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COMPLEXOMETRIC-SPECTROPHOTOMETRIC ASSAY OF TETRACYCLINES IN DRUG FORMULATIONS

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Summary—An accurate, rapid and very simple spectrophotometric method for the assay of tetracyclines (tetracycline.HCl, chlorotetracycline.HCl, demeclocycline, oxytetracycline.HCl and doxycycline) has been developed. The method is based on the complexation of iron(III) with tetracyclines in 0.001*M* sulphuric acid. It has been successfully applied to the assay of tetracyclines in drug formulations, and the interferences of excipients have been examined. The results have been statistically compared with those obtained by two standard methods and found to be very satisfactory.

The tetracyclines and their derivatives (tetracycline, oxytetracycline, chlorotetracycline, demeclocycline and doxycycline) are extensively employed as bacteriostatic antibiotic drugs. Various methods for their determination have been reviewed.[1] In the British Pharmacopoeia[2] a biological assay for tetracycline is given but it is an elaborate method not suitable for routine analysis. Iron(III) was early recommended in the U.S. Pharmacopeia[3] for the determination of oxytetracycline. A simple spectrophotometric method for the assay of tetracycline was recently described[1] but it is not suitable for the assay of other derivatives. Two spectrophotometric methods for the assay of some tetracycline derivatives have been described based on oxidation with ammonium vanadate[4] and sodium cobaltinitrite,[5] but neither is specific.

The present work is based on the ability of tetracyclines to form metal-ion complexes.[6,7] Chelation with cations such as iron(III), aluminium, copper(II), nickel, cobalt(II), zinc, vanadium(III), thorium, lanthanum, magnesium and calcium is well established.[8–18] It has been reported that iron(III) has higher affinity than other cations for chelation with tetracyclines *in vitro*.[6,8–14,19]

In the method presented here, tetracycline is added to iron(III) and the absorbance of the brown complex formed is measured.

EXPERIMENTAL

Apparatus

A Varian Model DMS 100 Spectrophotometer connected to a Varian Model DS 15 Data Station and a Hewlett–Packard Model 82905 B Printer was used for all absorbance measurements. Matched sets of 10-mm cells were used throughout.

Reagents

High-purity distilled water was used throughout. Stock solutions were prepared from analytical or pharmaceutical grade chemicals, and working solutions were prepared from these by appropriate dilution.

Ferric ammonium sulphate solution (1 mg/ml) was prepared by dissolving about 1 g, accurately weighed, in a litre of 0.001*M* sulphuric acid.

Tetracycline solution (1 mg/ml) was freshly prepared by dissolving the required amount in 0.001*M* sulphuric acid by warming, then cooling and making up to volume in a standard flask. For analysis of capsules, the contents of 10 were mixed and weighed and a quantity of the powder equivalent to 250 mg of tetracycline was accurately weighed out, and stirred with 200 ml of 0.001*M* sulphuric acid for 10 min, with warming, then the solution was filtered (Whatman No. 41 filter-paper), the paper was washed with hot 0.001*M* sulphuric acid and the filtrate and washings were diluted to volume with 0.001*M* sulphuric acid in a 250-ml standard flask after cooling to room temperature.

General procedure

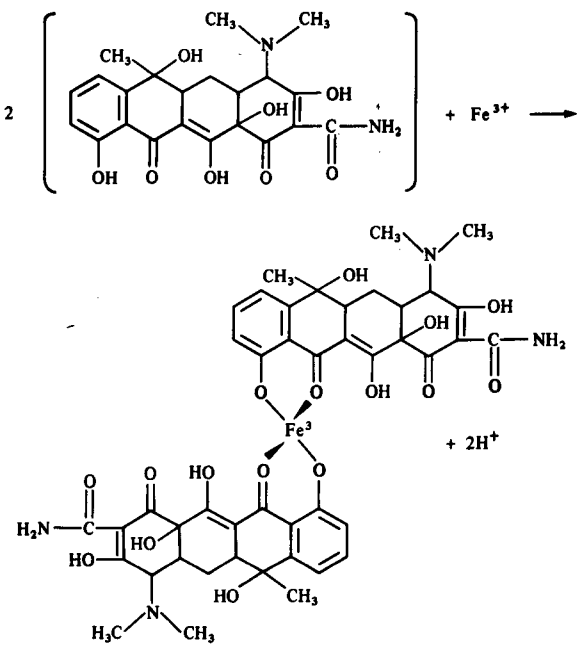
Place 10 ml of ferric ammonium sulphate solution and an appropriate amount of tetracycline solution in a 50-ml standard flask. Swirl and leave for 20 min for tetracycline hydrochloride but only 5 min for the other derivatives, then dilute to the mark with 0.001*M* sulphuric acid. Measure the absorbance at the appropriate wavelength against a reagent blank treated similarly.

