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Spectrophotometric Determination of Minocycline Using Zirconium(IV), *o*-Hydroxyhydroquinonephthalein and Fluoride Ions in the Presence of Sodium Dodecyl Sulfate[1)]

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A simple and rapid spectrophotometric method for the determination of minocycline was established using zirconium(IV), *o*-hydroxyhydroquinonephthalein and fluoride ions in the presence of sodium dodecyl sulfate. This method could be used to determine 5—40 μg/10 ml of minocycline; the Sandell sensitivity was estimated to be 0.0067 μg/cm^2 at 515 nm. This method was applied to the determination of minocycline hydrochloride in pharmaceutical preparations, and the recovery of minocycline added to human urine was studied.

Keywords——spectrophotometry; minocycline; zirconium(IV); *o*-hydroxyhydroquinonephthalein; fluoride ion; sodium dodecyl sulfate

Minocycline (MINO),[2)] a tetracycline antibiotic, has a broad therapeutic range and is widely used in clinical chemotherapy, but little work has been done on chemical methods for the determination of MINO.[3)] As tetracycline antibiotics contain an enolized hydroxy group of a 1,3-diketone, like alizarin derivatives, and react with metal ions such as thorium(IV), zirconium(IV) [Zr(IV)], uranium(VI), bismuth(III), aluminum(III), molybdenum(VI), *etc.*, several methods for the determination of tetracycline antibiotics using these metal ions have been reported.[4)] On the other hand, we have noticed that the coexistence of some drugs such as tubocurarine, papaverine, tetracycline, *etc.* in the color reaction among Zr(IV), *o*-hydroxyhydroquinonephthalein (Qn.Ph.) and fluoride ions in the presence of sodium dodecyl sulfate (SDS) shows a red shift (10—15 nm) as compared with the Zr(IV)–Qn.Ph.–fluoride ions complex in the absence of these drugs.

In this paper, a simple and rapid spectrophotometric determination of MINO using Zr(IV), Qn.Ph. and fluoride ions in the presence of SDS is described. The proposed method was applied to the determination of minocycline hydrochloride (MINO·HCl) in pharmaceutical preparations. Recovery of MINO added to human urine was also examined.

Experimental

Materials and Reagents——Standard MINO Solution: A stock solution (1.0×10^{-3} M, M = mol dm^{-3}) of MINO was prepared by dissolving MINO·HCl ($C_{23}H_{27}N_3O_7 \cdot HCl \cdot 2H_2O$, manifested potency, 840 μg/mg, Japan-Lederle, Ltd., Tokyo) in water, and the working solution was prepared by suitable dilution of this stock solution as required.

Zr(IV) Solution: A stock solution (1.0×10^{-2} M) of Zr(IV) was prepared by dissolving zirconium tetrachloride in water. The working solution (1.0×10^{-3} M) was prepared by suitable dilution of this stock solution as required.

Qn.Ph. Solution: A Qn.Ph. solution was prepared in 1.0×10^{-3} M methanol solution as described in a previous report.[5)]

Sodium Fluoride Solution: A 1.0×10^{-2} M aqueous solution of sodium fluoride was prepared.

SDS Solution: A 1.0% aqueous solution of SDS (Kishida Chemical Co., Ltd.) was prepared without purification.

Buffer Solution: A buffer solution of pH 2.3 was made by mixing appropriate amounts of 0.2 M hydrochloric

acid and 0.2 M sodium acetate (Walpole buffer solution) for pH adjustments.

All other materials and reagents were of analytical reagent grade. All the solutions were prepared with doubly distilled water.

Apparatus——A Shimadzu UV-240 recording spectrophotometer with 1.0-cm silica cells was used to take absorption spectra and for absorbance measurements. A Hitachi-Horiba F-7 glass electrode pH meter was used for pH measurements.

