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Kinetic Determination of Furazolidone and Furaltadone Based on Alkaline Hydrolysis Reaction

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A simple and rapid kinetic method for the determination of furazolidone or furaltadone is reported. It is based on the measurement of the rate of their alkaline hydrolysis reaction. Kinetic data are recorded spectrophotometrically at 425 or 430 nm, the maximum absorption wavelengths of the respective hydrolysis products. The calibration plots are linear in the range 1–20 μg/ml and the detection limits are 0.17 and 0.19 μg/ml for furazolidone and furaltadone, respectively. The precision of the method, expressed as the relative standard deviation, is 1.2% for 9.0 μg/ml furazolidone and 1.4% for 9.0 μg/ml furaltadone. Good recoveries have been obtained in applying the method to the analysis of furazolidone or furaltadone in pharmaceutical preparations and feed.

INTRODUCTION

Nitrofurans are chemotherapeutical agents well known as antimicrobian agents (from Dodd and Stillman studies) and widely used to fight common infections in humans and animals or characteristic infections of domestic animals or poultry (*1*).

One of the most frequently studied nitrofurans is furazolidone [3-(5-nitrofurfurylideneamino)-2-oxazolidinone], used as a coccidiostat in poultry and swine feeds. Another very similar compound, furaltadone [5-morpholinomethyl-3[5-nitro-furfurylideneamino]-2-oxazolidinone] is widely used in Spain in different veterinary formulations (*2*) but the number of papers about its analytical determination is limited.

Both compounds undergo a reaction of alkaline hydrolysis, giving rise to a colored product with a maximum absorption wavelength around 420 nm which subsequently disappears with time. This reaction has been used as the basis of spectrophotometric methods of determination of these compounds (*3–5*). In the first of these studies (*3*), the relationship between the solvent, the KOH concentration, and the absorption spectra of solutions of nitrofurazone, furazolidone, furaltadone, nitrofurantoin, and 1-[3-(5-nitro-2-furyl) allylideneamino] hydantoin is established, and a spectrophotometric method for identifying and determining the constituents of two component mixtures of these compounds in dimethylformamide or acetone medium in the presence of KOH is described. Another paper (*4*) describes a photometric method for the determination of furazolidone in air, by removing it by electrostatic filters, dissolving in DMF, mixing with KOH, and measuring the absorbance after 15 min at 550 nm. Finally, furazolidone (*5*) has

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been determined in pharmaceuticals containing this compound alone and with other drugs by spectrophotometric measurement of the orange color of the formed derivative with NaOH at 425 nm. The absorbance is measured after keeping the reaction mixture at 30°C for half an hour and Beer's law is obeyed in the range 5–30 μg/ml.

Taking into account the precise control of the measuring time, required by the variation of the rate of the hydrolysis reactions with the base concentration, as well as the later decomposition of the colored products formed, the developing of kinetic methods based on these reactions, which is the object of this paper, is of interest. Besides, it allows the spectrophotometric determination of these compounds in colored samples.

The proposed kinetic–spectrophotometric method follows the hydrolysis reaction, by recording the absorbance–time curves at the maximum absorption wavelength and obtaining the slope of the tangent to these curves at the beginning (tg α). The method has been successfully applied to the determination of furazolidone or furaltadone in pharmaceuticals and feeds.

Other spectrophotometric methods of furazolidone determination in pharmaceuticals involve the decomposition of furazolidone with 50% H_2SO_4 (*6*), reduction with Zn/HCl (*7*), or decomposition and later reaction of the obtained product with other compounds (*8, 9*). The official AOAC method for determining furazolidone in finished feeds involves the colorimetric detection of the phenylhydrazine HCl adduct (*10*). We have not found reported spectrophotometric methods to determine furaltadone in feeds and only the above-cited method (*3*) and another (*11*) based on the direct measurement of its absorbance at 259 nm have been proposed for its determination in pharmaceuticals.

EXPERIMENTAL

Reagents

Furazolidone and furaltadone obtained from Sigma Chemical Co. were used. Standard solutions of these compounds were prepared by dissolving the appropriate amount in DMF. Sodium hydroxide solutions of different concentrations were prepared from NaOH (Merck) and standardized against potassium phtalate acid. All other chemicals were of analytical reagent grade.

