



High Performance Liquid Chromatograph Nexera[™] lite

Simultaneous Determination of Functional Components in Coffee

N. Iwata

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



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 l.D., 3 μm)		
Column (PFPP):	Shim-pack Scepter PFPP-120 ^{*2}		
	(150 mm×4.6 mm l.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 D/NJ 227 21016 05 *2 D/NJ 227 21057 05 *2 D/NJ 227 24001 01			

*1 P/N: 227-31016-05 *2 P/N: 227-31057-05 *3 P/N: 227-34001-0

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 (%h3D) III Six Repeated Allalyses				
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		

2 Demoster bility (0/ DCD) in City Demoster d Arrelyn

Calibration Curves

The calibration curves for the five target compounds were highly linear, with coefficients of determination (r²) 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.



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 tenfold 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.		

Nexera, Shim-pack Scepter, and Shimadzu LabTotal are registered trademarks of Shimadzu Corporation in Japan and other countries.

HIMADZU

Shimadzu Corporation

Analytical & Measuring Instruments Division **Global Application Development Center**

mAU 270 nm 7.5 200 Peaks 1. Trigonelline 5.0 2. Pyrocatechol 150 2.5 3. Chlorogenic acid 0.0 4. Caffeine 100 7.0 7.5 8.0 min 50 Å. 0 0.0 2.5 7.5 10.0 5.0 min mAU 325 nm 100 -Peak 3. Chlorogenic acid 75 -50 25 0 0.0 2.5 5.0 7.5 10.0 min Fig. 4 Chromatograms of Coffee (Solid Line: Coffee, Dashed line: Standard Solution) Trigonelline Pvrocatechol mAL 400⁻ 150 — STD — STD 300-Coffe Coffee 100 200 50 100 0 0 300 200 300 200 nm nm Caffeine Chlorogenic acid <u>mAU</u> mAl – STD — STD 500 75 Coffee 50 250 25 0 0 200 300 200 300 nm nm Fig. 5 UV Spectra

Conclusion

mAU 250

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

- 1) van Dam R. M., Feskens E. J., Lancet 360, 1477-1478 (2002).
- 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

For Research Use Only. Not for use in diagnostic procedure. This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu See http://www.shimadzu.com/about/trademarks/index.html for details. Third party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not they

are used with trademark symbol "TM" or "®". The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.