Standard Procedure——A MINO solution containing 5—40 μg of MINO was placed in a 10-ml calibrated flask; to this were added 0.5 ml of 1.0×10^{-3} M Zr(IV) solution, 1.5 ml of 1.0×10^{-2} M sodium fluoride solution, 0.5 ml of 1.0% SDS solution, 3.0 ml of buffer (pH 2.3) solution and 1.0 ml of 1.0×10^{-3} M Qn.Ph. solution. The mixture was diluted to 10 ml with water and kept at 20—25 °C for 20 min. The absorbance of the Qn.Ph.–Zr(IV)–fluoride ions–MINO solution (solution A) was measured at 515 nm against the Qn.Ph.–Zr(IV)–fluoride ions solution (solution B).

Results and Discussion

Absorption Spectra

In Fig. 1, curves I, II and III show the absorption spectra of Qn.Ph.–fluoride ion, Qn.Ph.–Zr(IV)–fluoride ions (solution B) and Qn.Ph.–Zr(IV)–fluoride ions–MINO (solution A), respectively, in the presence of SDS at pH 2.3.

Solution A produced higher absorbance around 515 nm against water as compared with solution B. On the other hand, solution A and solution B in the absence of SDS were unstable and these solutions gradually deteriorated on standing. An addition of SDS as an anionic surfactant to these solutions stabilized them remarkably. In addition, the difference of absorbance at 515 nm between solutions A and B in the presence of SDS was greater than that in the absence of SDS. No difference of absorbance between solutions A and B in the absence of fluoride ion was observed.

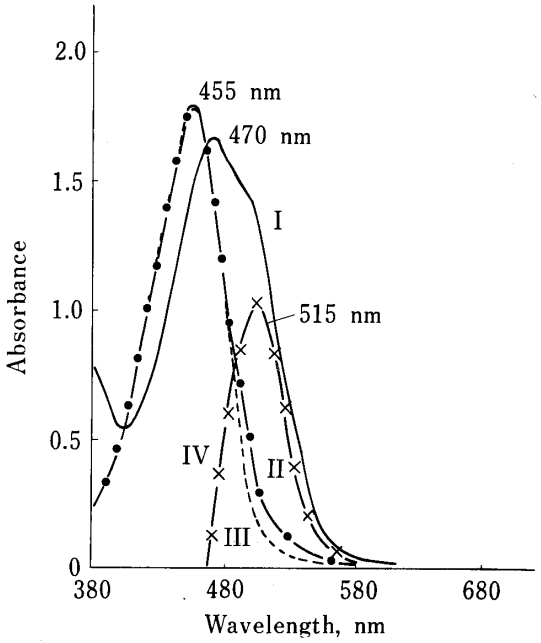

Fig. 1. Absorption Spectra of Qn.Ph.–Fluoride Ions, Qn.Ph.–Zr(IV)–Fluoride Ions and Qn. Ph.–Zr(IV)–Fluoride Ions–MINO Solutions in the Presence of SDS at pH 2.3

MINO, 274.5 μg/10 ml; Zr(IV), 3.0×10^{-5} M; Qn.Ph., 6.0×10^{-5} M; NaF, 9.0×10^{-4} M; SDS, 0.5 ml of 1.0% SDS solution/10 ml; reference, water.

Curve I (——), Qn.Ph.–Zr(IV)–fluoride ions–MINO; curve II (—●—), Qn.Ph.–Zr(IV)–fluoride ions; curve III (– – –), Qn.Ph.–fluoride ion; curve IV (—×—), curve I minus curve II.