RESULTS AND DISCUSSION

Mechanism

The tetracyclines were added to iron(III) solution in sulphuric acid of different concentrations. A brown soluble compound was always obtained, with each derivative giving a characteristic wavelength of maximum absorption, at 423 nm for tetracycline and 435 nm for the four derivatives examined. The maxima can be attributed to complex formation of iron(III) with the tetracyclines. When iron(II) was used no colour change was observed. The tetracycline structure contains numerous sites at which chelation with metal cations might occur, the most important of these being in the portion of the molecule which contains the two enolized 1,3-diketone groupings.[6,20–22] Such enol groups readily form six-membered rings with metal ions, with the two oxygen atoms as donors.[21,22] Job's method[23,24] showed that

the ratio of iron(III) to tetracycline is 1:2. This ratio has also been reported for combination of copper(II), nickel and zinc with tetracycline.[20] Accordingly, it is suggested that iron(III) chelates with tetracycline as follows:



All the iron(III) complexes thus formed are stable for 24 hr. Figure 1 shows that the absorbance of the tetracycline–iron(III) complex at 423 nm decreases as the sulphuric acid concentration is increased, becoming minimal at 0.1*M* acidity. In the sulphuric acid range 0.0005–0.002*M* the absorbance is almost constant and 0.001*M* acidity is optimal. At sulphuric acid concentrations >0.1*M* protonation of the tetracycline molecule is possible and an absorbance peak for the protonated form appears at 441 nm as in curve 9. At acidities lower than 0.0005*M*, hydrolysis of iron(III) is expected and the maximum is not as well defined (curve 1). For this reason, 0.001*M* sulphuric acid was selected as optimum.

Spectral data

Beer's law was found to hold over the range 10–200 μg/ml for all five compounds examined. Table 1 gives the molar absorptivities at the wavelength of maximum absorption.

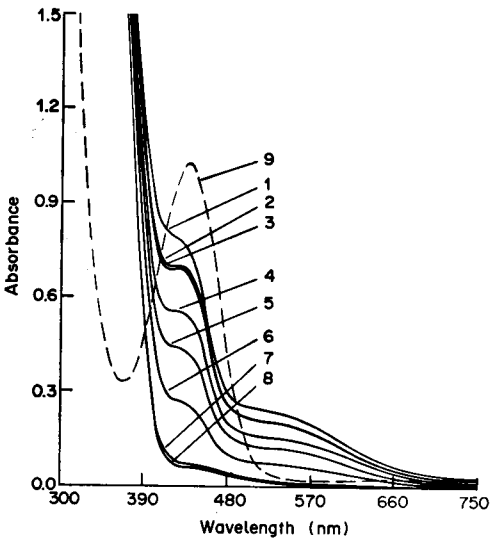


Fig. 1. Absorption spectra for reactants [3 mg of tetracycline + 6 mg of iron(III)] in 50 ml of solution, measured against a reagent blank at different sulphuric acid molarities: 1—0.0001; 2—0.0005; 3—0.001; 4—0.003; 5—0.005; 6—0.01; 7—0.05; 8—0.10; 9—2.

Applications

The method was applied to the assay of tetracyclines in drug formulations (commercial products randomly collected from local pharmacies). Typical results are given in Table 2 and show good agreement with those obtained by other methods. The sodium molybdate method was used for tetracycline, and the sodium cobaltinitrite method[5] for the other four compounds. The *t*-values (Table 3) showed no significant difference between the means obtained (95% confidence limit).

Interferences

The results for analysis of the compounds in drug formulations with the constituents listed in Table 2 indicate that the excipients usually present in dosage forms, such as starch, lactose and glucose, did not interfere. Troleandomycin, glucosamine.HCl and vitamin K_3 did not interfere with the determination of tetracycline. Thiamine, pyridoxine and folic acid did not interfere with the determination of oxytetracycline. Vitamins B_1, B_2, B_6, B_{12}, nicotinamide and calcium pantothenate did not interfere with the determination of tetracycline or oxytetracycline. Sulphamethizole and phenazopyridine.HCl, however,

