96758-98-4; **30**, 73940-61-1; **31**, 96758-99-5; **32**, 96759-00-1; **33**, 67-66-3; **34**, 630-18-2; **35**, 35120-10-6; **36**, 507-20-0; **37**, 96759-01-2; **38**, 75-97-8; **39**, 6163-64-0; **40**, 75-28-5; **41**, 115-11-7; **42**, 75-18-3; **43**, 75-65-0; **44**, 75-09-2; **45**, 78-93-3; **46**, 563-80-4; **47**, 56-23-5; **48**, 556-61-6; **49**, 1618-26-4; **50**, 6628-18-8; **51**, 67-64-1; **52**, 96759-02-3; **53a**, 96759-03-4; **53b**, 69412-78-8; **54/55** (isomer I), 96759-04-5; **54/55** (isomer II), 96759-14-7; **56**, 96759-05-6; **57a**, 96759-06-7; **57b**, 96759-07-8; **58a**, 96759-08-9; **58b**, 96790-92-0; **59**, 107-31-3; **60**, 67-56-1; **61**, 74-87-3; **62**, 115-10-6; **63**, 71-23-8; **64**, 67-63-0; **65**, 109-74-0; **66**, 78-82-0; **67**, 109-79-5; **68**, 124-68-5; **69**, 629-45-8; (*Z*)-1,1-bis(methylthio)-3,3-dimethylbutan-2-one *O*-(*N*-methylcarbamyl)oxime, 73926-58-6; methyl 3,3-dimethyl-2-oxobutanehydroximidothioate, 96759-09-0; 3,3-dimethyl-2-oxobutanehydroximidoyl chloride, 96759-10-3; 3-methyl-1-(methylthio)butan-2-one oxime, 96759-11-4; 3-methyl-1-(methylthio)butan-2-one, 39199-24-1; 1-chloro-3-methylbutan-2-one, 17687-63-7; 1-chloro-3,3-dimethylbutan-2-one *O*-(*N*-methylcarbamyl)oxime, 96790-93-1; 1-chloro-3,3-dimethylbutan-2-one oxime, 24046-91-1; 1-cyano-2,2-dimethylpropanal oxime, 96758-85-9; 2,2-dimethylpropanal oxime, 637-91-2; 3,3-dimethyl-1-hydroxybutan-2-one oxime, 96759-12-5; 1-acetoxy-3,3-dimethylbutan-2-one, 38559-25-0; 1,3-dimethylurea, 96-31-1; 1,1-dichloro-3,3-dimethylbutan-2-one, 22591-21-5; 3,3-dimethylbutan-2-one, 75-97-8; 3,3-dimethyl-1-(methylsulfonyl)butan-2-one, 61524-35-4; 3,3-dimethyl-1-(methylthio)butan-2-one, 39199-12-7; (*E*)-3,3-dimethyl-1-(methylthio)butan-2-one oxime, 96759-13-6; 1-chloro-3,3-dimethylbutan-2-one, 13547-70-1; (*Z*)-3,3-dimethyl-1-(methylthio)butan-2-one oxime, 39195-82-9; thiofanox, 39196-18-4.

Supplementary Material Available: Syntheses and spectral data (46 pages). Ordering information is given on any current masthead page.

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Received for review May 18, 1984. Revised manuscript received April 19, 1985. Accepted April 29, 1985.

A Rapid Method for the Determination of Olaquindox in Poultry Feeds by Derivative UV Spectrophotometry

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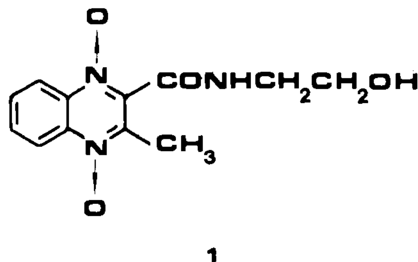
A simple method is described for the determination of the growth-promoting agent, Olaquindox, in poultry feeds. After filtration of the aqueous feed extract, the drug is directly quantified by 2nd derivative UV spectrophotometry. Recoveries from samples, fortified at levels 20–150 ppm, ranged between 96–103%. The proposed method is particularly recommended for everyday analysis of a large number of samples.

