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Application News

No. L530

High Performance Liquid Chromatography

Measurement of Purine Content in Foods Using HPLC

Purine generally means all nucleic acids, nucleotides, nucleosides, and purine bases that have a purine structure. In the body, purines are eventually metabolized into uric acid. Among purines in the body, about 20 % are from the foods we eat while the remaining 80 % are endogenous.

It is known that if the excretion and production of uric acid falls out of balance, uric acid accumulates in the body (resulting in high uric acid levels) and causes hyperuricemia as well as gout. On the other hand, researches in recent years have revealed that there is an association between mental illnesses and low levels of serum uric acid. It is therefore important to control the amount of dietary purine intake such as by implementing a low-purine diet for patients with hyperuricemia.

This article introduces analyses of purine content in foods using the "Prominence™-i" integrated high performance liquid chromatograph (hereinafter, Prominence-i).

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Analysis of a Mixed Standard Solution of Purine Bases

We analyzed a mixed standard solution of five purine bases (adenine, guanine, hypoxanthine, uric acid, and xanthine). The Asahipak GS-320HQ multimode column from Shodex was used as the analytical column. As the mobile phase, we mixed sodium dihydrogen phosphate dihydrate and phosphoric acid to make a mole ratio of 4:1 for preparing the sodium phosphate buffer. Fig. 1 shows the chromatogram of the mixed standard solution of purine bases (containing 10 mg/L of each standard) and Table 1 lists the analytical conditions.

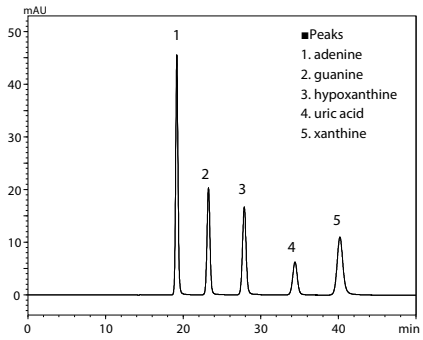

Fig. 1 Chromatogram of a Mixed Standard Solution of Purine Bases

Table 1 Analytical Conditions

System	:	Prominence-i 3D
Column	:	Shodex Asahipak GS-320HQ (300 mm L, 7.5 mm I.D., 6 µm)
Flow rate	:	0.6 mL/min
Mobile phase	:	150 mmol/L (sodium) phosphate buffer (pH 2.6)
Column temp.	:	35 °C
Injection volume	:	10 µL
Detection	:	PDA 260 nm

Calibration Curves

For each of the five purine bases, we created a calibration curve in the range of 0.1 to 20 mg/L. All calibration curves resulted in favorable linearities with coefficients of determination of 0.9999 or higher. Fig. 2 shows the calibration curves.

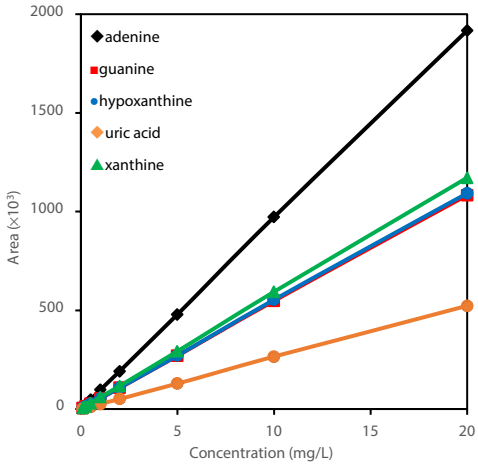

Fig. 2 Calibration Curves

Pretreatment

Tuna and broccoli were used as samples. The tuna sample was prepared from a slice available on the market and the broccoli sample was prepared by cutting out edible sections.

Fig. 3 illustrates the pretreatment protocol. Samples were first homogenized and then freeze-dried, after which they were hydrolyzed using perchloric acid (70 %) and then neutralized. Supernatants were taken and separated to make a sample without enzyme treatment (pretreatment A in Fig. 3) and a sample with enzyme treatment (pretreatment B in Fig. 3). The sample without enzyme treatment underwent centrifugal filtration while the sample with enzyme treatment underwent ultrafiltration before being injected into the HPLC. Since perchloric acid was used for hydrolysis, the purine bases were also broken down.

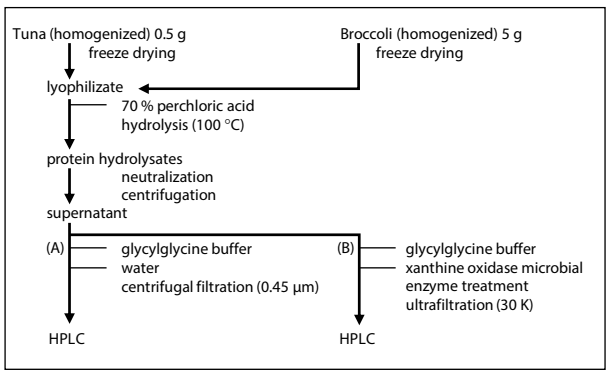

Fig. 3 Pretreatment Protocol

Analysis of Tuna

Meat and seafood are known to have a higher purine content compared to fruits and vegetables since they contain muscle tissue. Fig. 4 shows the chromatograms of tuna. The black line is the standard, the red line the sample without enzyme treatment, and the blue line the sample with enzyme treatment. Since purine bases are oxidized by the enzyme treatment, the elution position has shifted. Therefore, by comparing the chromatograms of the samples with and without enzyme treatment, peaks can be determined to be those purine bases if those peaks do not appear in the chromatogram of the sample with enzyme treatment. The difference in peak area values between the samples with and without enzyme treatment were used for quantitation.[1)]