Apparatus

A Beckman Instrument DU-50 spectrophotometer fitted with a thermostatized cell and a Selecta thermostat and connected to an IBM PC-XT 286 and an Olivetti DM-282 printer was used. The Beckman Data Leader software was used for treatment of data and analysis by linear regression of the absorbance–time curves.

Procedures

Determination of Furazolidone or Furaltadone

Samples are prepared, in the measuring cell, by pipetting 1.5 ml of deionized water; an appropriate volume (smaller than 0.5 ml) of furazolidone or furaltadone

solution in DMF, containing up to 50 μg of these compounds; DMF, if necessary, to complete 0.5 ml; and 0.5 ml of 0.5 *M* NaOH solution. The absorbance–time curves are obtained at 25°C, at 425 nm for furazolidone and at 430 nm for furaltadone. The slopes of the tangents to these curves at the beginning are calculated by linear regression analysis by using the Data Leader software (*12*) and the furazolidone or furaltadone concentration is obtained by using appropriate calibration graphs.

Determination of Furazolidone or Furaltadone in Pharmaceuticals

Determination in tablets. Five tablets are carefully pulverized and a representative amount is weighed, dissolved in DMF, filtered, washed and diluted to a known volume with DMF. Suitable aliquots of thus obtained solutions, smaller than 0.5 ml, are used to carry out the analysis according to the above-described procedure.

Determination in suspensions. Suitable aliquots of formulations are weighed and dissolved in DMF, filtered, and diluted to a known volume with DMF, and analysis is undertaken as above.

Determination in solutions. Suitable aliquots of solutions are diluted to a known volume with DMF and samples are prepared according to the procedure for determination of furazolidone or furaltadone.

Determination in Feeds

About 10 g of finely ground feeds are accurately weighed and carefully stirred with 40 ml of DMF, for 30 min. The extracts are centrifugated and filtered and the residues are washed with DMF and diluted to a final volume of 50 ml. Suitable aliquots of these solutions are used to proceed with the analysis according to the above-described procedure.

RESULTS AND DISCUSSION

As mentioned in the Introduction, furazolidone and furaltadone undergo alkaline hydrolysis reactions, which give rise to orange-colored products with maximum absorption wavelengths around to 420 nm. However, these products are unstable and their spectra change with time, as can be observed in Fig. 1, in which the changes of the absorption spectrum of a furazolidone sample at pH 13.4 over periods of time can be noted. Because of this the spectrophotometric determination of these compounds on the basis of these reactions requires precise control of the measurement of time. To develop a kinetic spectrophotometric method, samples are prepared, in the same measurement cell, to reduce the time after mixing the reagents and before measuring absorbance and, in this way, to obtain a more accurate measurement of the initial reaction rate.

At first, we studied the influence of sodium hydroxide concentration, by preparing samples with variable amounts of 2 *M* NaOH and 2 *M* KCl, deionized water to make a 2.5-ml volume, and 20 μl of 0.800 g/liter furazolidone or 1.00 g/liter furaltadone solutions. In Fig. 2, some of the absorbance–time (*A–t*) curves obtained for furaltadone are shown. As we observe in Fig. 3, the slope of the tangent to these curves (tg α), which gives us the initial rate of the reaction, changes

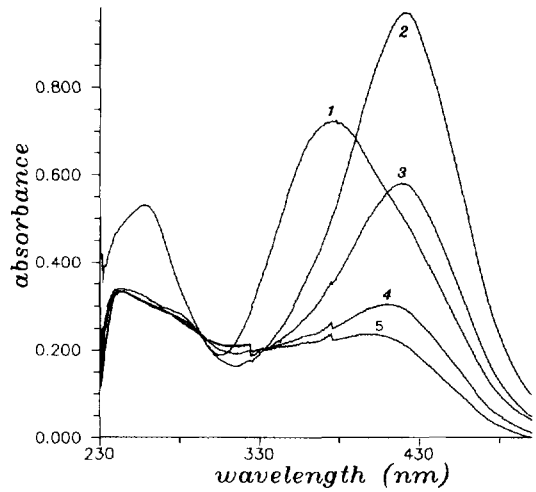

FIG. 1. Evolution of the absorption spectrum of a 10 μg/ml furazolidone solution at pH 13.4. Time (min): curve 1, 0; curve 2, 10; curve 3, 30; curve 4, 50; curve 5, 60.