Olaquindox [2-[*N*-(2-hydroxyethyl)carbamoyl]-3-methylquinoxaline 1,4-dioxide] (**1**) is a growth-promoting agent added to pig and broiler chick diets at levels of 10–150 mg/kg.



1

The initially described spectrophotometric method (Knapstein, 1977), for the determination of Olaquindox in mixed feeds, is rather time consuming, since it involves a prior cleanup of the crude feed extract by thin-layer chromatography to eliminate interfering substances. On the other hand, methods of analysis based on high-pressure liquid chromatography (HPLC) are faster but they still require liquid–liquid partition of the extract, before injecting it on to the column (Rückemann et al., 1979; Bories, 1979). A rapid HPLC procedure, not employing extract purification, has been reported recently (Thente and Anderson, 1982); however, HPLC column clogging should be expected after a certain number of injections.

In a study, conducted in our laboratory, on the performance evaluation of broiler chicks fed with Olaquindox, a method capable of estimating various levels of this additive in feeds has been developed that permits the direct quantitation of Olaquindox in the crude feed extract by second derivative UV spectrophotometry.

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0021-8561/85/1433-0907$01.50/0 © 1985 American Chemical Society

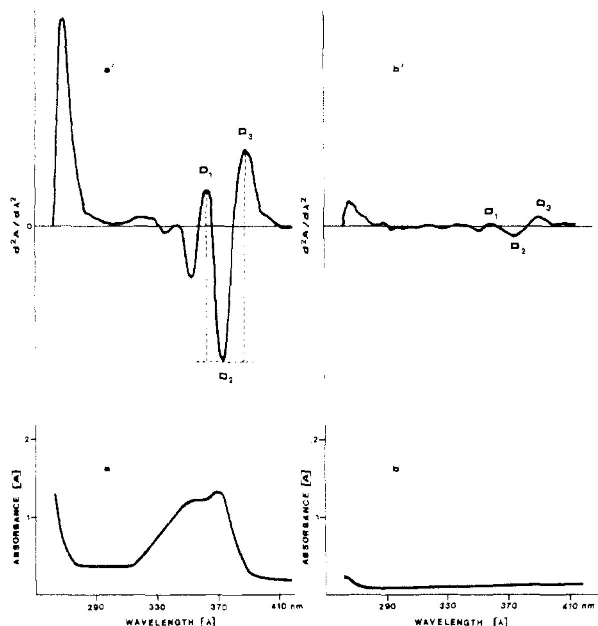

Figure 1. Normal (a, b) and 2nd derivative (a′, b′) UV spectra of Olaquindox standard solutions (2.3 and 0.23 μg/mL).

Figure 2. Second derivative spectrum of feed extract containing Olaquindox.

As it is known (Hager, 1973; O'Haver and Green, 1976) a 1st or 2nd derivative UV spectrum is the graphic representation of the differential quotient 1 or 2, respectively, over the wavelength range of interest, where A = absorbance and λ = wavelength.

$$\frac{dA}{d\lambda} \quad (1)$$

$$\frac{d^2A}{d\lambda^2} \quad (2)$$

This absorbance differentiation results in the transformation of a normal spectrum into a series of sharp maxima and minima that can be used for qualitative purposes. Quantitation is usually accomplished by measurement of the distance, in the ordinate direction, between a characteristic maximum and an adjoining minimum (peak-to-peak height).

MATERIALS AND METHODS

Instrumentation. A Perkin-Elmer Model 512 double beam spectrophotometer with 1-cm quartz absorption cells was used for all measurements. Derivative UV spectra were produced by electronic differentiation of the spectrophotometer output signal and monitored on a Perkin-Elmer Model 165 chart recorder. Electronic differentiation of the output signal was accomplished by a Perkin-Elmer Model 200-0507 Derivative Accessory permitting a selection of six (1, 2, ..., 6) time constants (sensitivities). Since positive as well as negative signals were to be expected in derivative spectra, the recorder pen had to be set at 50% full scale deflection before scanning. Second derivative spectra were obtained in the wavelength range of 330–410 nm at a scanning speed of 120 nm/min with monochromator slit set at 3 nm and time constant 6.