Adenine, guanine, and hypoxanthine were separated and detected from the tuna sample. Based on the obtained results, the total purine content in 100 g of tuna was 205.0 mg. If the total amount of these purine bases were to be metabolized into uric acid, the uric acid amount, in other words the uric acid equivalent amount, was 251.9 mg/100 g. The content of each purine base, the total purine content, and the uric acid equivalent are summarized in Table 2.

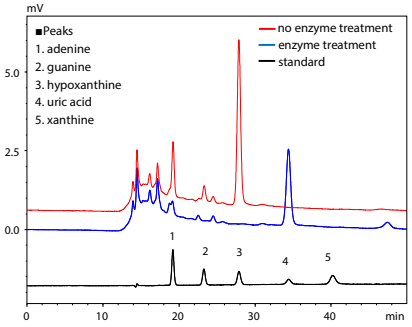

Fig. 4 Chromatograms of Tuna

Table 2 Individual Purine Base Content, Total Purine Content, and Uric Acid Equivalent (mg/100 g)

	Adenine	Guanine	Hypoxanthine	Xanthine
Tuna	17.0	12.4	175.6	0.0
Broccoli	25.9	32.0	0.4	0.0

	Total Purine Content	Uric Acid Equivalent
Tuna	205.0	251.9
Broccoli	58.3	68.3

Analysis of Broccoli

Fig. 5 shows the chromatograms of broccoli. The black line is the standard, the red line the sample without enzyme treatment, and the blue line the sample with enzyme treatment. Adenine, guanine, and hypoxanthine were separated and detected from the broccoli sample. Based on the obtained results, the total purine content in 100 g of broccoli was 58.3 mg. The uric acid equivalent was 68.3 mg/100 g. The content of each purine base, the total purine content, and the uric acid equivalent are summarized in Table 2.

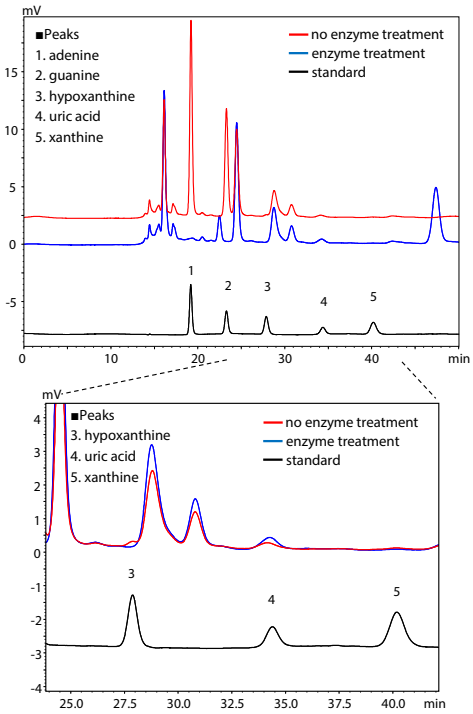

Fig. 5 Chromatograms of Broccoli

Confirmation Using UV Spectra

Using the photodiode array (PDA) detector originally equipped in the Prominence-i 3D, the process of enzyme treatment can be observed.

Fig. 6 shows the UV spectra of adenine at the elution position, which was eluted at about 20 min as shown in Fig. 4. The black line is the standard, the red line the sample without enzyme treatment, and the blue line the sample with enzyme treatment. Regarding the peaks detected around the retention time of adenine in Fig. 4 for the sample with enzyme treatment, adenine was not observed in the spectrum. Therefore, these peaks are likely to be due to impurities.

As demonstrated, the PDA detector is effective for monitoring the process of enzyme treatment and the existence of purine bases.

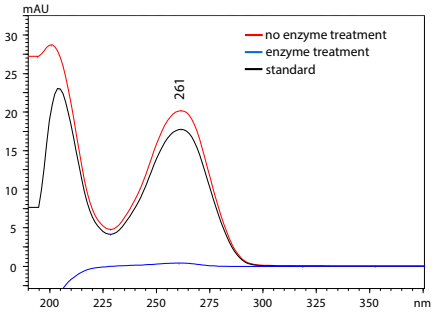

Fig. 6 Spectra of the Adenine Elution Position

<References>
1) Kaneko K, Yamanobe T and Fujimori S, Biomed Chromatogr, 23, 858-864 (2009)

This article was created with the collaboration of Professor Kiyoko Kaneko of the Faculty of Pharmaceutical Sciences at Teikyo University.

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