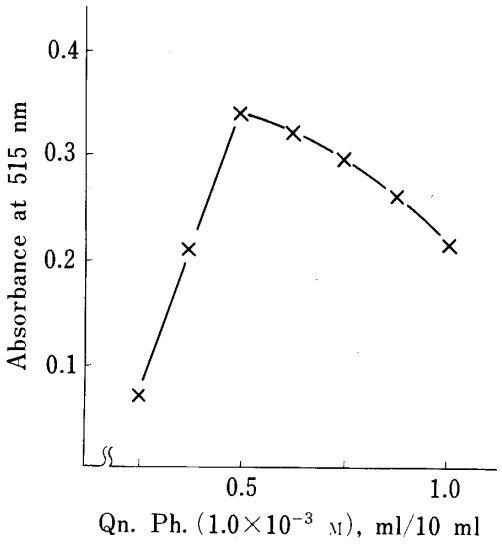

Fig. 2. Effect of Qn.Ph. Concentration

MINO, 22.9 μg/10 ml; Zr(IV), 5.0×10^{-5} M; NaF, 1.5×10^{-3} M; SDS, 0.5 ml of 1.0% SDS solution/10 ml; pH 2.3; reference, solution B.

Effects of pH and Surfactant

The effect of pH was examined. The maximum and constant absorbance was obtained between pH 2.0 and 2.7. Walpole buffer (0.2 M hydrochloric acid–0.2 M sodium acetate buffer) solution was found to be satisfactory for pH adjustments.

Compared with nonionic surfactants (0.5 ml of 1.0% solution/10 ml) such as polyvinyl alcohol or cationic surfactants (1.0 ml of 1.0×10^{-2} M solution/10 ml) such as cetyltrimethylammonium chloride, the use of an anionic surfactant gave superior sensitivity. SDS, an anionic surfactant, was the most effective dispersion agent; the maximum absorbance was obtained with 0.5 ml of 1.0% SDS solution.

Accordingly, subsequent measurements were carried out by addition of 3.0 ml of the buffer (pH 2.3) solution and 0.5 ml of 1.0% SDS solution in the final volume of 10 ml.

Effects of Qn.Ph., Zr(IV) and Fluoride Ions

The effect of the amount of Qn.Ph. on the absorbance of solution A at 515 nm against solution B was examined at various Qn.Ph. concentrations, the amounts of Zr(IV), fluoride ions and MINO being kept constant. The maximum absorbance was obtained when the molar ratio of Qn.Ph. to Zr(IV) was 1 : 2. The result is shown in Fig. 2. Further, the molar ratio of Zr(IV) to Qn.Ph. in the reaction was found to be 1 : 2 in the presence and absence of MINO by using the molar ratio method.

The effect of the amount of fluoride ion was next examined, the amount of Zr(IV) being kept constant. The absorbance of solution A at 515 nm against solution B gave the maximum absorbance value when fluoride ion was added at about 30-fold molar excess over Zr(IV).

Accordingly, all further work was carried out with 5.0×10^{-5} M Zr(IV), 1.0×10^{-4} M Qn.Ph. and 1.5×10^{-3} M sodium fluoride solutions in the final volume of 10 ml.

Stability and Calibration Curve

The maximum and constant absorbance of solution A at 515 nm against solution B was obtained when the solutions were kept at 20—25 °C for longer than 15 min. The absorbance

TABLE I. Effect of Various Substances on the Assay

Substance	Added (μg/10 ml)	Mol ratio (substance/MINO)	Absorbance at 515 nm
—	—	—	0.343
Fe(III) (Sulfate)	2.8	2	0.378
Th(IV) (Nitrate)	58.0	5	0.418
Cu(II) (Nitrate)	31.8	10	0.292
Zn(II) (Chloride)	65.4	20	0.343
Ca(II) (Chloride)	40.1	20	0.343
Oxalic acid	162.1	10	0.343
Uric acid	168.8	20	0.343
Hippuric acid	895.9	100	0.343
Taurine	625.7	100	0.343
Creatinine	113.1	20	0.343
Ascorbic acid	176.1	20	0.343
Caffeine	194.2	20	0.343
Ampicillin	349.4	20	0.343
Lactose	3423.0	200	0.343
Human albumin	250.0	—	0.418
Streptomycin	145.4	5	0.232
Diphenhydramine	255.4	20	0.245