Chemicals. Standard aqueous solutions of 1, 3, 5, and 7 μg/mL of Olaquindox, provided by the firm Bayer Leverkusen (Germany), were prepared by appropriate diluting of a 100 μg/mL solution of this compound. All solutions were prepared daily and kept in the dark.

Procedure. A quantity of 10 g of the finely ground (1 mm) feed was transferred to a 500-mL Erlenmeyer flask. A volume (200 mL) of distilled water was added, and the flask was stoppered and shaken for 30 min under exclusion of daylight. The resultant slurry was filtered through a Whatman No. 42 filter paper and the first 10–20 mL of the filtrate was discarded. Second derivative UV spectrum of the collected filtrate was recorded against distilled water, according to the prementioned conditions. Quantitation was made by measuring the D_1D_2 height (Figure 1) and comparing it to the heights obtained from the spectra of Olaquindox standard solutions (calibration curve).

Table I. Raw Data and Regression Equations of Calibration Curves for Olaquindox Determination by 2nd Derivative Spectrophotometry

concn of standard, μg of Olaquindox/mL	D_1D_2 height,[a] mm mean ±SD (% RSD) (n = 6)	D_2D_3 height,[b] mm mean ±SD (% RSD) (n = 6)
0	0.0 ± 0.0	0.0 ± 0.0
1	23.7 ± 0.6 (2.5%)	30.5 ± 0.7 (2.3%)
3	75.4 ± 1.7 (2.2%)	92.4 ± 1.8 (1.9%)
5	121.8 ± 0.9 (0.7%)	147.8 ± 1.2 (0.8%)
7	171.8 ± 1.7 (1.0%)	206.1 ± 4.0 (1.9%)

[a] Regression equation: $y = 0.017 + 24.538x$. Correlation coefficient $r = 0.999$. [b] Regression equation: $y = 1.346 \pm 29.379x$. Correlation coefficient $r = 0.999$.

RESULTS AND DISCUSSION

The performance of 2nd derivative vs. normal UV spectrophotometry in the identification of Olaquindox is shown in Figure 1. When the derivative spectrum of a 2.3 μg/mL of aqueous solution of this compound is recorded, a minimum (D_2) and two maxima (D_1, D_3) appear, corresponding respectively to the maximum and to the inflection points of the conventionally recorded analytical band. The appearance of such extremes enhances the resolution so that, while the normal spectrum of a furthermore diluted (0.23 μg/mL) Olaquindox solution gives absolutely no information, its 2nd derivative spectrum really does.

Owing to this improved resolution and band discrimination, 2nd derivative spectra permit quantitation of Olaquindox to be made directly in the crude feed extract (Figure 2), where normal UV spectrophotometry is of no practical use due to the expected overlap of the analytical spectral band by interferring bands of other absorbing compounds.

Although both D_1D_2 and D_2D_3 heights were in good linear relation to Olaquindox concentration (Table I) the former was chosen to be measured, since the latter was

Table II. Precision and Accuracy Data for the Determination of Olaquindox in Feeds by 2nd Derivative Spectrophotometry

Olaquindox added, ppm	Olaquindox found,[a] ppm	recovery, %	% RSD
20.0	20.7 ± 1.4	103	7
30.0	31.0 ± 1.1	103	3
60.0	59.5 ± 1.7	99	3
90.0	87.4 ± 0.7	97	1
120.0	116.2 ± 1.2	97	1
150.0	143.8 ± 1.7	96	1

[a] Mean of six replicates ±SD.

