Journal of Chromatography, 558 (1991) 125–130
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 364

Column liquid chromatographic determination of carbadox and olaquindox in feeds

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(First received November 6th, 1990; revised manuscript received April 3rd, 1991)

ABSTRACT

A column liquid chromatographic method for simultaneous determination of carbadox and olaquindox in swine feeds is described. The drugs were extracted from feeds with carbon tetrachloride–dimethylformamide (80:20) at 60°C for 30 min. The extract was mixed with water (25:45). After centrifugation the aqueous layer was chromatographed on a reversed-phase column using gradient elution and ultraviolet detection at wavelengths of 305 and 262 nm. Recoveries from samples fortified at levels of 20–50 ppm were $92 \pm 9\%$ for carbadox and $93 \pm 6\%$ for olaquindox (means ± standard deviations, $n = 71$).

INTRODUCTION

Growth promoters are widely used in the production of animal protein. The additives carbadox [methyl-3-(2-quinoxalinylmethylene)-N^1,N^4-dioxide] and olaquindox[2-(N-2'-hydroxyethylcarbamoyl)-3-methylquinoxaline-N^1,N^4-dioxide] are used as such in swine feeds in the European Community. It is recommended that there should not exceed 50 mg per kg of complete feed, except that olaquindox can reach 100 mg/kg in milk substitutes [1,2].

The published methods concern the determination of either carbadox [3-13] or olaquindox [14–18] in feeds. High-performance liquid chromatography (HPLC) is chosen because this technique permits the detection of low-level concentrations of carbadox (10–24 μg/kg) [3–5] as well as olaquindox (0.3–1 mg/kg) [14,15].

The present study aims to offer a view of the use of these additives by Portuguese enterprises and, at the same time, to present a method that permits the simultaneous determination of the two growth promoters.

0021-9673/91/$03.50

EXPERIMENTAL

Chemicals and reagents

Carbadox and olaquindox were kindly provided by Pfizer (Lisbon, Portugal) and Lusifar (Lisbon, Portugal), respectively. N,N-Dimethylformamide (DMF) and carbon tetrachloride were reagent grade from Merck (Darmstad, Germany). Methanol (LiChrosolv, Merck)–water purified via Milli-Q (Millipore, Bedford, MA, USA) was used as the mobile phase.

Apparatus

A Gilson liquid chromatograph (Villiers-le-Bel, France) equipped with two Model 302 pumps, a mixing chamber, Model 116 a UV dual-wavelength detector set at 305 nm for carbadox and 262 nm for olaquindox, a Model 7125 injection valve (Rheodyne, Cotati, CA, USA) with a 20-μl loop, and a Versapack C_{18} reversed-phase column, 250 × 4.1 mm I.D., 10 μm particle size (Alltech, Deerfield, IL, USA), were used. The chromatographic system was controlled by an Apple II_e personal computer (Cupertino, CA, USA) with proper software and data registration. The mobile phase was degassed by ultrasonication (Bandelin Sonorex RK 100, Berlin, Germany). A Moulinex blender (Lisbon, Portugal) with a steel blade and a 40-mesh tammy was used to grind and to sieve the sample. The extraction process was performed with a Selecta Agimatic N magnetic mixer (Barcelon, Spain), a G_1 glass filter (100 μm porosity), and a Selecta Macrotronic centrifuge.

Standard solutions

Weigh accurately 50.0 mg of carbadox into a 100-ml volumetric flask and dissolve in DMF (Solutions of carbadox and olaquindox are light-sensitive. Protect standard solutions and sample extracts from direct sunlight or artificial light. Conduct the analytical process under diffused lighting or use brown glass.) This stock solution is stable for several months when kept in the dark at 4°C. To 100-, 50-, 25- and 20-ml volumetric flasks, introduce 2 ml of stock solution and complete the volume with DMF. Then add 10 ml of each previous solution to a 50-ml volumetric flask and adjust the volume with carbon tetrachloride. These solutions contain respectively 2, 4, 8 and 10 μg of carbadox per ml.

