SHIMADZU
Excellence in Science

Application News

No.L450

High Performance Liquid Chromatography

Analysis of Orotic Acid in Yogurt

Orotic acid, a heteroaromatic compound discovered in whey, is also referred to as orotate, and uracil-6-carboxylic acid. In the past, it was also called vitamin B_{13}, but since it is synthesized *in vivo*, it is no longer considered to be a vitamin. Orotic acid is a major precursor of the nucleic acid pyrimidine, and has been attracting attention due to its diverse physiological effects. In Japan, it has been formulated into cosmetics and pharmaceuticals sold as "class 3 OTC drugs", namely OTC drugs with minimum risk of side effects. On January 23, 2012, the Pharmaceutical and Food Safety Bureau of the Japanese Ministry of Health, Labour and Welfare published a notification (No. 0123-3) regarding "a partial revision of the standards for a range of pharmaceutical products", stating that orotic acid, orotic acid potassium salt, and magnesium salt were added to the list of items specified as "ingredients that are not considered as drugs unless drug efficacy is advocated". As a result, its increased use in health foods, nutritional supplements, etc. can be expected.
Here, we introduce an example of analysis of orotic acid in yogurt by reversed-phase chromatography using a Shimadzu Prominence HPLC system.

Analysis of Standard Solution

Fig. 1 shows the structural formula of orotic acid. Due to its extremely high polarity, retention is minimal when using reversed-phase chromatography. Although retention may be increased by preparing the mobile phase using a buffer solution that does not contain an organic solvent, the retention time will gradually decrease when using a typical reversed-phase column. Therefore, in this analysis, we used the Hydrosphere C18 column designed by YMC Co. Ltd., featuring a deactivated silica surface and elevated hydrophilicity of the stationary phase, thereby permitting significant retention even when using a 100 % aqueous buffer solution as the mobile phase. In this application, we used only an acidic buffer solution as the mobile phase. Fig. 2 shows a chromatogram obtained from analysis of an orotic acid standard solution (10 mg/L), and Table 1 shows the analytical conditions used.

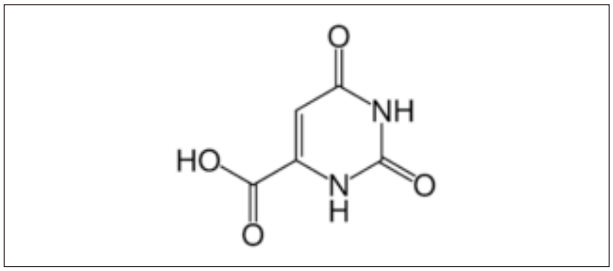

Fig. 1 Structure of Orotic Acid

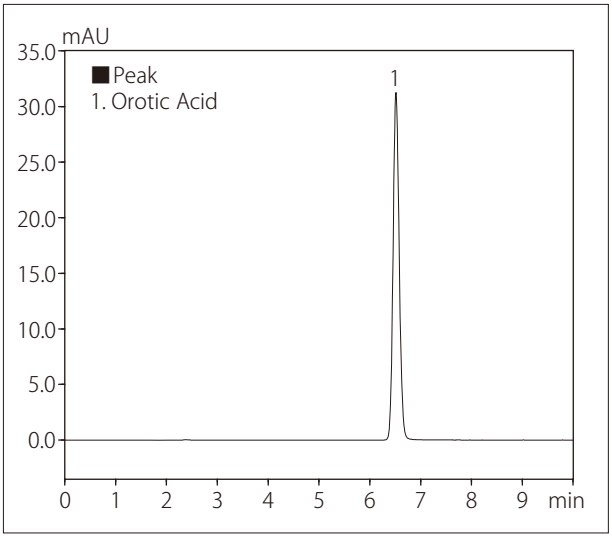

Fig. 2 Chromatogram of Standard Solution of Orotic Acid (10 mg/L, 10 µL injected)

Table 1 Analytical Conditions

Column	: Hydrosphere C18 (150 mm L. × 4.6 mm I.D., 5 µm, YMC Co., Ltd.)
Mobile Phase	: 10 mmol/L Phosphate (Sodium) Buffer (pH 2.6)
Flowrate	: 1.0 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 10 µL
Detection	: SPD-20A at 280 nm

Analysis of Low Concentration Standard Solution

Fig. 3 shows the chromatogram obtained using a 10 µL injection of orotic acid (0.1 mg/L). Table 2 shows the area and retention time repeatability from six repeat injections. The SIL-20AC was used as the autosampler, and excellent repeatability was obtained even with a low concentration sample.