MINO, 22.9 μg/10 ml; Zr(IV), 5.0×10^{-5} M; Qn.Ph., 1.0×10^{-4} M; NaF, 1.5×10^{-3} M; SDS, 0.5 ml of 1.0% SDS solution/10 ml; pH 2.3; reference, solution B.

was constant for at least 2 h. This method was found to be applicable to the determination of MINO in the concentration range of 5—40 μg in a final volume of 10 ml. The Sandell sensitivity was estimated to be 0.0067 $\mu g/cm^2$ for MINO at 515 nm, and the apparent molar absorptivity was calculated to be $5.5 \times 10^4 l \cdot mol^{-1} \cdot cm^{-1}$. When 6 sample solutions containing 22.9 μg/10 ml of MINO were determined by the standard procedure, the coefficient of variation was 0.53%.

Interference

Various substances were examined for interference. Though large amounts of metal ions such as iron(III), copper(II), thorium(IV), molybdenum(VI) and tin(IV) interfered with the determination of MINO, most other metal ions and anions tested did not interfere with the reaction in the presence of a 20- to 200-fold excess of MINO.

Among the substances tested, amino acids, creatinine, hippuric acid, uric acid, ascorbic acid, caffeine, lactose, ampicillin, urea, *etc.* did not interfere with the reaction in 20- to 200-fold excess over MINO. Large amounts of human albumin caused an increase in the absorbance at 515 nm, and large amounts of diphenhydramine or streptomycin caused a decrease. These results are summarized in Table I.

Composition of the Complex

As described above, the molar ratio of Zr(IV) to Qn.Ph. in the presence and absence of MINO was found to be 1 : 2 by using the molar ratio method. The results are shown in Fig. 3. The molar ratio of MINO to Zr(IV) was found to be 1 : 2 by the molar ratio method, and the fluoride ion to Zr(IV) ratio was 1 : 4 in the presence and absence of MINO (molar ratio method). The results are shown in Fig. 4.

Thus, it was concluded that the mixed complex formed in this reaction system may be expressed as (MINO) (ZrF_4) $(Qn.Ph.)_2$.

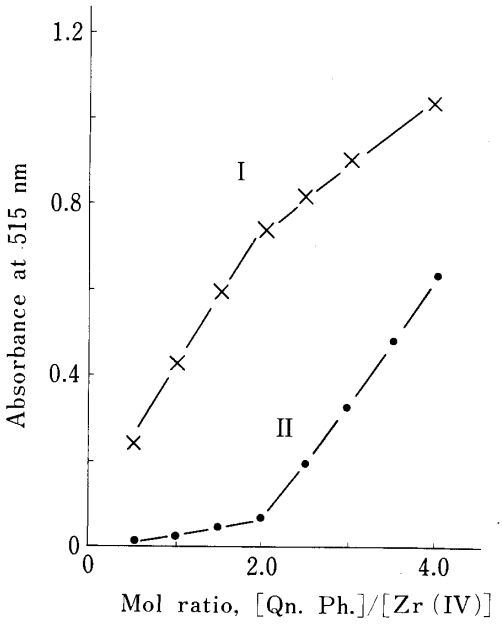

Fig. 3. The Ratio of Qn.Ph. to Zr(IV) by the Molar Ratio Method

Zr(IV), 2.0×10^{-5} M; Qn.Ph., 1.0×10^{-3} M × ml/10 ml; NaF, 6.0×10^{-4} M; SDS, 0.5 ml of 1.0% SDS solution/10 ml; pH 2.3; reference, reagent blank.
Curve I (—×—), in the presence of MINO (5.0×10^{-5} M, 228.4 μg/10 ml); curve II (—●—), in the absence of MINO.

