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o-Phthalaldehyde post-column derivatization for the determination of gizzerosine in fish meal by high-performance liquid chromatography

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ABSTRACT

A method for the fluorimetric determination of gizzerosine by high-performance liquid chromatography was developed. *o*-Phthalaldehyde reagent without thiol was used for derivatization. A higher selectivity for gizzerosine was obtained with this reagent than with *o*-phthalaldehyde reagent containing thiol. This method will be useful for the determination of gizzerosine in fish meal.

INTRODUCTION

Gizzerosine [2-amino-9-(4-imidazolyl)-7-azanonanoic acid] from fish meal has been found to be the cause of gizzard erosion in chicks[1]. It is assumed that gizzerosine is formed by the reaction between the ε-amino group of lysine and histidine when heated[1]. It is now important to be able to determine gizzerosine in fish meal for quality control purposes.

Fluorimetric detection of gizzerosine after reaction with *o*-phthalaldehyde and high-performance liquid chromatography (HPLC) has been reported[2–4]. The *o*-phthalaldehyde reagent contained thiol, as frequently used for the detection of amino acids and amines. However, the selectivity for gizzerosine is not satisfactory and the purification procedure before injection is time consuming. In this work we used *o*-phthalaldehyde reagent without thiol in order to obtain higher selectivity.

EXPERIMENTAL

Apparatus

A Shimadzu LC-9A high-performance liquid chromatograph equipped with an RF-535 fluorimetric detector, a SIL-6B sample injector and a CTO-6A column oven were used. Another LC-9A pump was used to deliver reaction reagent. The reaction tube set in the CTO-6A oven was a stainless-steel tube (7 m × 0.3 mm I.D.).

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Chemicals

Distilled water was of HPLC grade. *o*-Phthalaldehyde was of biochemical grade and citric acid, boric acid, phosphoric acid, sodium hydroxide and polyoxyethylene lauryl ether were of analytical-reagent grade. All reagents were purchased from Wako (Osaka, Japan). Gizzerosine standard and fish meal were kindly donated by the National Federation of Agricultural Cooperative Associations (Tokyo, Japan).

Chromatographic conditions

The strong cation exchanger Shim-pack ISC-07/S1504 (150 mm × 4 mm I.D.), obtained from Shimadzu (Kyoto, Japan), was used. The mobile phase was 30 m*M* sodium borate buffer (pH 9.8) at a flow-rate of 0.4 ml/min. The *o*-phthalaldehyde reagent was 15 m*M* citric acid containing 0.08% *o*-phthalaldehyde and 0.4% polyoxyethylene lauryl ether; the ether was used to keep the surface of the reaction tube clean. The flow-rate was 0.2 ml/min. The column temperature and reaction temperature were both 45°C.

Sample pretreatment

A 200-mg amount of fish meal was hydrolysed with 2 ml of 6 *M* hydrochloric acid at 110°C for 22 h. The sample was then filtered and evaporated under reduced pressure to remove the acid. The residue was dissolved in 2 ml of 10 m*M* sodium phosphate buffer (pH 2.6) and 200 μl of the solution were applied to a Bond Elut C_{18} column (catalogue No. 607101, packing volume 100 mg) obtained from Analytichem International (Harbor City, CA, U.S.A.) and the eluate was collected. A 200-μl volume

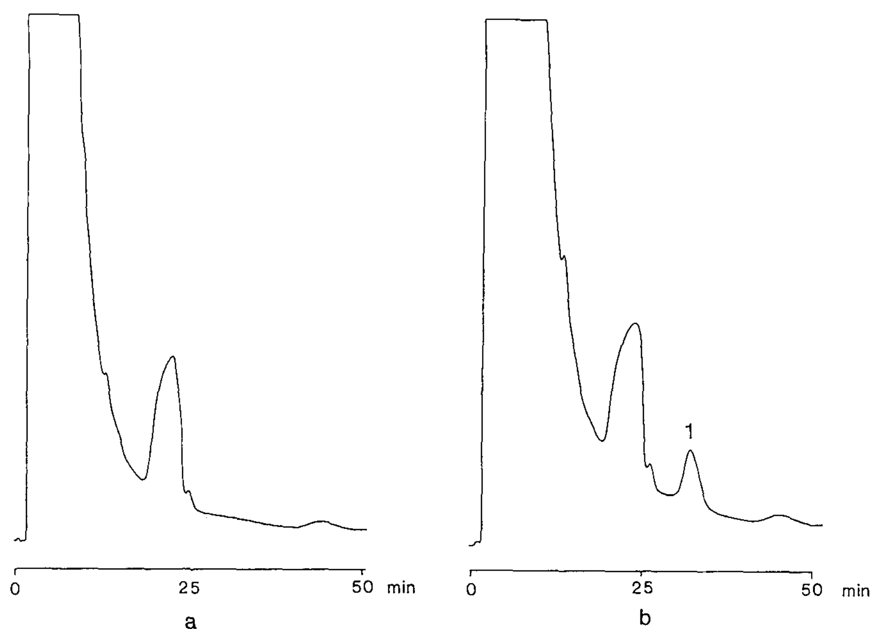

Fig. 1. Chromatograms of fish meal samples containing 65% of crude protein obtained using *o*-phthalaldehyde reagent as described. (a) Blank; (b) with 10 ppm of gizzerosine added. Peak 1 = gizzerosine.

Fig. 2. Chromatograms of a fish meal sample and a standard sample using *o*-phthalaldehyde reagent containing thiol. (a) Fish meal (blank) (same sample as in Fig. 1a); (b) standard sample. Peak 1 = gizzerosine (10 ng), corresponding to 10 ppm in fish meal.

of water was then applied and the eluate was collected in the same vial. A 20-μl volume of the eluate (400 μl) was injected.

RESULTS AND DISCUSSION

The optimum wavelengths were 320 nm (excitation) and 410 nm (emission). The optimum reaction pH was *ca.* 8.0. Chromatograms of fish meals (crude protein 65%) are shown in Fig. 1 (a) blank; (b) with 10 ppm of gizzerosine added]. Another fish meal sample (crude protein 60%) was tried and no interference peak was observed. Fish meals that induced heavy gizzard erosion in chicks contained 20 ppm of gizzerosine[2]. The detection limit was *ca.* 0.5 ppm in fish meal. The detector response was linear up to 1000 ng. The linear regression equation for the calibration graph was $y = 3912x - 331$ ($r = 0.999$), where y = peak area (μVs)and x = amount of sample (ng). The mean retention time and recovery at 10 ppm were 30.2 min and 98.2% ($n = 5$), respectively, with relative standard deviations of 1.8% and 1.5%, respectively. The reagent is selective to gizzerosine, compared with the *o*-phthalaldehyde reagent containing thiol.

The aminoethylimidazolyl group of gizzerosine is assumed to react with *o*-phthalaldehyde and produce a fluorescent compound[5]. Fig. 2 shows the chromatograms (standard sample and fish meal) obtained using *o*-phthalaldehyde reagent containing thiol. An interference peak was observed.

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