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Note

High-performance liquid chromatographic determination of Olaquindox® in feeds

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2-(N-2-Hydroxyethylcarbamoyl)-3-methylquinoxaline-1,4-dioxide, or Olaquindox (Bayo-N-Ox)®*, is a growth-promoting substance used in various European countries as an additive to pig feed. A spectrophotometric method, involving prior purification by thin-layer chromatography, has been described for its determination in feeds[1]. The increasing use of high-performance liquid chromatography (HPLC) in the determination of drugs and additives in feeds, and the possible widespread use of this technique in feed-industry routine control laboratories, has led to the development of a fast, accurate and reproducible HPLC method of analysis for Olaquindox in such samples. The strong UV absorption of this compound makes it detectable with a conventional single-wavelength (254 nm) detector. Because of the polar character of Olaquindox, an efficient reversed-phase chromatography stage is used as the ultimate purifying step. Moreover, this polarity permits the use of liquid–liquid partition with a non-polar solvent to remove interfering substances, *e.g.*, lipids and pigments. Interference from other commonly used additives has also been examined.

MATERIALS AND METHOD

Apparatus

Equipment and conditions were as follows: homogeniser (Ultra-Turrax Polytron); high-pressure liquid chromatograph (Spectra-Physics 3500 B, Orsay, France), with single pump and controlled flow-rate, equipped with a 8300 UV detector (dual flow-cells with a 1-mm bore and a 10-mm light path; volume 10 μl; detector attenuation: 0.04 a.u.f.s.; 1-mV universal recorder with chart speed 5 mm/min; pre-packed stainless-steel column (250 mm × 21 mm I.D.) of Spherisorb ODS C_{18} particle size 5 μm (Spectra-Physics); mobile-phase flow-rate: 1.2 ml/min; Valco-N60 injection valve with 10-μl sample loop.

Reagents

The following reagents were used: solvents: acetonitrile, 2,2,4-trimethylpentane (isooctane) and methanol (all analytical grade); potassium carbonate, analytical grade; standard Olaquindox, analytical grade; mobile phase: methanol–water (5:95) de-gassed under vacuum just before use.

* Bayer AG registered trade-mark.

Procedure

Olaquindox is very sensitive to light, and the entire procedure must be performed in the dark or under inactinic light.

Weigh 10 g of feed containing 50 ppm of olaquindox into a 150-ml centrifuge tube, then add 50 ml of acetonitrile and 3 g of potassium carbonate. Homogenise for a few minutes, then centrifuge at 2500 *g* and transfer 10 ml of the clear supernatant liquid to a 30-ml separating-funnel. Add 10 ml of isooctane, shake for a few minutes, set aside for 30 min, discard the isooctane phase, and evaporate the acetonitrile phase to dryness under vacuum. Dissolve the residue in 10 ml of methanol, then inject 10 μl of this solution into the chromatograph using the 10-μl sample loop.

RESULTS AND DISCUSSION

Analytical parameters

The retention time of Olaquindox is *ca.* 3 min (Fig. 1), and the response of the detector is linear for amounts from 0 to 500 ng. Thus, by using the apparatus at maximum sensitivity (0.005 a.u.f.s.), 1 ng of Olaquindox can be detected easily.

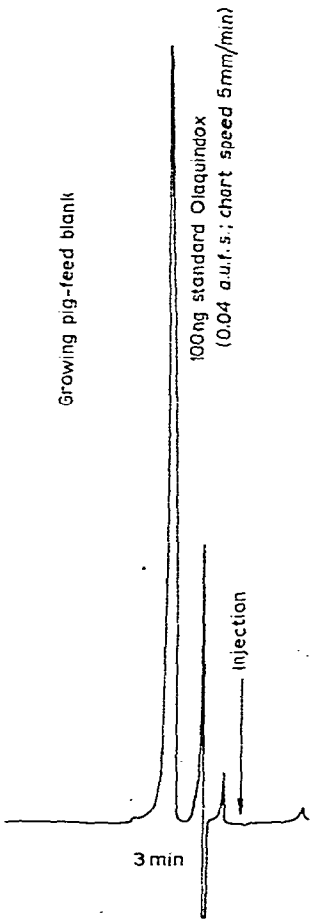

Fig. 1. Typical HPLC chromatogram obtained from standard Olaquindox.

