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Note

Ion-pair high-performance liquid chromatographic assay of levamisole in biological fluids

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The discovery and early development of the broad-spectrum anthelmintic drug tetramisole (2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]thiazole) was described in 1966 [1, 2]. The compound was found to be effective against numerous gastrointestinal and pulmonary nematodes in a variety of domestic animals and against at least two nematodes infesting the intestinal tract of man [2].

Levamisole is the laevo isomer of tetramisole and has fewer side-effects [3]. Methods for its determination in plasma have been described, based on polarographic assay [4], thin-layer chromatography [5] and gas chromatography [6]. Recently a high-performance liquid chromatographic (HPLC) method has been proposed for controlling veterinary anthelmintic preparations [7]. The aim with the method described here was to cover the same concentration range in biological samples as with the gas chromatographic method, but with a simpler sample handling procedure. The described HPLC method requires a single extraction step using a small volume of serum (0.5 ml), followed by ion-pair chromatography on a reversed phase, and is specifically designed for pharmacokinetic studies.

EXPERIMENTAL

Apparatus

A Model 202 chromatography with a U6K injector and a Model 6000A pump (Waters Assoc., Milford, MA, U.S.A.) was used, equipped with a Schoeffel GM 770 variable-wavelength UV detector and a 250 × 4.6 mm I.D. column packed with μBondapak C_{18} (particle size 10 μm) (Waters Assoc.).

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Reagents

The reagents were of analytical grade (Prolabo, Paris, France). Levamisole was purchased from Specia (Paris, France).

Standards

A stock standard solution containing 1 mg/ml of levamisole in methanol was prepared. The solution was diluted with mobile phase in order to obtain 1, 0.1 and 0.01 μg in a constant injection volume (10 μl). Standard solutions were stored at 4°C until use.

Operating conditions

The mobile phase was 0.2% acetic acid in water—methanol—heptane sulphonic acid (55:45:2) and the flow-rate was 2 ml/min (inlet pressure 200 bar). Before use the mobile phase was degassed by applying a vacuum to the solvent reservoir for approximately 5 min.

Detection was effected at 225 nm. The system was operated at ambient temperature (18—20°C) and the retention time for levamisole was 3.8 min. A typical chromatogram for 100 ng of levamisole injected directly into the column with a detection sensitivity of 0.01 a.u.f.s. is shown in Fig. 1.

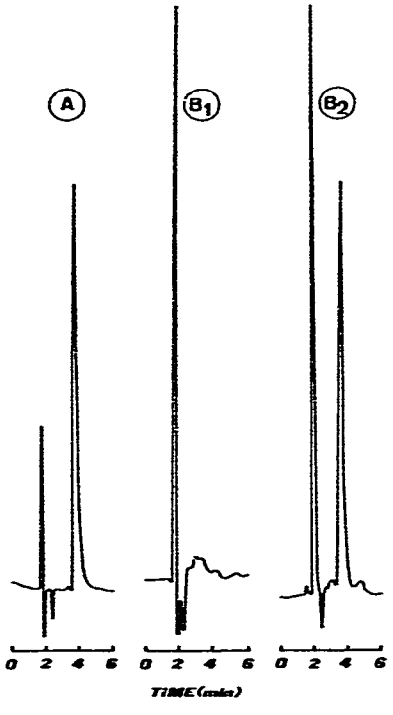

Fig. 1. Typical chromatograms: (A) corresponding to an injection of 0.1 μg of levamisole (0.01 a.u.f.s.) and (B) obtained on analysis of sheep blank plasma sample (B_1) and of plasma from sheep administered levamisole (B_2).

Extraction procedure

One millilitre of 0.1 *N* sodium hydroxide solution and 10 ml of chloroform were added to 0.5 ml of plasma, urine or mucus. The tubes were shaken for 10 min and centrifuged at 8400 *g* for 10 min. The organic phase was separated by filtration on phase-separating paper (Whatman 1PS) and evaporated to dryness under a stream of nitrogen gas to prevent oxidation. The residue was dissolved in 100 μl of eluent and the entire solution was injected into the column.