To prepare standard solutions of olaquindox, proceed as for carbadox.

Sample preparation

Weigh 10.0 g of a sample, previously ground and sieved by the Moulinex blender, into a 250-ml flask and add a magnetic bar, 20 ml of DMF and 60 ml of carbon tetrachloride. Adapt an air condensator and put in the magnetic mixer set to 500 rpm at 60°C for 30 min. Then cool, filter through G_1 under vacuum and wash the residue with a minimum of carbon tetrachloride. Transfer to a 100-ml volumetric flask and adjust the volume with carbon tetrachloride [19]. Introduce 25 ml of the filtered extract into a centrifuge tube, add 45 ml of water, stir vigorously for 2 min, and centrifuge for 5 min at 320 *g*.

Chromatography

Inject the anterior aqueous layer into the chromatographic system. Elution was

performed by gradient, starting with methanol–water (15:85) until $t = 4$ min, increasing linearily to 50:50 until $t = 6$ min, maintaining 50:50 till $t = 10$ min, and returning linearly to 15:85 till $t = 12$ min. Flow-rate was 1.5 ml/min.

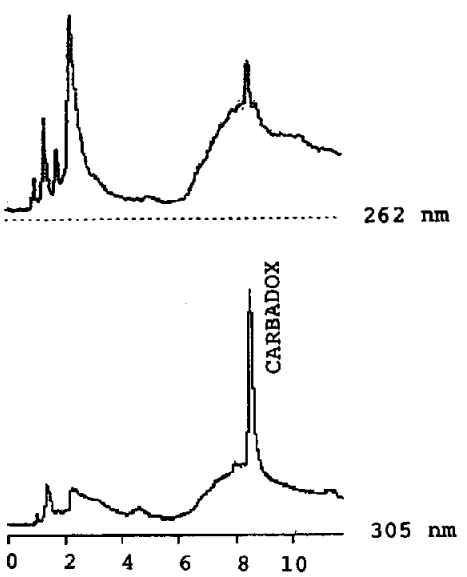
Peak areas were interpolated in a calibration curve obtained from similarly treated standard solutions. Calibration curves had coefficients of correlation of 0.997 and 0.998 for carbadox and olaquindox, respectively.

RESULTS AND DISCUSSION

Chromatograms of carbadox and olaquindox samples are presented in Figs. 1 and 2, respectively. Fig. 3 presents chromatograms of a sample without carbadox and olaquindox (Fig. 3A) and the same sample supplemented with 20 mg/kg of the two growth promoters (Fig. 3B), showing good separation of the compounds and the absence of interfering components, while the sensitivity is quite satisfactory.

The recoveries of carbadox and olaquindox from fortified feed samples, based on peak areas, are given in Table I. In this table it can be seen, for instance, that a sample without carbadox (quantity existent = 0.00 mg/kg) spiked with 20.00 mg/kg (quantity added = 20.00 mg/kg) has a recovery of 100% (mean recovery of carbadox, $n = 3$). The precision of the method was analysed by repeated determinations of carefully prepared feed samples. The intra-assay coefficient of variation (C.V.) of three samples ranged from 1.3 to 3.9% for carbadox and from 1.0 to 3.6% for olaquindox. Interassay C.V. at the different concentrations ranged from 4.9 to 9.1% for carbadox and from 2.2 to 6.5% for olaquindox.

Apparently, at the concentration levels studied, the recovery is independent both of the quantity with which the samples were supplemented and of the concentration of



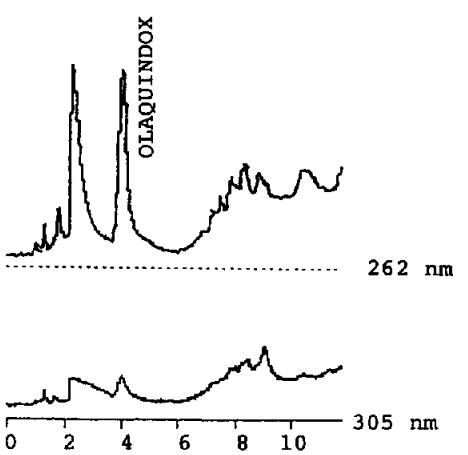


Fig. 1. Chromatograms of a sample with carbadox (22.58 mg/kg).