Table 1. Analytical appraisal for compounds (in pure form) investigated

Generic name	Supplier	λ_{max}, nm	ϵ, $l.mole^{-1}.cm^{-1}$
Tetracycline.HCl	Lederle	423	4646
Demethylchlorotetracycline	Lederle	435	5190
Chlorotetracycline.HCl	Lederle	435	5533
Oxytetracycline.HCl	Pfizer	435	5240
Doxycycline	Pfizer	435	4922

Table 2. Results for all drugs investigated by the proposed method and/or the sodium molybdate[a] and sodium cobaltinitrite[b] methods

Drug proprietary name, and supplier	Generic name	Nominal composition, *mg*	Proposed method: Found per capsule, *mg**	Proposed method: Error, %†	Standard method, found, *mg*
Tetrerba, Carlo Erba	Tetracycline	250 tetracycline.HCl	262	+4.9	262[a]
Uropol, Bristol-Myers	Tetracycline	125 tetracycline.HCl 250 sulphamethizole 50 phenazopyridine.HCl	429	+243	302[a]
Dumocycline, Dumex	Tetracycline	250 tetracycline.HCl	257	+2.6	258[a]
Tetrambezim, Lepetit	Tetracycline	250 tetracycline.HCl	251	+0.5	253[a]
Sigmamycin, Pfizer	Tetracycline	167 tetracycline.HCl 83 troleandomycin	155	−7.0	155[a]
Achromycin, Lederle,	Tetracycline	250 tetracycline.HCl	249	−0.3	249[a]
Latycin, Biochemie	Tetracycline	250 tetracycline.HCl 250 glucosamine.HCl 2.5 Vit. B_1 2.5 Vit. B_2 2.5 Vit. B_6 2.5 Vit. B_{12} 75 Vit. C 0.5 Vit. K_3 25 nicotinamide 5 calcium pantothenate	149	−40	263[a]
Vibramycin, Pfizer,	Doxycyclin	100 doxycycline	102	+1.5	102[b]
Urobiotic, Pfizer	Oxytetracycline.HCl	125 oxytetracycline.HCl 250 sulphamethiazole 50 phenazopyridine.HCl	478	+282	330[b]
Terramycin S.F., Pfizer	Oxytetracycline.HCl	250 oxytetracycline.HCl 2.5 thiamine 2.5 riboflavin 25 niacinamide 5 calcium pantothenate 0.5 pyridoxine 0.375 folic acid 1 Vit. B_{12} 75 ascorbic acid	173	−31	381[b]
Terramycin, Pfizer	Oxytetracycline.HCl	250 oxytetracycline	259	+4.0	260[b]
Demeclocycline, Lederle	Demethylchloro-tetracycline	pure analytical reagent (another batch)	—	−1.4	—
Chlorotetracycline	Chlorotetracycline.HCl	pure analytical reagent (another batch)	—	−0.5	—

*Mean of 7 determinations.
†Difference from nominal content.

Table 3. Statistical comparison of the results obtained (7 replicates) by the proposed method with results obtained by the sodium molybdate[1] and/or sodium cobaltinitrite[5] method

Drug proprietary name	Recovery ± standard deviation, %: Method*	Method†	Method§	*t* Calculated‡
Tetrerba	104.8 ± 0.9	105.0 ± 0.2	—	0.38
Dumocycline	102.6 ± 0.6	103.2 ± 0.2	—	1.71
Achromycin V	99.8 ± 0.7	99.5 ± 0.4	—	0.81
Tetrambezim	100.5 ± 0.7	101.2 ± 0.4	—	1.75
Sigmamycin	93.0 ± 0.2	93.0 ± 0.8	—	0.51
Vibramycin	101.6 ± 0.4	—	102.0 ± 0.5	0.68
Terramycin	103.6 ± 0.6	—	104.1 ± 0.8	0.56
Demeclocycline	99.1 ± 0.2	—	99.5 ± 0.2	1.10
Chlorotetracycline.HCl	95.3 ± 0.6	—	94.9 ± 0.5	0.43

*Proposed method.
†Sodium molybdate method.
§Sodium cobaltinitrite method.
‡Theoretical value = 2.45 ($P = 0.05$).

gave a large positive error in the determination of both tetracycline and oxytetracycline.

Vitamin C also interfered with the determination of tetracycline and oxytetracycline, but with a negative systematic error. This may be due to decrease in the iron(III) concentration by reduction with ascorbic acid, but addition of extra iron(III) failed to eliminate the interference.

Conclusion

This method is accurate, simple, rapid and suitable for routine analysis of all commonly used tetracyclines. There is no interference from most compounds added to drug formulations containing tetracyclines.

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