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Note

High-performance liquid chromatographic method for the simultaneous determination of oxolinic, nalidixic and piromidic acids in cultured fish

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Oxolinic acid (OA), nalidixic acid (NA) and piromidic acid (PA) are synthetic antibacterial agents widely used in human and piscine medicine. Although numerous methods for their determination in pharmaceutical products and biological specimens have been reported[1–12], there are only a few methods for the simultaneous determination of these compounds[13,14]. We have established a rapid, sensitive and accurate method which eliminates the need for an internal standard.

EXPERIMENTAL

Chemicals

OA, NA and PA were generously supplied by Tanabe Seiyaku (Osaka, Japan), Daiichi Seiyaku (Tokyo, Japan) and Dainippon Seiyaku (Osaka, Japan), respectively. All reagents were of analytical grade (Wako, Osaka, Japan).

Apparatus and conditions

The high-performance liquid chromatographic (HPLC) system consisted of a Tri-rotar VI pump, an Uvidec-100 VI UV–VIS detector (JASCO, Tokyo, Japan), a Model 7125 injector (Rheodyne, Berkeley, CA, U.S.A.) and a CR-3A integrator (Shimadzu, Kyoto, Japan). The wavelength was first set at 254 nm, and after 6.5 min, it was immediately changed to 280 nm by a programming technique. Analysis was performed on a 7-μm Nucleosil C_8 column (250 mm × 4.0 I.D. mm) at ambient temperature. The mobile phase was acetonitrile–methanol–0.1 *M* citric acid (containing 0.01 *M* tetra-*n*-butylammonium bromide) (2:4:4), flow-rate 1.0 ml/min.

Analytical procedure

A 5-g amount of homogenized tissue was extracted with 50 ml of methanol, and centrifuged for 10 min at 2300 *g*. The residue was re-extracted using the same procedure. The methanol was transferred to a separating funnel, and 200 ml of a succinic acid buffer solution (pH 4), 4 g of sodium chloride and 50 ml of ethyl acetate were added. The solution was shaken gently, and extracted twice with ethyl acetate. The pooled extracts were rinsed with water, and extracted twice with 50 ml of a

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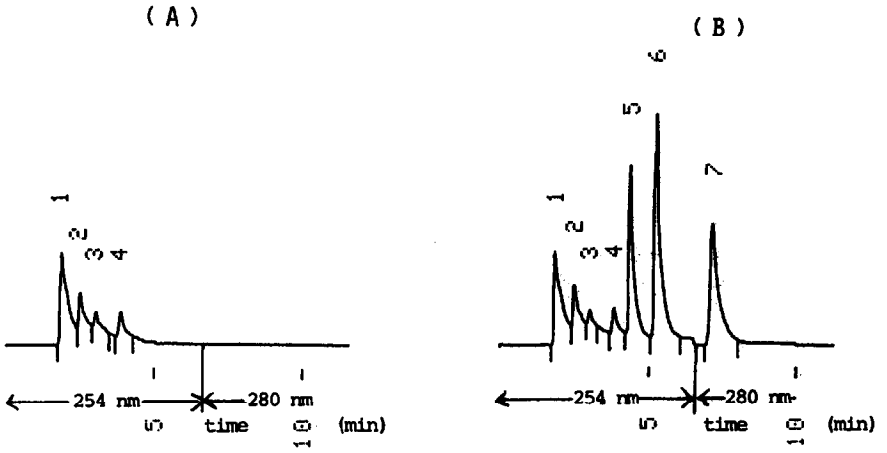

Fig. 1. Chromatograms of extracted eel: (A) blank eel sample; (B) eel sample spiked with 0.2 μg/g of both OA and PA and 0.4 μg/g of NA. Peaks: 5 = OA, 6 = NA and 7 = PA (peaks 1–4 are unidentified).

borate buffer solution (pH 10). The pooled aqueous layers were acidified to pH 4 with 1 *M* hydrochloric acid and extracted twice with 30 ml of ethyl acetate. The organic layer was rinsed with water, dried with anhydrous sodium sulphate, evaporated just to dryness with a rotary evaporator and dissolved in 5 ml of the mobile phase. A 20-μl aliquot of the solution was injected for HPLC analysis.

RESULTS AND DISCUSSION

The separation of OA, NA and PA was carried by reversed-phase ion-pair chromatography and wavelength programming. Fig. 1 shows typical chromatograms of eel and eel spiked with OA, NA and PA. The retention times were 4.4 min for

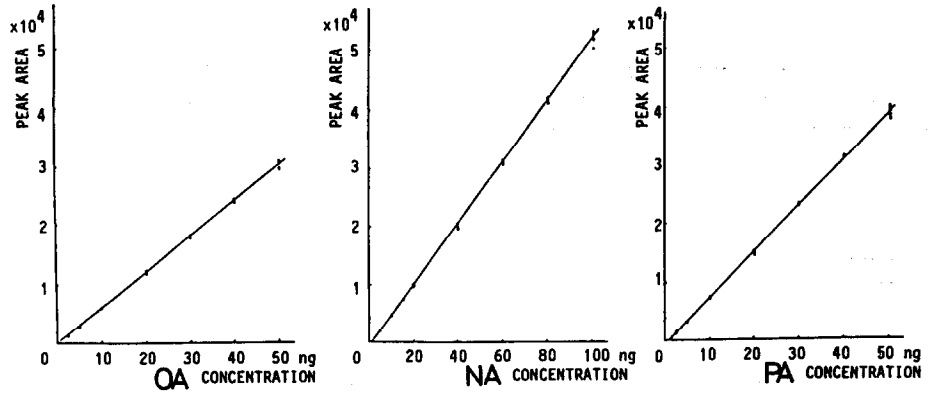

Fig. 2. Calibration graphs for OA, NA and PA. The correlation coefficients of the lines are r = 0.999, 0.999 and 0.998 for OA, NA and PA, respectively.

TABLE I

RECOVERIES OF OA, NA AND PA FROM CULTURED FISH

n = 5; C.V. = coefficient of variation.

Compound	*Recovery (%)**	*C.V. (%)*
OA (1 μg/g)	93.0 ± 1.5	1.6
NA (2 μg/g)	83.9 ± 3.9	4.6
PA (1 μg/g)	83.0 ± 5.4	6.5

* Mean ± S.D.

OA, 5.2 min for NA and 7.2 min for PA. OA, NA and PA were found not to interfere with the assay. Fig. 2 shows that the calibration graphs were linear from 2.5 to 50 ng for OA and PA, and from 5 to 100 ng for NA (0.02 a.u.f.s.). The limit of detection was 1 ng for each compound (0.005 a.u.f.s.). Recovery values are listed in Table I. The recovery examination was performed by analyzing samples (eel, n = 5) spiked with 1 μg/g of both OA and PA, and 2 μg/g of NA. The coefficients of variation were 1.6% for OA, 4.6% for NA and 6.5% for PA. The sensitivity and reproducibility are sufficient to determine the residual drugs in cultured fish.

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