogenic acid, 4. Caffeine 5. Caffeic acid

Fig. 2 Chromatograms of Mixed Standard Solution (10 mg/L each)

Table 1 Analytical Conditions

System:	Nexera lite
Column (C18):	Shim-pack Scepter C18-120*1 (150 mm×4.6 mm I.D., 3 µm)
Column (PFPP):	Shim-pack Scepter PFPP-120*2 (150 mm×4.6 mm I.D., 3 µm)
Flowrate:	1.0 mL/min
Mobile Phase:	A) 20 mmol/L (Sodium) phosphate buffer (pH 2.6) B) Acetonitrile
Time Program:	0 %B (0.00-1.00 min)→10%B (4.00 min) →20 %B (10.00-12.00 min) →70 %B (12.01-13.00 min) →0 %B (13.01-18.00 min)
Mixer:	180 µL
Column Temp.:	25 °C
Injection Volume:	5 µL
Vial:	SHIMADZU LabTotal™ for LC 1.5 mL, Glass*3
Detection (PDA):	Ch1 : 270 nm, Ch2 : 325 nm (SPD-M40)

*1 P/N: 227-31016-05 *2 P/N: 227-31057-05 *3 P/N: 227-34001-01

■ Repeatability

Table 2 shows the reproducibilities (%RSD) of the retention time and the peak area of mixed standard solution of 1 mg/L for each compound in six repeated analyses.

Table 2 Repeatability (%RSD) in Six Repeated Analyses

Compound	Retention time	Peak area
Trigonelline	0.08	0.65
Pyrocatechol	0.05	0.50
Chlorogenic acid	0.07	0.14
Caffeine	0.06	0.15
Caffeic acid	0.05	0.21

■ Calibration Curves

The calibration curves for the five target compounds were highly linear, with coefficients of determination (r^2) of 0.99999 or greater. Fig. 3 shows the calibration curves of trigonelline and pyrocatechol. Table 3 shows the concentration ranges of calibration curves and the coefficients of determination for all the target compounds.

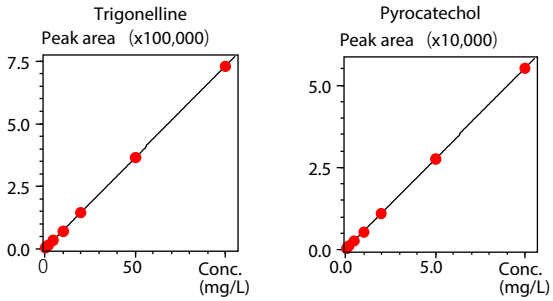


Fig. 3 Calibration Curves

Table 3 Concentration Ranges of Calibration Curves and Coefficients of Determination (r^2)

Compound	Conc. range (mg/L)	r^2
Trigonelline	1-100	0.99999
Pyrocatechol	0.1-10	0.99999
Chlorogenic acid	1-100	0.99999
Caffeine	1-100	0.99999
Caffeic acid	0.1-10	0.99999

■ Analysis of Coffee

Ten grams of commercial ground coffee beans were extracted with 150 mL of boiling water to form the sample. The sample was filtered through a 0.2 µm membrane filter and diluted ten-fold with ultrapure water before HPLC analysis.

Chromatograms of the coffee are shown in Fig. 4 and the analytical results in Table 4. In this table, "Concentration" means the concentration after sample preparation. Fig. 5 shows UV spectra of the sample and the standard solution. The UV spectral similarity to the reference compounds suggests that the target compounds and respective contaminants in the coffee were appropriately separated.

Table 4 Analytical Results (N=6)

Compound	Concentration (mg/L)	%RSD
Trigonelline	21.5	0.08
Pyrocatechol	1.0	1.05
Chlorogenic acid	21.3	0.07
Caffeine	79.2	0.05
Caffeic acid	N.D.	N.D.

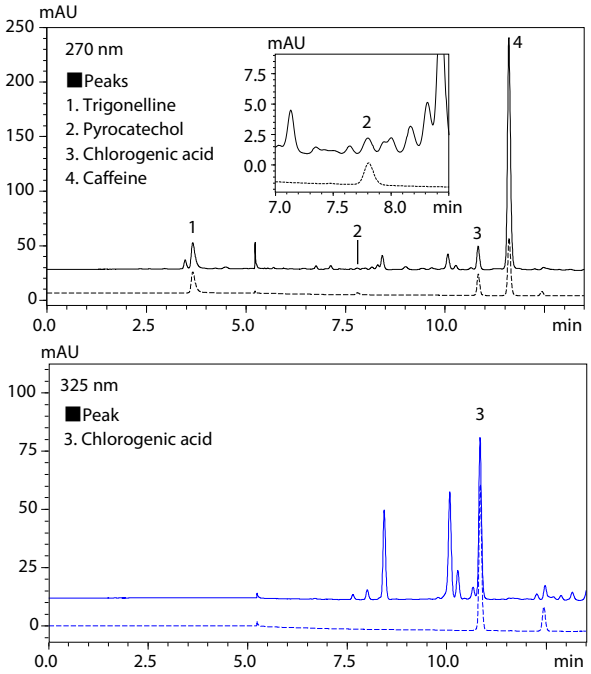


Fig. 4 Chromatograms of Coffee
(Solid Line: Coffee, Dashed line: Standard Solution)

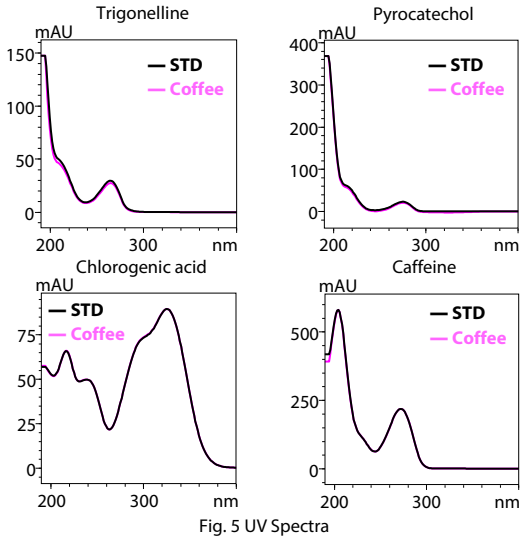


Fig. 5 UV Spectra

■ Conclusion

A method for simultaneous analysis of functional component with different physical properties in coffee was developed. Using a Shim-pack Scepter PFPP, even basic compounds such as trigonelline were adequately retained and separated from contaminants. The method described in this article is expected to contribute to research and development in food engineering including the study for functional components.

References

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2) Poole R., Kennedy O. J., Roderick P., Fallowfield J. A, Hayes P. C., Parkes J., *BMJ* 359, j5024 (2017).
3) Fukuyama K., Kakio S., Nakazawa Y., Kobata K., Funakoshi-Tago M., Suzuki T., Tamura H., *Mol. Nutr. Food Res.* 62, e1800238 (2018).
4) Farid M.M., Yang X., Kuboyama T., Tohda C. *Scientific Reports* 10, 16424 (2020).

01-00280-EN First Edition: Feb. 2022

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