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Note

Rapid high-performance liquid chromatographic method for the determination of oxolinic acid in chicken plasma

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Oxolinic acid (OA; 5-ethyl-5,8-dihydro-8-oxo-1,3-dioxolo[4,5-*g*] quinoline-7-carboxylic acid) is an antibacterial drug used for humans and animals. It is one of the derivatives of nalidixic acid, an antibacterial drug used in man (Fig. 1).

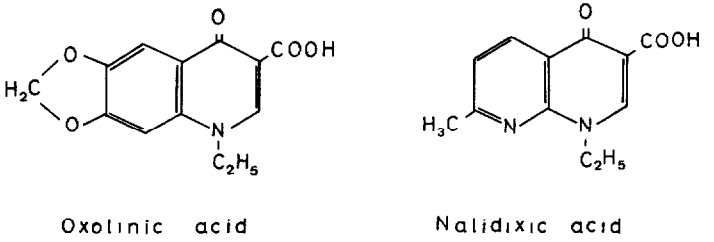

Fig. 1. Structures of oxolinic acid and nalidixic acid, the internal standard.

Recently, OA was reported to show toxicities on the central nervous system, such as motor activity increase, irritability and stereotyped behaviour [1, 2]. Thus, the determination of OA in blood is needed for pharmaceutical and clinical studies. Several methods to determine OA, such as bioassay [3, 4], fluorometry [5] and high-performance liquid chromatography (HPLC) [6], were described. However, the bioassay and fluorometry methods are poor in selectivity and the HPLC procedure has a long pretreatment and is very complex.

The purpose of the present work was to develop a rapid and simple HPLC assay procedure for the determination of OA in chicken plasma.

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EXPERIMENTAL

Chemicals

OA and nalidixic acid, as internal standard, were kindly supplied from Tanabe Seiyaku (Osaka, Japan). Ekicrodisc 13CR (0.45-μm disposable filter) was supplied by Gelman Science Japan (Tokyo, Japan). All other reagents were of analytical grade.

Chromatography

The liquid chromatography system consisted of a Tri Rotar V pump (Japan Spectroscopic Co., Hachioji, Japan), a Rheodyne 7125 injector (Rheodyne, Cotati, CA, U.S.A.) with 20-μl loop and a Uvidec-100V variable-wavelength UV detector (Japan Spectroscopic Co.) set to 260 nm. The detector sensitivity was 0.04 a.u.f.s. The column was Nucleosil C_8, 5 μm particle size, 250 × 4.6 mm I.D. (Macherey-Nagel, Düren, F.R.G.), maintained at 36°C. Peak areas were quantified by means of a Hewlett-Packard 3390A integrator (Yokokawa Hewlett-Packard, Tokyo, Japan) with 4 mV f.s.d. The mobile phase consisted of methanol—0.1 *M* citric acid—acetonitrile (6:7:1). The flow-rate was 1.0 ml/min.

Standard curves

Standard samples for determination of OA were prepared for each series of runs by spiking known amounts of OA to drug-free plasma, 0.01—16 μg/ml.

Extraction procedure

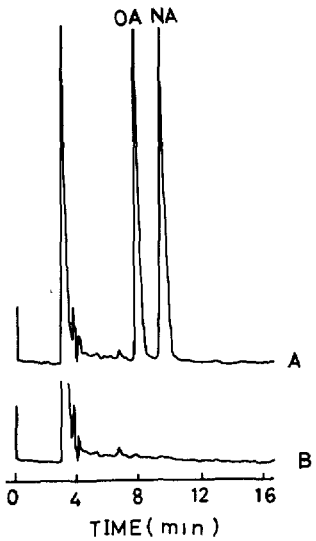
To 0.5 ml of plasma, 10 ml of acetonitrile were added. After mixing for 10 min on a mechanical shaker and adding 10.0 ml of internal standard solution (0.2 μg/ml nalidixic acid in acetonitrile), the organic layer was filtered by Ekicrodisc 13CR (0.45-μm disposable filter) and transferred to a 100-ml flask. The organic layer was evaporated to dryness at 40°C. The residue was dissolved in 500 μl of methanol and an aliquot of 20 μl was injected into the HPLC system. The ratio of peak areas (OA/internal standard) was measured.

Animal study

A healthy chicken (body weight 1.2 kg) was fasted 17 h prior to and 12 h after oral administration of 20 mg/kg OA with 4 ml of water. A 1-ml sample of blood was collected in a heparinized tube from the sub-wing vein at 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 h after oral administration of OA. After centrifugation, the plasma was separated and frozen until determination.

RESULTS AND DISCUSSION

Typical chromatograms of blank plasma and a plasma sample from a chicken after oral administration of OA (20 mg/kg) are shown in Fig. 2. The retention times for OA and nalidixic acid (internal standard) in a C_8 reversed-phase column were 7.6 and 9.1 min, respectively. On the other hand, using a C_{18} reversed-phase column, the peak of OA was the same but the peak of nalidixic acid appeared later and was broader than with the C_8 column.



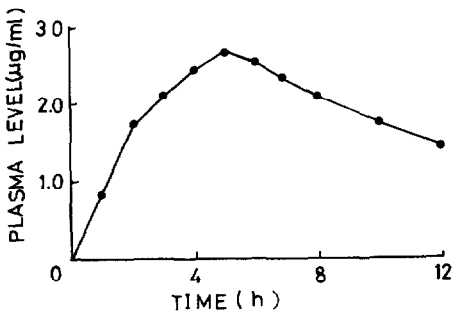


Fig. 2. Typical chromatograms of plasma preparation. (A) Plasma sample from a chicken after oral administration of OA (20 mg/kg); (B) blank plasma. Peaks: OA = oxolinic acid; NA = nalidixic acid (internal standard).

Fig. 3. Plasma concentration—time profile for a chicken given a single 20 mg/kg oral dose of oxolinic acid.

TABLE I

RECOVERY, INTRA- AND INTER-DAY COEFFICIENTS OF VARIATION (C.V.) OF OXOLINIC ACID IN PLASMA

Added (μg/ml)	Recovery ± C.V. (%) (n = 8)	Intra-day C.V. (%) (n = 6)	Inter-day C.V. (%) (n = 6)
0.8	98.9 ± 8.3	1.54	0.84
4.0	98.0 ± 3.6	0.38	2.90

Over the OA concentration ranges studied (0—16.0 μg/ml in plasma), linearity of response was found to be good ($r > 0.99$). The detection limit for OA in plasma was ca. 10.0 ng/ml. The recovery of OA and intra- and inter-day variations determined at two different concentrations (0.8 and 4.0 μg/ml) are summarized in Table I. The recovery of OA was almost complete and the variation in OA determination was very small. The present method was applied to the determination of OA in chicken plasma.

Fig. 3 shows the plasma concentration—time profile for a 20 mg/kg dose of OA orally administered to a chicken. The elimination half-life was 7.1 h and the peak plasma level was reached (2.72 μg/ml) at 5 h. Similar plasma concentration—time data have previously been reported [3, 4].

In summary, the HPLC method presented here provides a selective, reliable and reproducible method for the rapid determination of OA in chicken plasma. The method does not require time-consuming or complex extraction or derivatization techniques. I have found the method suitable for the pharmacokinetic studies of OA in chicken.

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