Table III. Recovery from Feed Samples Containing 60 ppm of Olaquindox and Various Levels of Other Additives

additive	level in feed, ppm	% recovery of Olaquindox[a]
Amprolium (+ Ethopabate)	125 (+8)	96
Carbadox	50	102
Clopidol	125	98
Erythromycin thiocyanate	200	95
Furaltadone	500	102
Furazolidone	150	102
Monensin	125	96
Nitrofurazone	125	108
Oxytetracycline	500	98
Ronidazole	100	96
Sulfamethazine	200	102
Sulfaquinoxaline	125	96
Tetracycline	500	98
Tylosin	200	102

[a] Mean of two replicates.

significantly influenced by the copresence in the feed of other additives examined.

To evaluate the precision and accuracy of the method, 10-g samples of a poultry feed, fortified with 1 mL of Olaquindox solutions at levels ranging from 20–150 ppm, were analyzed according to the procedure. The results based on six independent determinations at each fortification level are summarized in Table II.

The estimated recovery and relative standard deviation values (RSD) were found to be comparable to those reported for the determination of Olaquindox with the prementioned methods. It appears, however, that a fairly large RSD (7%) should be expected when analyzing samples with an Olaquindox content of 20 ppm, due to matrix effects. In such a case and because of the procedure's simplicity, the method of standard addition may be conveniently applied to compensate for those effects. This alternative was successfully applied on a sample fortified with as low as 10 ppm.

Possible interferences with Olaquindox analysis from the presence of each of the additives shown in Table III were also examined; the determination was significantly affected by Nitrofurazone only, due to overlapping of the 2nd derivative spectra. The presence of this additive at a 125 ppm level resulted in an Olaquindox recovery of 108%, whereas much poorer recovery values (120%) were obtained when Nitrofurazone was added at a 300 ppm level.

In conclusion, the current study shows that the use of 2nd derivative spectrophotometry provides a rapid, accurate, and precise method for the determination of Olaquindox in feeds. Its advantages, i.e., minimal sample preparation, no need for costly reagents and instrumentation, render this method valuable for routine analysis.

Registry No. Olaquindox, 23696-28-8; amprolium, 121-25-5; carbadox, 6804-07-5; clopidol, 2971-90-6; erythromycin thiocyanate, 7704-67-8; furaltadone, 139-91-3; furazolidone, 67-45-8; monensin, 17090-79-8; nitrofurazone, 59-87-0; oxytetracycline, 79-57-2; ronidazole, 7681-76-7; sulfamethazine, 57-68-1; sulfaquinoxaline, 59-40-5; tetracycline, 60-54-8; tylosin, 1401-69-0; ethopabate, 59-06-3.

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Received for review April 2, 1985. Accepted July 5, 1985.

Identification of Toxic Alkaloids from the *calcaratus* Subspecies of *Lupinus arbustus*

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A recent chemical study of the previously uninvestigated *Lupinus arbustus* subspecies *calcaratus* resulted in the isolation and characterization of the novel bipiperidyl–indole alkaloid gramodendrine. The present study describes the identification of three additional structurally diverse alkaloids from the title plant. These include the quinolizidine base lusitanine, the piperidine derivative ammodendrine, and the indole alkylamine gramine. The total alkaloid fraction as well as the first three of the above individual alkaloids were tested for gross locomotor activity and for rotorod performance in mice. Pharmacologic data indicated that gramodendrine and ammodendrine were moderately potent (300 mg/kg, ip) in reducing spontaneous motor activity (30% of control) and in causing central nervous system depression (50% of control).

In the Rocky Mountain region of North America, acute toxicoses and death of livestock grazing on certain species of the legume genus *Lupinus* has been attributed to the quinolizidine alkaloid content of these plants (Couch, 1937; Kingsbury, 1964; Keeler, 1975). The age and species of lupine together with the species of animal contribute to a variation in the symptoms and severity of poisoning (Couch, 1937; Beath et al., 1953). Recently, it was shown that the ingestion of certain lupine species by pregnant

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0021-8561/85/1433-0909$01.50/0 © 1985 American Chemical Society