Fig. 2. Chromatograms of a sample with olaquindox (32.85 mg/kg).

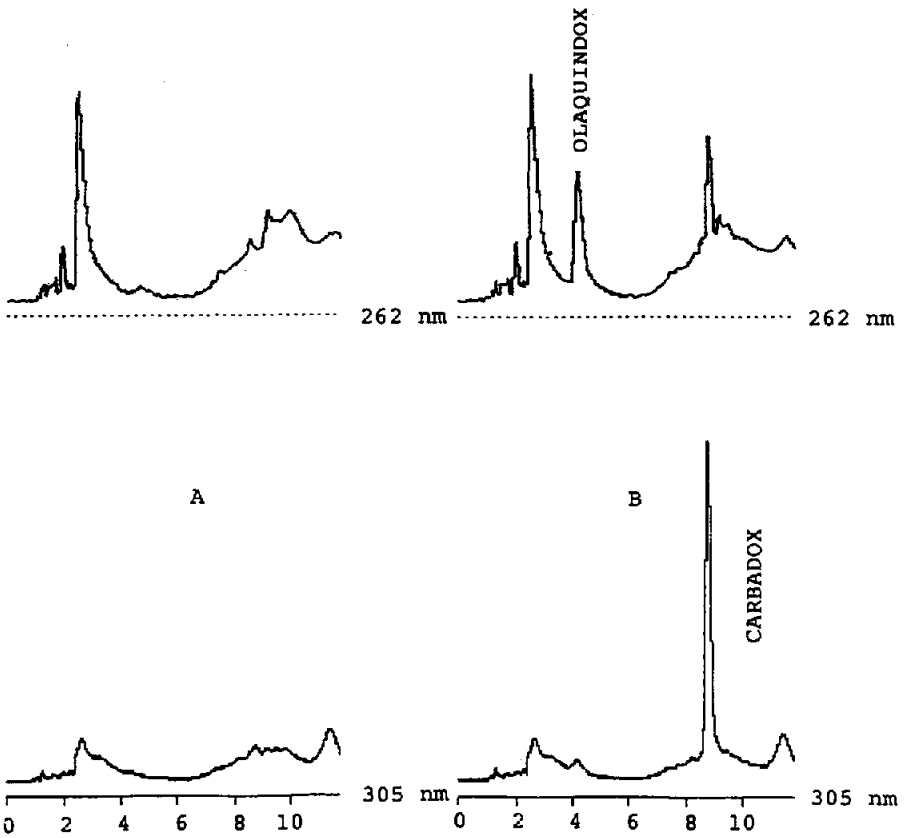


Fig. 3. Chromatograms of a representative sample without carbadox or olaquindox (A), and the same sample supplemented with 20 mg/kg of the two growth promoters (B).

TABLE I

MEAN RECOVERIES (%) OF CARBADOX AND OLAQUINDOX IN SPIKED FEED SAMPLES ($n = 3$)

Growth promoters	Quantity existent (mg/kg)	Quantity added (mg/kg)			
		20.00	30.00	40.00	50.00
Carbadox	0.00	100	99	94	94
	20.37	100	95	103	98
	49.66	72	81	84	85
Olaquindox	0.00	100	89	88	91
	15.94	99	95	101	101
	44.62	93	84	89	94