Determination in feeds

Concentration steps were unnecessary in the procedure described, because of the high sensitivity of detection; interfering substances were therefore not concentrated. In order to avoid over-loading the column with substances (such as lipids) that could decrease its efficiency, liquid–liquid partition against an apolar solvent was used; interfering lipid-soluble pigments were also removed at this stage.

Different types of standard feeds were tested [chicken feed for its high lipid content, and rabbit feed for its strong pigment colour (due to alfalfa)] in order to reproduce more extreme conditions than those normally met with pig feed. Interference was either non-existent or very limited (see Fig. 2). The recovery and the reproducibility of the method were evaluated from 10 consecutive determinations on

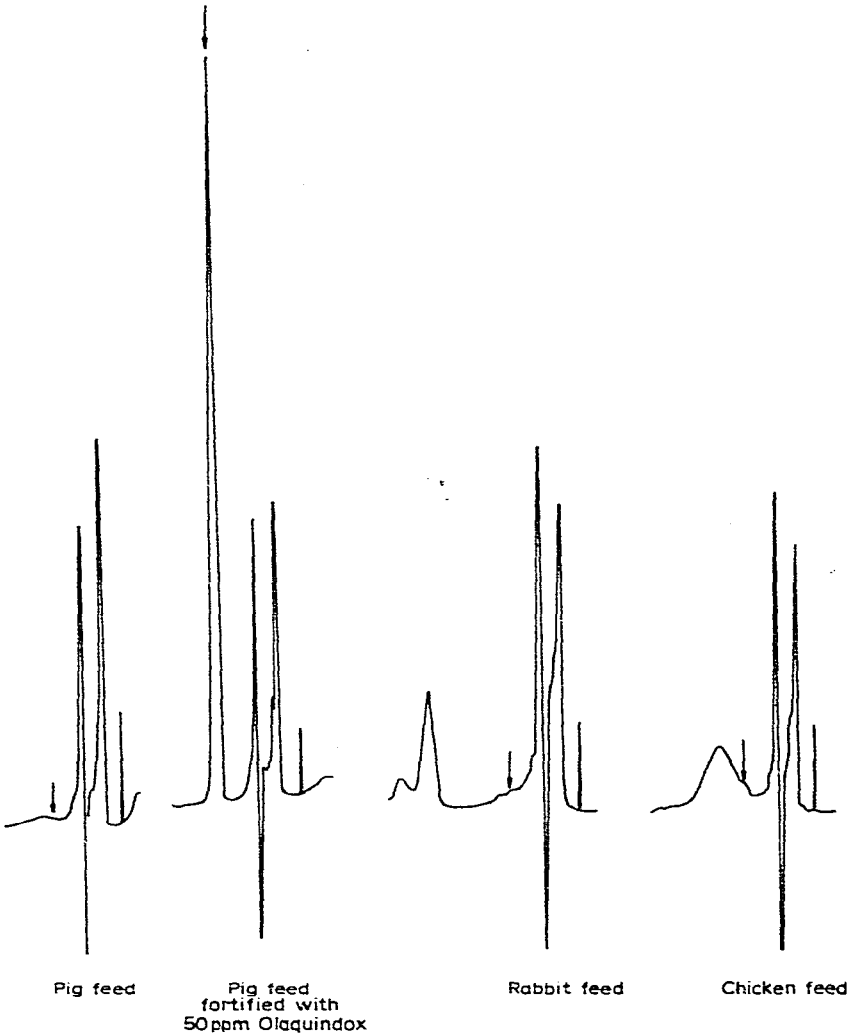

Fig. 2. Chromatograms from various standard feeds and fortified pig feed; the arrows indicate the retention time of Olaquindox.

standard pig feed fortified with 50 ppm of Olaquindox; the mean concentration measured was 48 ppm, *i.e.*, recovery was 96 ± 4% (S.D.)

The final amount of Olaquindox injected (100 ng) gives full-scale response with an attenuation of 0.04 a.u.f.s. Detection of the lower recommended dosage (10 ppm for finishing pigs), involving injection of 20 ng, gave satisfactory results, without further amplification of the signal.

The determination was unaffected by any pig-feed additive authorised for use within the E.E.C.

REFERENCES

1 H. Knapstein, *Landwirtsch. Forsch.*, 30 (1977) 94.