Calibration graphs

The recovery of levamisole added to sheep serum or mucus in the concentration range 0.1—1 μg was 70 ± 4%.

Analyses of the standards with or without extraction showed in both instances a high correlation between the concentration (x) and peak height (y). The equation of a typical calibration graph from 0.1 to 1 μg/ml was $y = 79.51x + 0.76$ and the correlation coefficient was 0.999.

When calibration graphs were constructed on five different days for a levamisole range of 0.1—1 μg/ml an excellent linear relationship was obtained each time. The slopes of the calibration graphs were reproducible, with a coefficient of variation of 3.5%.

RESULTS AND DISCUSSION

Ion-pair chromatography was performed by adjusting the pH of the mobile phase so that the sample was present in its ionic form (pH 3.5). A strongly ionic counter ion with a strong lipophilic group attached (heptanesulphonic acid; Waters Assoc.) was used.

The procedure described permits the rapid and selective determination of levamisole in biological fluids. Extraction with chloroform results in a clear extract that can be injected directly into the liquid chromatographic column without further purification. No interference from endogenous substances was observed. 4-Hydroxylevamisole, the major metabolite of levamisole, was also separated with a retention time of 2.8 min.

The method has been utilized in pharmacokinetic studies of levamisole in sheep. Preliminary results for plasma and mucus levels were obtained for intramuscular administration in two sheep. Each animal received a dose of 15 mg/kg and samples of blood were taken for 6 h and 2, 3, 4 and 6 h after the administration. Nasal mucus was collected on cotton plugs, which were weighed, rinsed and analysed for levamisole. A peak concentration (5.08 μg/ml) in plasma was observed 40 min after treatment (Fig. 2). The mean half-life of levamisole was about 1.8 h, and after 24 h a mean concentration of 0.05 μg/ml was still measurable.

Maximal nasal mucus levels were obtained 2 and 3 h after administration of the drug, and were 5—10 times greater than plasma concentrations. This demonstrates the ability of levamisole to diffuse from the blood into the pulmonary and bronchial secretions in which the drug exerts its anthelmintic activity against pulmonary worms. In the mucus fluid levels are effectively higher than the lethal concentration (2—6 μg/ml) as determined against selected strains of Trichostrongyles [8] or Ostertagia [9].

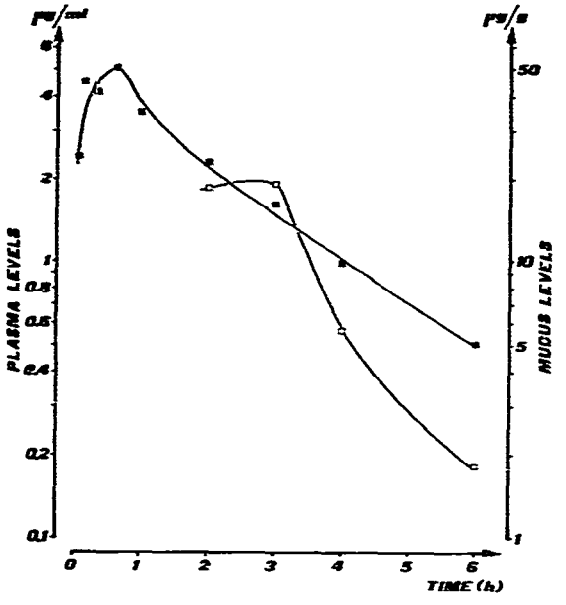

Fig. 2. Plasma (▪) and mucus (▫) levels of levamisole after intramuscular administration of 15 mg/kg of levamisole to a sheep.

The lowest concentration of levamisole that can be determined in 0.5 ml of serum with acceptable precision was 0.05 μg/ml. Approximately 20—25 samples can be conveniently assayed in 1 day by one analyst.

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