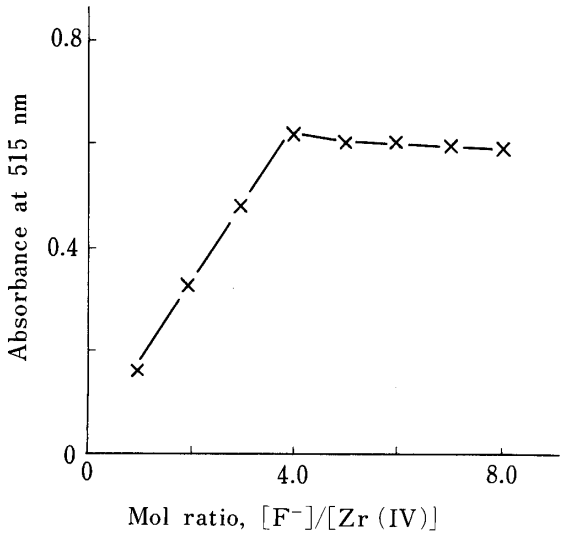

Fig. 4. The Ratio of F^- to Zr(IV) by the Molar Ratio Method

MINO, 2.0×10^{-5} M (91.5 μg/10 ml); Zr(IV), 2.0×10^{-5} M; Qn.Ph., 4.0×10^{-5} M; NaF, 1.0×10^{-3} M × ml/10 ml; SDS, 0.5 ml of 1.0% SDS solution/10 ml; pH 2.3; reference, MINO–Zr(IV)–Qn.Ph. solution.

Table II. Analytical Results for MINO·HCl in Pharmaceutical Preparations

Samples	MINO·HCl		Recovery[a] (%)	CV[b] (%)
	Calcd (mg)	Found[a] (mg)		
Capsules[c]	100	100.0	99.6	1.1
Granules[c]	20	19.6	97.6	1.5

a) Average recovery from 5 determinations.
b) Coefficient of variation.
c) Capsules (≒270 mg); granules (≒1000 mg).

Table III. Determination of MINO Added to Human Urine

MINO		Recovery[a] (%)	CV[b] (%)
Added (μg)	Found[a] (μg)		
13.5	12.6	93.5	2.5
22.9	22.1	96.6	2.0

a) Average recovery from 5 experiments.
b) Coefficient of variation.

Application to Pharmaceutical Preparations

This method was applied to the determination of MINO·HCl in pharmaceutical preparations. MINO·HCl was determined according to the following procedure. An aliquot of capsules or granules was ground in a mortar to a fine powder; the powder, was exactly weighed, and dissolved in water by shaking. The solution was filtered, and then the MINO·HCl content was determined according to the standard procedure. The results are given in Table II.

Recovery of MINO Added to Human Urine

Recovery of MINO added to human urine was examined.[6] Satisfactory results were obtained in the following way. Exactly 10 ml of human urine containing 20—160 μg/ml of MINO was adjusted to about pH 2 with 1 M hydrochloric acid solution. The solution was diluted to 20 ml with water. A requisite volume (less than 0.5 ml) of the sample solution was taken and MINO content was determined according to the standard procedure. The results are shown in Table III.

Conclusion

A simple and rapid spectrophotometric determination of MINO based on the formation of Qn.Ph.–Zr(IV)–fluoride ions–MINO mixed complex in the presence of SDS was developed. Beer's law was obeyed in the range of 5—40 μg of MINO in a final volume of 10 ml. The apparent molar absorptivity was calculated to be 5.5×10^4 l·mol^{-1}·cm^{-1} for MINO at 515 nm.

This proposed method was applied successfully to the determination of MINO·HCl in pharmaceutical preparations. Recovery of MINO added to human urine was good. This method may also be applicable to biological samples, and to determination of other tetracycline antibiotics.

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pharmaceutical preparations.

References and Notes

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6) Urinary excretion of MINO is regarded as small compared with that of other tetracycline antibiotics, but nevertheless, urine was chosen as a convenient biological sample.