Application Note

420008X

High-Speed Separation of ATP and its Degradation Products by Ultra High-performance Liquid Chromatography and its Application to Evaluation of Degree of Freshness of Fish Meat

Introduction

ATP(adenosine triphosphate) in fish meat is decomposed as time elapses following the route shown below.

ATP → ADP(adenosine diphosphate) → AMP(adenosine monophosphate) → IMP(inosinic acid) → Ino(inosine) → Hypo(hypoxanthine)

There is a K value which indicates the degree of freshness of fish meat, and is defined as follows.

K value(%)=[(Ino + Hypo) / (ATP + ADP + AMP + IMP + Ino + Hypo)] x 100

It is known that fish meat can be used for sashimi if K value is less than 20% and can be used for cooking and processing if K value is 20 - 60%.

In this report, the degree of freshness of fish meat is measured by calculating K values using Ultra High-performance Liquid Chromatography (UHPLC). In addition, the time course of K values, i.e., degradation of the freshness, was measured. K value was calculated using the free calculation function in the ChromNAV CDS.

Keyword : UHPLC,ATP, ADP, AMP, IMP, Ino, Hypo, 2.0 µm, C18 column, UV-Vis detector

Experimental

Equipment.

Pump:	X-LC 3185PU x 2
Degasser:	X-LC 3080DG
Mixer:	X-LC 3180MX
Column oven:	X-LC 3067CO
Autosampler:	X-LC 3159AS
Detector:	X-LC 3070UV

Conditions.

Column:	X-PressPak AQ-C18-W (3.0 mmID x 50 mmL, 2.0 µm)
Eluent A:	100 mM Phosphate buffer (pH 4.2)
Eluent B:	100 mM Phosphate buffer (pH 4.2)/Acetonitrile (50/50)
Gradient condition:	(A/B), 0 min(100/0)→ 2.2 min(100/0)→ 6.0 min(50/50)→ 7.0 min(50/50)→ 7.05 min(100/0) 1 cycle; 10 min
Flow rate:	0.6 mL/min
Column temp.:	30ºC
Wavelength:	260 nm
Injection volume:	1 µL
Standard sample:	ATP and degradation products 1.0 µg/mL each

Results

Fig. 1 shows chromatogram of adenosine nucleotides. Analysis time was shortened approximately 1/8 times as compared with conventional HPLC without sacrificing the resolution of each component.

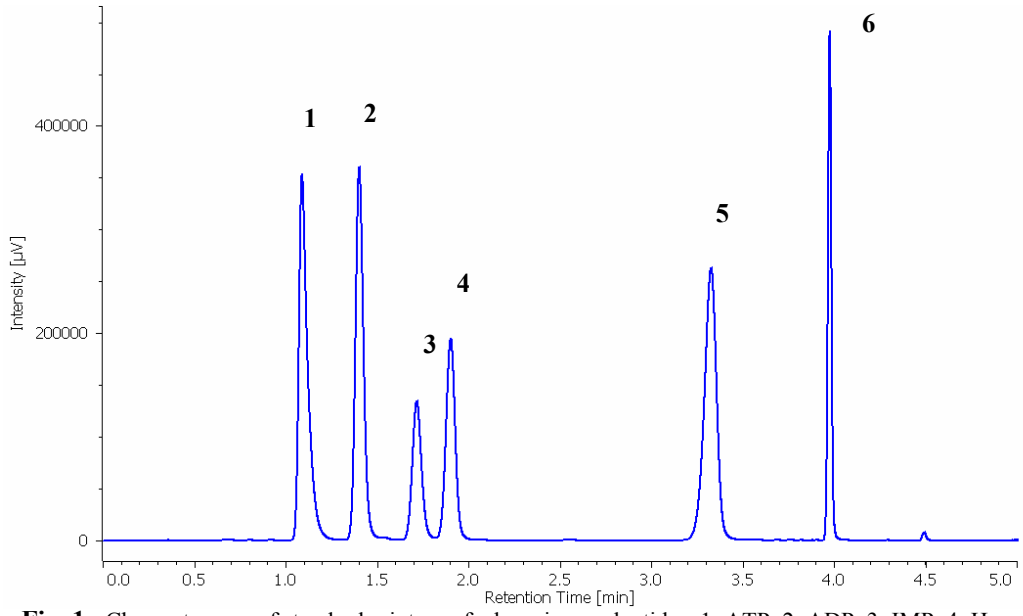

Fig. 1. Chromatogram of standard mixture of adenosine nucleotides 1: ATP, 2: ADP, 3: IMP, 4: Hypo, 5: AMP, 6: Ino

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Fig. 2 shows a chromatogram of adenosine nucleotides in sashimi grade tuna fish meat two days after purchase. Target six components are detected without any interference by contaminants. This sample's K value was 8 %.