Table 2 Repeatability (n=6)

	Retention time	Area
%RSD	0.01	0.86

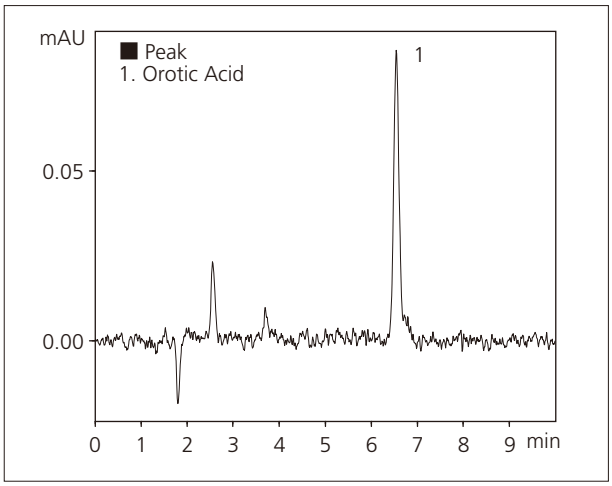

Fig. 3 Chromatogram Obtained with Low Concentration Standard Solution (0.1 mg/L, 10 µL injected) Linearity

Linearity

Fig. 4 shows the calibration curve obtained using concentrations from 0.1 to 10 mg/L. The calibration curve displays excellent linearity, with a correlation coefficient (R^2) greater than 0.9999.

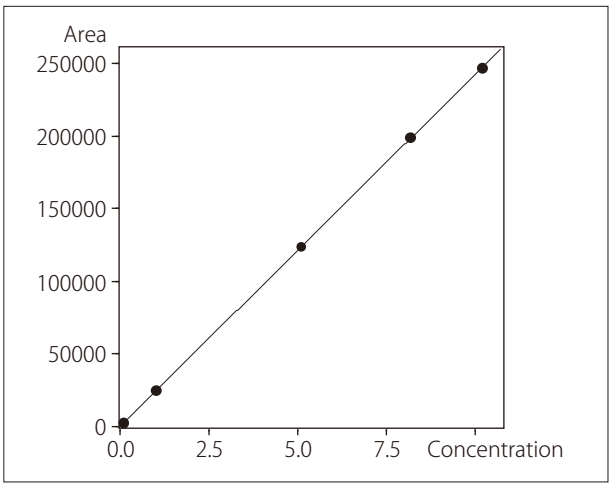

Fig. 4 Linearity (0.1 - 10 mg/L, 10 µL injected)

Analysis of Yogurt

Fig. 5 shows a chromatogram of yogurt, and Fig. 6 shows that of a yogurt drink.
After filtering the sample through an ultrafiltration membrane and then diluting it ten times with water, a 10 µL injection was made. The orotic acid content in the yogurt was found to be about 75 mg/L, and in the yogurt drink, about 35 mg/L.

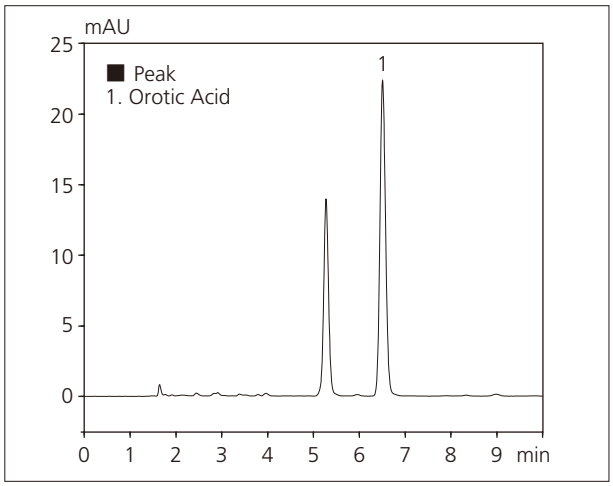

Fig. 5 Chromatogram of Yogurt (10 µL injected)

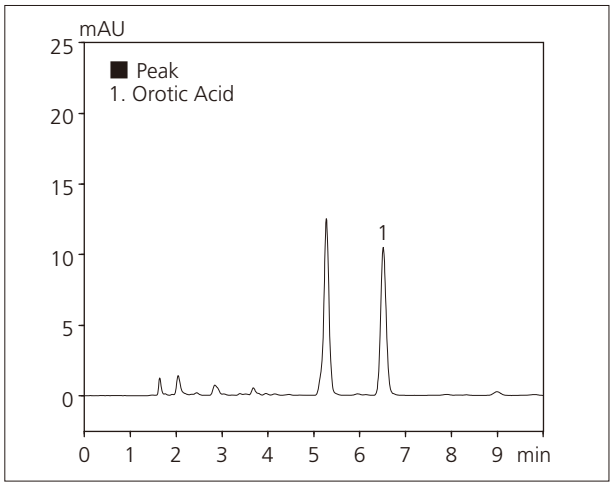

Fig. 6 Chromatogram of Yogurt Drink (10 µL injected)

First Edition: Aug. 2013

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/