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Note

Liquid chromatographic determination of cyadox in medicated feeds and in the contents of the porcine gastrointestinal tract with fluorescence detection

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Cyadox (2-formylquinoxaline-N^1,N^4-dioxide cyanoacetylhydrazone) (see Fig. 1), manufactured by SPOFA (Prague, Czechoslovakia), is used in Eastern Europe as a feed additive for pigs, calves and poultry. Like the other quinoxaline N^1,N^4-dioxides used in veterinary practice, such as carbadox and olaquindox, cyadox also has a growth-promoting effect[1,2].

For a comparative study of pharmacological and toxicological properties, weaned pigs were fed carbadox, olaquindox and cyadox by in-feed medication[3]. To evaluate the efficacy of these drugs against one of the possible causative agents of swine dysentery (*Treponema hyodysenteriae*), a high-performance liquid chromatographic (HPLC) method for the determination of cyadox in feed and gastrointestinal samples had to be developed, analogous to the previously published method for carbadox[4].

This paper describes a method for the determination of cyadox in feed and gastrointestinal samples. As cyadox exhibits native fluorescence (Fig. 2), fluorescence detection was chosen. Cyadox was extracted with acetone and the extract was filtered. An aliquot of the extract was evaporated to dryness and the residue was dissolved in the mobile phase.

EXPERIMENTAL

Apparatus and reagents

A Kratos Spectroflow 400 liquid chromatograph and a Promis autosampler, equipped with a Perkin-Elmer PE 3000 fluorescence spectrophotometer and a Spectra

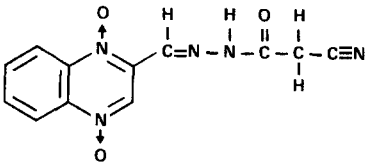

Fig. 1. Structure of cyadox.

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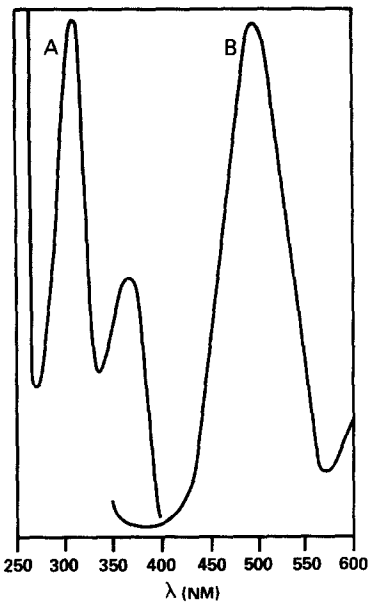

Fig. 2. Fluorescence spectrum of cyadox, 1 μg/ml in water–acetonitrile (85:15). (A) Excitation spectrum, emission at 487 nm. (B) Emission spectrum, excitation at 310 nm. Slit width excitation and emission, 15 and 20 nm, respectively. Scan speed, 30 nm/min.

Physics SP-4290 integrator were used. The mobile phase was water–acetonitrile (85:15) at a flow-rate of 0.4 ml/min. The chromatographic column consisted of two Chromsep HPLC columns, total length 200 mm × 3 mm I.D., packed with LiChrosorb RP-18, particle size 7 μm, connected with a Chromsep guard column packed with a 30–40 μm pellicular reversed-phase material (Chrompack, Middelburg, The Netherlands). Under these conditions the retention time of cyadox was about 10.5 min. The excitation and emission wavelengths were 310 and 487 nm, respectively. The injection volume was 20 μl.

For calibration, a stock solution containing 250 μg ml/of cyadox was prepared in dimethyl sulphoxide and standard solutions containing 0.25, 0.5, 1.0 and 2.0 μg /ml of cyadox were prepared by appropriate dilution with the mobile phase. Cyadox (purity 96.4%) was generously donated by Ing. L. Huda (Chemapol, Prague, Czechoslovakia).

As cyadox is light sensitive, all manipulations were carried out in a darkened room using low actinic glassware.

Feeding of cyadox to pigs

Thirty weaned piglets, aged 7 weeks, were divided into five groups of six pigs. Each group was housed separately and treated for 6 weeks with a medicated feed containing 19, 42, 82, 185 or 335 mg/kg of cyadox. In addition, one group of six pigs received unmedicated feed. Feed and drinking water was administered *ad libitum*.