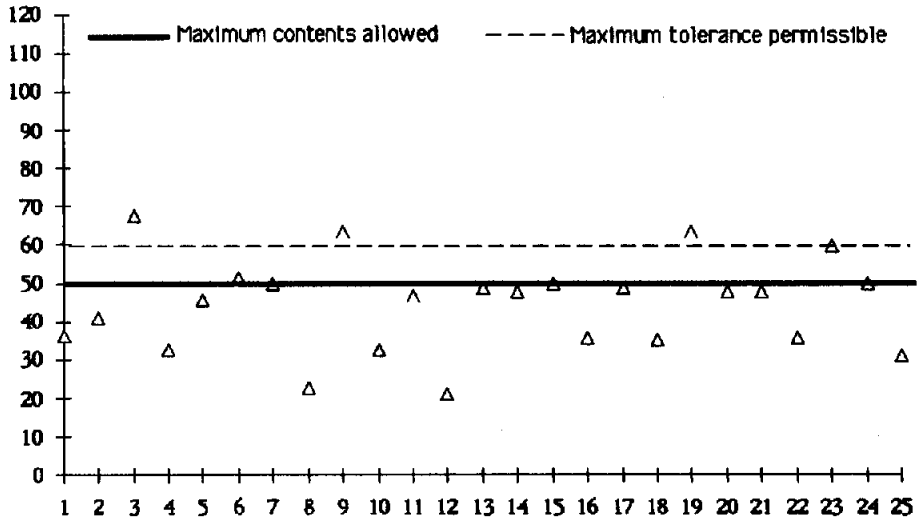


Fig. 4. Distribution of carbadox concentrations in feed samples. *y*-axis: concentration in mg/kg; *x*-axis: samples.

the additives previously determined in the samples. The results also indicate that this method is quite acceptable for routine determination of carbadox and olaquindox in feeds.

Various commercial swine feeds were sampled as random and analysed for their carbadox and olaquindox content of the 71 samples obtained, 25 contained carbadox.

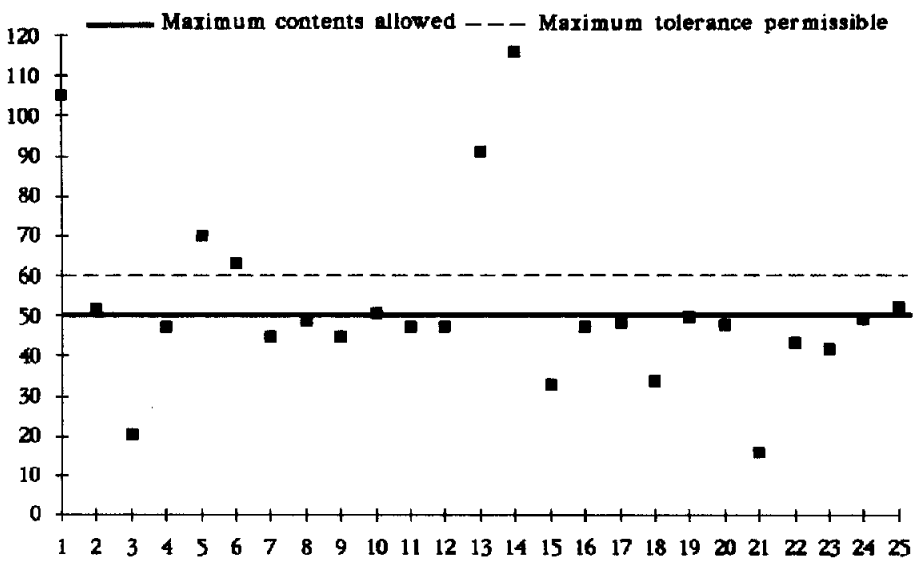


Fig. 5. Distribution of olaquindox concentrations in feed samples. *y*-axis: concentration in mg/kg; *x*-axis: samples.

25 contained olaquindox, and 20 contained neither. Only one sample contained both promoters, which under Portuguese law is not permitted [20].

Figs. 4 and 5 show the contents of carbadox and olaquindox in 50 samples containing at least one of the additives, verifying that the samples do not exceed either the maximum limit allowed or the tolerance of 20%. In general, it can be concluded that the use of carbadox as a growth promoter in Portuguese feeds obeys Portuguese rules. However, the use of olaquindox should be subjected to rigid vigilance since the limit is frequently trespassed.

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