linearly with NaOH concentrations in the range studied, for both reactions. It suggests a partial reaction order of 1 with respect to hydroxile ions. A 0.1 *M* NaOH concentration was determined to be best for later experiments, which provides an appreciable linear interval in the *A–t* curves.

The ionic strength influence has been studied by preparing samples in a manner similar to that in the preceding study, with variable amounts of 2 *M* KCl solution. A slight decrease of hydrolysis reaction rate with ionic strength has been observed for both furazolidone and furaltadone, a foreseeable result for the reaction between a neutral and a charged specie (*13*). The solubility of these compounds in DMF (>50 g/liter) is considerably higher than that in other solvents. Hence,

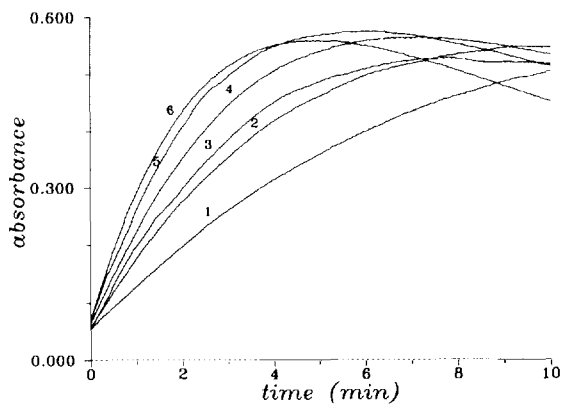

FIG. 2. Absorbance–time curves for the hydrolysis reaction of furaltadone (8 μg/ml) at different NaOH concentrations, by preparing the samples in the measurement cell: [NaOH], *M*: curve 1, 0.037; curve 2, 0.059; curve 3, 0.073; curve 4, 0.091; curve 5, 0.110; curve 6, 0.128.

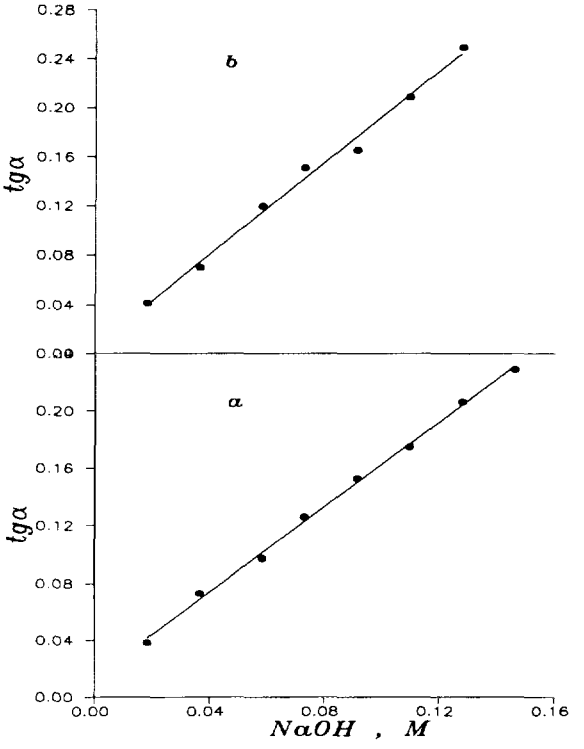

FIG. 3. Influence of NaOH concentration on the hydrolysis reaction rate of (a) furazolidone (6.4 μg/ml) and (b) furaltadone (8 μg/ml).

solubilities of furazolidone and furaltadone in different solvents are, respectively, acetone, 0.095 and 14.31 g/liter; methanol, 0.22 and 1.83 g/liter; methyl isobutyl ketone, 0.32 and 2.30 g/liter; ethyl acetate, 0.35 and 2.85 g/liter; isopropyl ether, 0.04 and 0.06 g/liter; chloroform, 0.37 and 3.95 g/liter; and water, 0.10 and 0.56 g/liter.