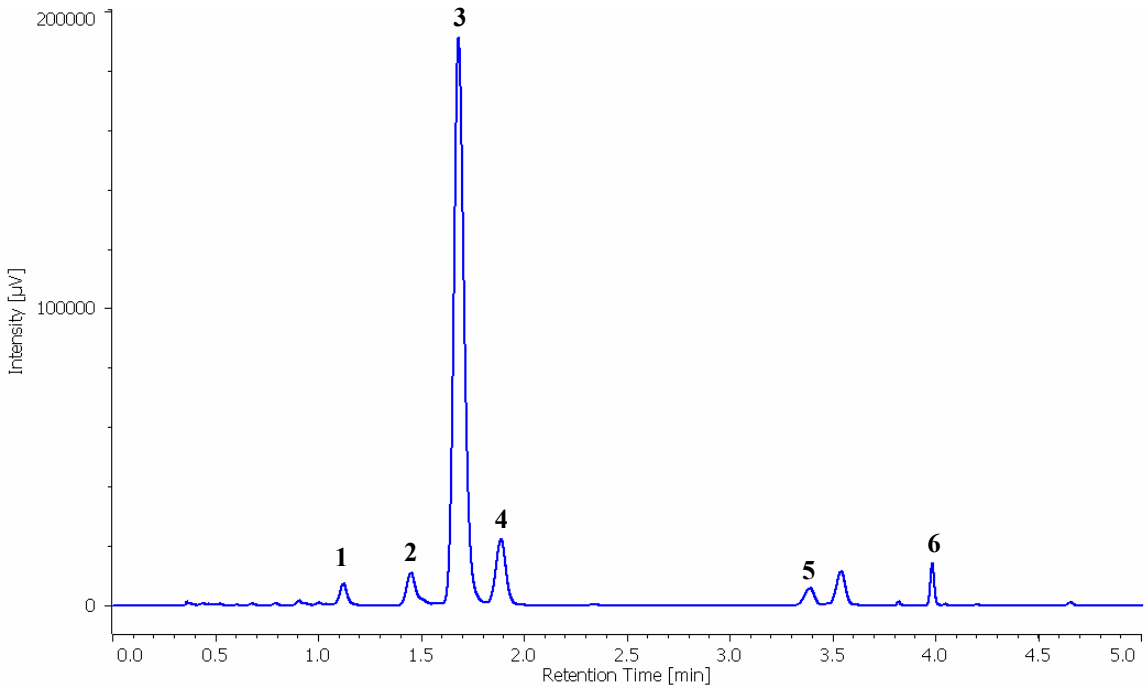

Fig. 2. Chromatogram of adenosine nucleotides in sashimi grade tuna fish meat (stored in a refrigerator at 4ºC for two days after purchase). 1: ATP, 2: ADP, 3: IMP, 4: Hypo, 5: AMP, 6: Ino

Preparation. 0.4 M perchloric acid aqueous solution(20 mL) was added to tuna fish meat (2.5 g) and homogenized. Then 2 M potassium carbonate aqueous solution(1 mL) was added to the obtained supernatant (5 mL) and applied to centrifugal separation. Obtained supernatant was filtered with 0.2 μm membrane filter.

Fig. 3 shows the time course of the degradation of tuna fish meat.

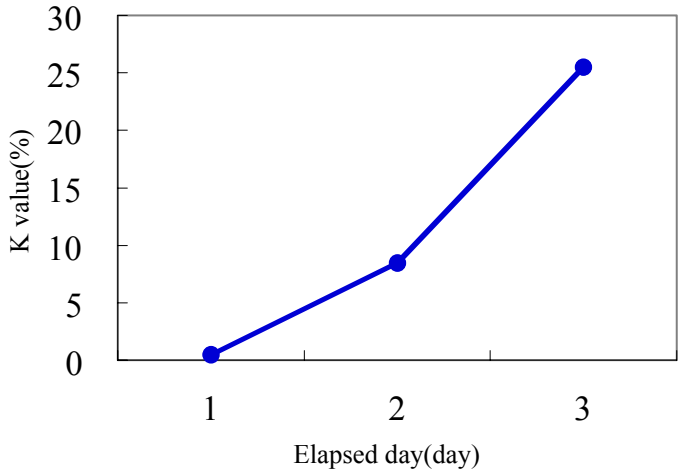

Fig. 3. Time course of the degradation of tuna fish meat

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