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Technical note

Simultaneous determination of ascorbic acid and dehydroascorbic acid in fish tissues by high-performance liquid chromatography

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Abstract

An high-performance liquid chromatographic method with post-column derivatization has been developed for the simultaneous determination of ascorbic acid (AA) and dehydroascorbic acid (DHAA) in fish tissues. Extracted AA and DHAA were separated by a Shim-pack SCR-1O1H column within 20 min, reacted with sodium hydroxide containing sodium borohydride and monitored at 300 nm. The detection limits for both AA and DHAA were 0.1 μg/ml.

1. Introduction

Various methods for the determination of ascorbic acid (AA) and dehydroascorbic acid (DHAA) have been proposed [1–4]. However, these methods are very time-consuming. Recently Yasui and Hayashi described the simultaneous analysis of AA and DHAA in tomato juice by ion-exclusion high-performance liquid chromatography using post-column derivatization with 100 m*M* sodium hydroxide containing 100 m*M* sodium borohydride as reagent [5]. However, since their method can not completely separate AA and DHAA from other substances in fish tissues, we have tried to develop an analytical method for the simultaneous determination of AA and DHAA in fish tissues. The analytical method developed can separate AA and DHAA from other compounds and requires a total run-time of 20 min and a 0.5-g amount of liver or a 0.2-ml volume of plasma.

2. Experimental

2.1. Equipment

Isocratic analyses were performed with a Model LC-6A pump (Shimadzu, Kyoto, Japan), equipped with a Model 7125 injector (Rheodyne, Cotati, CA, USA). The flow-rate of the mobile

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phase (5 m*M* oxalic acid) was 0.8 ml/min. Temperature of the column and the reaction coil ("j" type piping kit; Shimadzu) were controlled at 30°C by a Model CTO-6A column oven (Shimadzu). Chromatographic separations were performed on an ion-exclusion column, Shim-pack SCR-1O1H (particle size, 10 μm; 300 × 7.9 mm I.D.; Shimadzu). The post-column reagent (100 m*M* sodium hydroxide containing 100 m*M* sodium borohydride) was delivered at a flow-rate of 0.6 ml/min by a Model LC-6A pump (Shimadzu). The mixed effluent was monitored at 300 nm with a Model SPD-10A UV–Vis detector (Shimadzu). For integration, a Model C-R4A Chromatopac (Shimadzu) was used.

2.2. Materials

DHAA was obtained from Aldrich (Milwaukee, WI, USA). AA, metaphosphoric acid and oxalic acid were of analytical grade and obtained from Wako Pure Chemical Industries (Osaka, Japan). All other reagents were of analytical grade.

2.3. Samples

Liver and blood were collected from cultured carp and yellowtail. Liver (0.5 g) was homogenized with 10 ml of 5% metaphosphoric acid and 0.2 ml of plasma was mixed with 0.2 ml of 10% metaphosphoric acid. Then these samples were centrifuged at 6000 *g* at 0°C for 10 min. The supernatants obtained were filtered for deproteination by Molcut II (Nihon Millipore Kogyo K.K., Tokyo, Japan) and 10-μl aliquots of the filtrate were analyzed by HPLC.

3. Results and discussion

AA and DHAA in the liver of yellowtail were determined by the method of Yasui and Hayashi [5], which is similar to our method except for the column used (Shimpack SCR-1O2H, particle size 7 μm; 300 × 8 mm I.D; Shimadzu Co.) and the mobile phase (2 m*M* perchloric acid). However, as shown in Fig. 1A, the baseline was not stable and separation of DHAA and AA from unknown peaks was incomplete. Thus we used the Shim-pack SCR 101H column which might give a stronger hydrophobic interaction with a given component than the Shim-pack SCR-102H column. Moreover, we used oxalic acid as the mobile phase because of its chelating effect to prevent loss of AA by complexing with metals. As shown in Fig. 1B, a stable baseline was obtained and DHAA, AA and unknown peaks were clearly separated from each other. Both calibration graphs were linear, for AA in the range 0.1–20 μg/ml and for DHAA in the range 0.1–10 μg/ml, with correlation coefficients of 0.999 ($n = 4$). The detection limits for both AA and DHAA were 0.1 μg/ml. The coefficients of

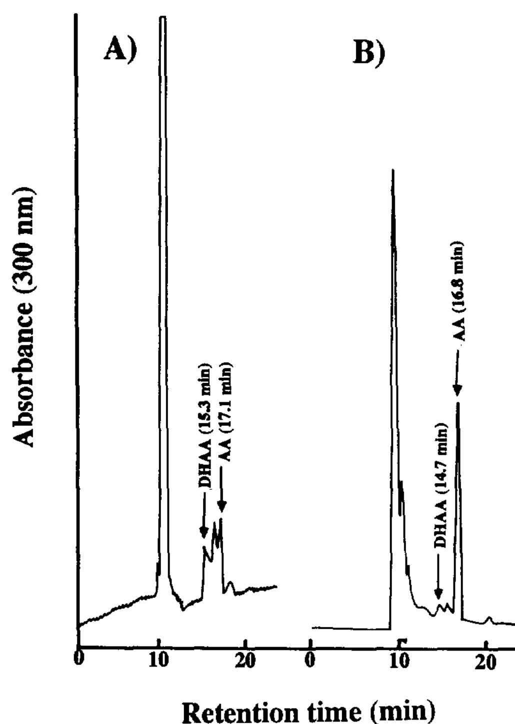

Fig. 1. Chromatograms of ascorbic acid and dehydroascorbic acid. (A) Column, Shim-pack SCR-1O2H; mobile phase, 2 m*M* perchloric acid; sample, yellowtail liver (AA = 107 μg/g, DHAA = 16 μg/g). (B) Column, Shim-pack SCR-1O1H; mobile phase, 5 m*M* oxalic acid; sample, yellowtail liver (AA = 154 μg/g, DHAA = 16 μg/g). The samples used in (A) and (B) were obtained from two different yellowtails.

Table 1
Contents of ascorbic acid, dehydroascorbic acid in the plasma and liver of yellowtail and carp

		Concentration (mean ± S.D.)			
		Plasma (μg/ml)		Liver (μg/g)	
		AA[a]	DHAA[b]	AA	DHAA
Yellowtail	($n = 3$)	5.2 ± 2.0	1.4 ± 1.1	87 ± 6.0	9.1 ± 1.2
Carp	($n = 3$)	6.00 ± 0.18	0.82 ± 0.02	141 ± 12	13 ± 0.5

[a] Ascorbic acid.
[b] Dehydroascorbic acid.

variation for AA and DHAA in the carp liver were 2.15% and 2.66% ($n = 4$), respectively. The recoveries of AA and DHAA added to carp liver were 99.9 ± 7.6% and 86.4 ± 3.0% ($n = 3$), and those of AA and DHAA added to yellowtail plasma were 93.3 ± 3.7% and 101.5 ± 3.4% ($n = 3$), respectively. Pretreatment of this method is very simple and the reproducibility and recovery of the method are satisfactory. AA and DHAA contents in the liver and plasma of yellowtail and carp can be determined by this method (Table 1). In addition, this method only requires a total run time of 20 min and a 0.5-g amount of liver or a 0.2-ml volume of plasma. Therefore the present method may provide a useful tool for studying the physiological, biochemical and nutritional significance of ascorbic acid in fish.

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