For this reason, we prepared the samples in the presence of DMF and used the solvent to extract furazolidone or furaltadone from their formulations.

The influence of the DMF proportion was studied by obtaining the A–t curves

TABLE 1
Calibration Graphs for the Kinetic–Spectrophotometric Determination of Furazolidone or Furaltadone

Compound	Linear regression equation	r	RSD (%) 4 μg/ml	RSD (%) 9 μg/ml	LOD (μg/ml)	LOQ (μg/ml)
Furazolidone	$tg\ \alpha = 4.93 \times 10^{-2}FZ^a + 1.0 \times 10^{-3}$	0.9995	1.9	1.2	0.17	0.57
Furaltadone	$tg\ \alpha = 4.44 \times 10^{-2}FD^a - 1.06 \times 10^{-2}$	0.9996	3.4	1.4	0.19	0.63

[a] FZ, FD: Furazolidone and furaltadone concentration in μg/ml.

TABLE 2
Determination of Furazolidone or Furaltadone in Pharmaceutical Formulations

Pharmaceutical formulation	Composition	Determination of furazolidone: Furazolidone claimed (mg/g or mg/ml)	Furazolidone found*	Furazolidone by HPLC
Saleton	(Per tablet) Furazolidone, 50 mg Difenoxilate hydrochloride, 2.5 mg Neomycin sulfate, 3.5 mg Atropine sulfate, 0.025 mg	272	271.9 ± 2.7	270.80 ± 0.39
Desinvag solution	(Per 100 ml) Furazolidone, 1 mg 50% Benzalkonium chloride, 4 ml	0.01	0.0103 ± 0.0003	
Enteromicine	(Per tablet) Furazolidone, 250 mg Neomycin sulfate, 250 mg Electrolites Cl^- 474 mg Na^+ 346 mg K^+ 52 mg Ca^{2+} 18 mg Mg^{2+} 15 mg	84.6	83.5 ± 0.33	84.46 ± 0.44
		Determination of furaltadone: Furaltadone claimed (mg/g or mg/ml)	Furaltadone found*	Furaltadone by HPLC
Panotile solution	(Per ml) Furaltadone hydrochloride, 4.5 mg Polymixin B sulfate, 10,000 I.U. Neomycin sulfate, 3.5 mg Fluorocortisone acetate, 1.0 mg Didocaine hydrochloride, 40.0 mg	4.5	4.60 ± 0.04	4.40 ± 0.04
Altabactine suspension	(Per 200 ml) Furaltadone, 4.16 g Chloroamphenicol, 4.16 g Neomycine sulfate, 10.0 g Valeramide sulfate, 41.50 g Magnesium chloride, 16.66 g	19.6	18.96 ± 0.47	18.40 ± 0.74

* Each value is the mean of three determinations.

at the absorption maximum wavelengths of furazolidone or furaltadone for each DMF proportion. We found that the initial reaction rates slightly increase with DMF concentration and a 20% proportion was determined to be best for later experiments. With greater proportions of DMF bubbles appear. The measurement wavelengths, with this DMF proportion, are 425 nm for furazolidone and 430 nm for furaltadone.

The influence of temperature has been investigated between 15 and 40°C. We found an exponential increase of tg α with temperature and a linear variation of ln tg α with $1/T$. The slope of the obtained straight lines give us activation energy values of 4.8 and 4.3 kJ/kmol for furazolidone and furaltadone reactions, respectively. On the basis of these investigations, further experiments were carried out at 25°C.

Due to the temperature increment observed in the DMF and water mixture, the furazolidone or furaltadone solutions in DMF, additional DMF to make a 20% concentration, if necessary, and the deionized water are mixed and allowed to cool, before the NaOH solution is added.