After 6 weeks the animals were fasted for 16 h and then had access to their rations; 3 h later the animals were killed and gastrointestinal samples at defined

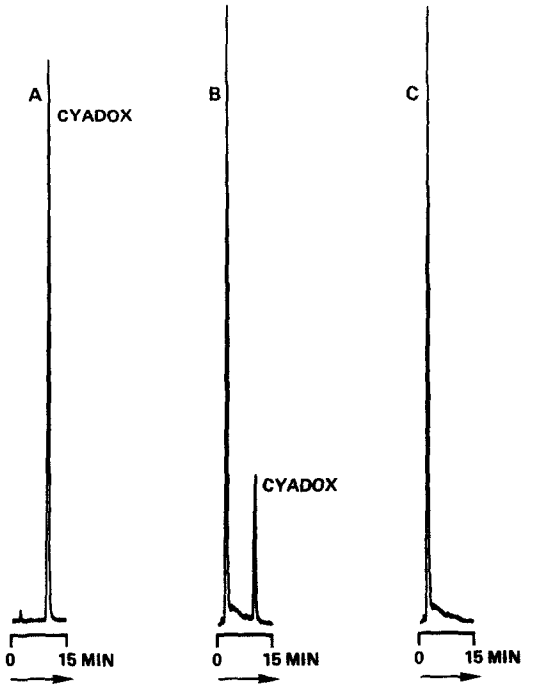

Fig. 3. (A) Cyadox standard solution, 2.0 μg/ml. (B) Sample of duodenal content of a pig fed with feed containing cyadox; the in-feed concentration was 50 mg/kg. (C) Sample of duodenal content of an untreated pig.

locations (see caption to Fig. 4) were taken at autopsy. The feed and gastrointestinal samples were stored at −20°C until analysis for cyadox.

Sample preparation

Samples of 0.1–2.0 g of freeze-dried gastrointestinal content or medicated feeds were weighed into 50-ml glass-stoppered Erlenmeyer flasks. After 20 ml of acetone had been added, the flasks were swirled on a shaking machine for 90 min. The extract was centrifuged at 300 *g* for 15 min and an aliquot was evaporated under a stream of nitrogen. The residue was dissolved in the mobile phase to yield solutions containing 0–2 μg/ml of cyadox. A 20-μl volume was injected for HPLC analysis.

RESULTS AND DISCUSSION

As cyadox exhibits native fluorescence, a very simple method of analysis was developed without a time-consuming clean-up and possible poor recoveries. The chromatograms showed no interference from matrix material (Fig. 3). The response for cyadox was linear in the range 0–2 μg/ml, with a correlation coefficient of $r = 0.998$ ($n = 4$). The limit of detection for a 1-g sample is *ca.* 0.1 μg of cyadox. Spiking of a control gastrointestinal sample with cyadox resulted in good recoveries (Table I).

TABLE I

RECOVERIES OF CYADOX IN SPIKED CONTROL DUODENAL SAMPLES

Cyadox added (μg/g)	*Recovery (%)*
250	82
100	77
50	88
25	76
12.5	75
2.5	79

During a period of 3 weeks, all gastrointestinal samples were analysed in duplicate as described. The peak areas of freshly prepared standard solutions did not change significantly during this period. On the basis of the cyadox profiles along the porcine gastrointestinal tract resulting from the feeding experiment (Fig. 4), an evaluation of the prophylactic efficacy of cyadox against enteral pathogens was made. In *Treponema hyodysenteriae*, one of the causitive agents associated with swine dysentery (dysentery Doyle), the cyadox concentrations in different parts of the gastroin-

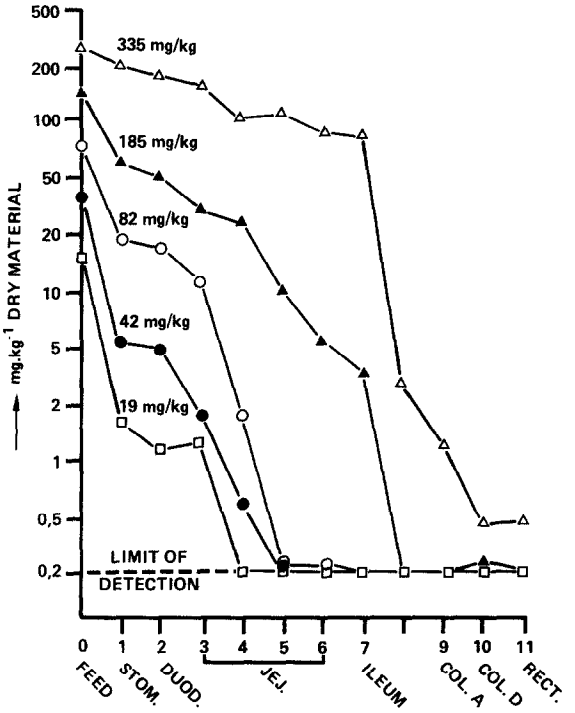

Fig. 4. Results of the determination of cyadox in feed and in gastrointestinal contents of 30 pigs fed medicated feed (mean of each treated group). Each sample was analysed in duplicate. 0 = feed sample; 1 = stomach; 2 = duodenum; 3 = jejunum, 75–125 cm from duodenum; 4 = jejunum, 50 cm around the centre; 5 = jejunum, 125–75 cm before ileum; 6 = jejunum/ileum, at junction; 7 = ileum; 8 = caecum; 9 = colomn ascendens; 10 = colon descendens; 11 = rectum.

testinal tract were interrelated with minimal inhibiting concentrations (MIC), as known from literature for this species. This showed that for cyadox in doses of 25 mg/kg or higher a prophylactic efficacy can be expected.

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