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Technical note

High-performance liquid chromatographic analysis of ascorbyl-2-phosphate in fish tissues

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Abstract

L-Ascorbic-2-phosphate magnesium salt (APM) in fish tissues was determined by high-performance liquid chromatography. APM extracted with 5% metaphosphoric acid was separated by a LiChrospher 100 RP18 column within 20 min. The detection limit was 0.1 μg/g tissue.

Keywords: Ascorbyl-2-phosphate; Ascorbic acid; Vitamins

1. Introduction

Because L-ascorbic acid is very labile, stable derivatives of this vitamin have been synthesized. L-Ascorbyl-2-phosphate Mg (APM), one of these derivatives, is more stable than L-ascorbic acid in fish feeds and has vitamin C activity equal to ascorbic acid in many species of fish [1–5]. Several methods for determination of APM in fish tissues have been proposed [4,5]. However, these methods cannot completely separate APM from other substances in fish tissues. Therefore, we have developed an analytical method for determination of APM in fish tissues, which can separate APM from other materials in 20 min with as little as 0.1 g of tissue.

2. Experimental

2.1. Equipment

Isocratic analyses were performed with a Model LC-6A pump unit (Shimadzu, Kyoto, Japan), equipped with a 7125 injector (Rheodyne, Cotati, CA, USA). Chromatographic separation was performed using a LiChrospher 100 RP18 column (250×4 mm I.D., 5 μm, Kanto, Tokyo, Japan). The effluent was monitored at 254 nm with a Model SPD-10A UV–Vis detector (Shimadzu). For integration, a Model C-R4A Chromatopac (Shimadzu) was used.

2.2. Materials

APM was a provided by Dr. M. Ukawa (Marubeni Shiryo). *n*-Octylamine and metaphosphoric acid were

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of analytical grade and obtained from Wako (Osaka, Japan). Acid phosphatase was purchased from Sigma (St. Louis, MO, USA). All other reagents were also of analytical grade.

2.3. Sample preparation

Liver was collected from carp *Cyprinus carpio* (average body weight 100 g) with (50 mg/100 g) or without APM in the diet. Liver and intestine were also collected from yellowtail *Seriola quinqueradiata* (average body weight 500 g) with APM (20 mg/100 g) in the diet. All samples were kept at −80°C until analysis. Liver and intestine (0.1–0.5 g) were homogenized with 10 ml of 5% metaphosphoric acid, and centrifuged at 6000 *g* at 0°C for 10 min. The supernatant was filtered for deproteinization by Molcut II (Nihon Millipore, Tokyo, Japan) and 10 μl of the filtrate was analyzed by HPLC.

2.4. High-performance liquid chromatography

A 10-μl volume of the extract was injected onto the column. The mobile phase consisted of 50 m*M* KH_2PO_4 containing 5% (v/v) acetonitrile and 0.0475% *n*-octylamine, and its pH was adjusted to 2.2 with phosphoric acid. The flow-rate was 1 ml/min.

3. Results and discussion

Miyasaki et al. [3] have analyzed APM in various tissues of rainbow trout *Oncorhynchus mikiss* using a Shodex RS pak DE-613 column with mobile phase pH 4.6. We analyzed the extract obtained from the liver of carp with or without APM in the diet by their method. As shown in Fig. 1b, there were a few peaks with retention times near APM. Similarly, using a LiChrospher 100 RP18 column, there were peaks with retention times near APM (data not shown). As shown in Fig. 1d, when the pH of mobile phase was decreased to 2.2, there was no peak with a retention time similar to APM. Analyzing extracts obtained from the liver of carp with APM in the diet, APM was detected in the extract and clearly separated from other substances (Fig. 1e). With both correlation coefficients of 0.998 ($n=5$), the calibration graph was linear in the range of 0–20 μg/ml. The R.S.D. (10 μg/ml) was 2.15% ($n=4$). The recovery of APM (0.05 mg/ml) added to carp liver was 99.9±7.6% ($n=3$). The limit of detection was less than 0.1 μg/g. To confirm the absence of compounds co-eluting with APM, the extract was incubated with acid phosphatase at 37°C for 1 h. As shown in Fig. 2, this treatment resulted in the disappearance of the APM peak.