Furazolidone or Furaltadone Calibration Graphs

Under the optimum conditions established, calibration graphs were obtained for furazolidone or furaltadone. Good linearity was obtained in both instances in a concentration interval of 1 to 20 μg/ml. In Table 1, the relevant data for calibration graphs have been summarized, as well as the relative standard deviation (RSD) values obtained in the analysis of 11 samples, with 4.0 or 9.0 μg/ml of each one of these compounds, and the detection (LOD) and determination (LOQ) limits (*14*). It must be noted that even though detection limits of 0.17 and 0.19 μg/ml have been found, for furazolidone and furaltadone, respectively, the effective detection limits, in keeping with the 20% proportion of DMF selected, are 0.85 and 0.95 μg/ml, respectively, for furazolidone or furaltadone solutions in DMF.

TABLE 3
Determination of Furazolidone or Furaltadone in Feeds

	Determination of furazolidone	
Sample	Furazolidone added (%)	Furazolidone found (%)*
Pig feed	0.005	0.0047 ± 0.0015
	0.050	0.0489 ± 0.0050
Rabbit feed	0.050	0.0490 ± 0.0040
	Determination of furaltadone	
Sample	Furaltadone added (%)	Furaltadone found (%)*
Pig feed	0.005	0.0046 ± 0.0019
	0.050	0.0480 ± 0.0030
Rabbit feed	0.050	0.0487 ± 0.0040

* Each value is the mean of three determinations.

Applications

The proposed methods for furazolidone or furaltadone analysis have been applied to the determination of these compounds in pharmaceutical formulations and feeds, according to the above-mentioned procedures. The results obtained are in Tables 2 and 3. It must be emphasized that although the analyzed formulations contain other kinds of drugs, as well as excipients, the results obtained are very much in agreement with the levels claimed by the manufacturer and with the results obtained by HPLC.

In the analysis of feed samples, spiked with different levels of these compounds, the simplicity and good results obtained are worthy of note, despite the very high initial absorbance values of the samples prepared for the analysis.

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REFERENCES

1. Dámaso, D.; Moreno-López, M.; Daza, R. M. *Antibióticos y quimioterápicos. Uso clínico.* Grutesa, Madrid, 1984.
2. Guía de productos zoosanitarios. Veterindustria, Madrid, 1990.
3. Egert, V. E.; Giller, S. A.; Lielzalve, A. A.; Shimanskaya, M. V. *Izv. Akad. Nauk. Latv. SSR, Ser. Khim.*, 1963, **5**, 531–540.
4. Bake, M. Y.; Izrailet, L. I. *Gig. Sanit.*, 1980, **8**, 60–61 (C.A. 94:89288m).
5. Briswas, A.; Ghosh, S. *Indian J. Pharm. Sci.*, 1980, **42**(2), 60–62.
6. Andreeva, A. T.; Devyatkina, I. A.; Kozlova, L. M. *Nek. Probl. Biofarm. Farmakokinet. Tezisy Dokl. Nauchn. Konf.*, 1973, 65–66 (C.A. 80:63897v).
7. Hassan, S. M.; Sharaf El-Din, M.; Belal, F.; Sultan, M. *J. Pharm. Pharmacol.*, 1988, **40**(11), 798–800.
8. Moraleva, M. K.; Kovalenko, L. I.; Gorelova, D. A. *Nek. Probl. Biofarm. Farmakokinet.*, 1973, 78–80 (C.A. 80:124807q).
9. Ivakhenko, P. N.; Chigarenko, L. S. *Farmatsiya*, 1976, **25**(2), 29–32.
10. Williams, S. *AOAC Official Methods of Analysis*, 14th ed. 1984, 42.075.
11. Kurinnaya, N. V. *Farm. Zh.*, 1972, **27**(4), 35–39 (C.A. 77:143846f).
12. Data Leader Software Package. Beckman, Fullerton, CA, 1987.
13. Kopanica, M.; Stara, V. Kinetic method in chemical analysis. In *Comprehensive Analytical Chemistry* (Wilson and Wilson, Eds.), 13th ed., p. 31. XVIII, Ed. Elsevier, Amsterdam, 1983.
14. Nomenclature, symbols, units and their usage in spectrochemical analysis. *Spectrochim. Acta*, 1978, **33B**, 242.