APM concentrations in the liver and intestine of yellowtail and carp can be determined by this method (Table 1). In addition, since this method can

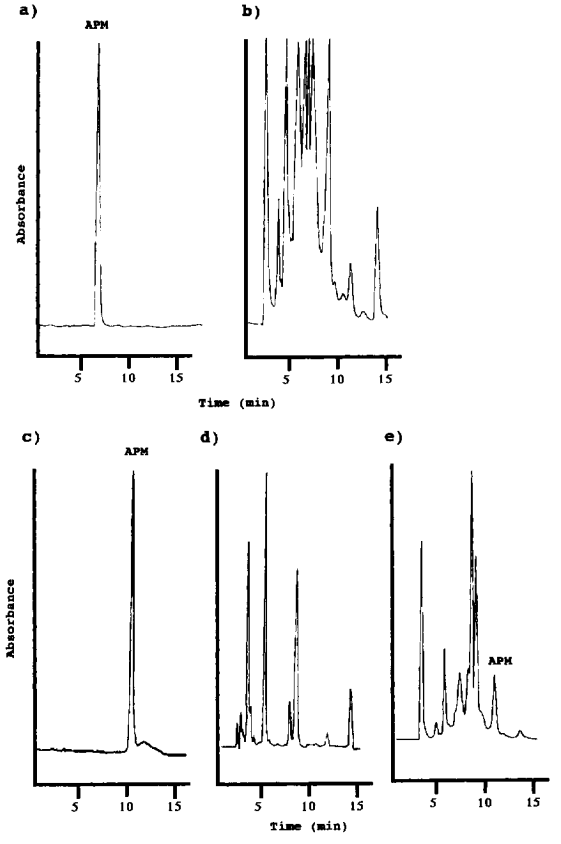

Fig. 1. Chromatograms of APM. (a) Column, Shodex RS pak DE-613; pH of mobile phase, 4.6; sample, APM standard (10 μg/ml). (b) Column, Shodex RS pak DE-613; pH of mobile phase, 4.6; sample, the liver of carp without APM in the diet. (c) Column, LiChrospher 100 RP18; pH of mobile phase, 2.2; sample, APM standard (10 μg/l). (d) Column, LiChrospher 100 RP18; pH of mobile phase, 2.2; sample, same as used in (b). (e) Column, LiChrospher 100 RP18; pH of mobile phase, 2.2; sample, the liver of carp with APM in the diet (50 mg/g).

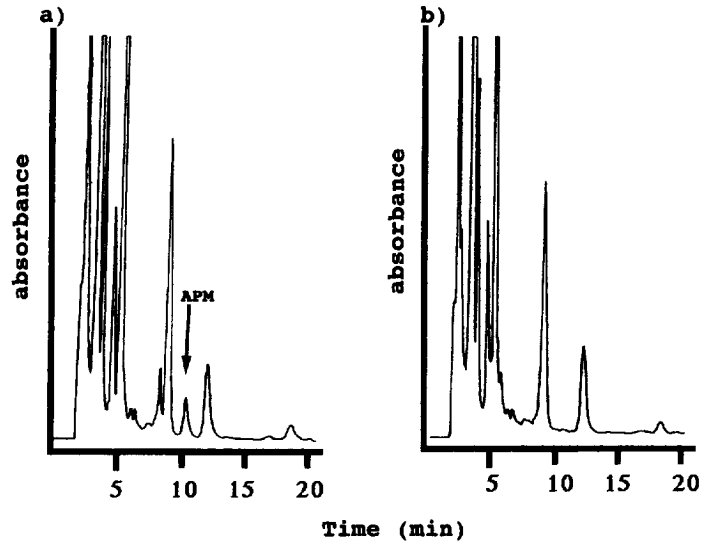

Fig. 2. (a) Chromatogram of extract obtained from the liver of yellowtail fed on diet with APM (50 mg/g). (b) Chromatogram of the extract incubated with acid phosphatase (10 U/ml) at 37°C for 1 h. Analytical conditions as for Fig. 1e.

Table 1
APM concentration in the liver and plasma of yellowtail and carp with or without APM in the diet

	n	APM in diet (mg/100 g)	Liver (mean±S.D.) (μg/g)	Plasma (mean±S.D.) (μg/ml)
Yellowtail	4	20	68.0±25.9	7.2±3.4
Carp	3	0	ND	ND
Carp	3	50	39.1±31.1	ND

ND=not detected; <0.1 μg/g or 0.1 μg/ml.

be carried out in 20 min with 0.1 g of tissue, it may be useful for studying the nutritional significance of APM in fish.

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