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Spectrophotometric Determination of Cephalexin and Ampicillin using *o*-Hydroxyhydroquinonephthalein and Palladium (II)[1]

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A new sensitive, rapid and simple spectrophotometric method for the determination of cephalexin (CEX) and ampicillin (AB–PC) was established using *o*-hydroxyhydroquinonephthalein (Qn. Ph.) and palladium(II) [Pd(II)] at a low concentration of cetyltrimethylammonium chloride (CTAC) in weak acidic media. This method is based on the fact that the intensity of the absorption peak of Qn.Ph.-Pd(II) complex at 630 nm is decreased significantly by the addition of CEX or AB–PC, and the decrease in the absorbance is proportional to the concentration of CEX or AB–PC. The method could be used in the concentration range up to —17 μg/10 ml of CEX and —14 μg/10 ml of AB–PC, where the spectrophotometric sensitivities were estimated to be 0.0015 $\mu g/cm^2$ for CEX and 0.0012 $\mu g/cm^2$ for AB–PC for an absorbance unit of 0.001 at 630 nm, and was applied to the determination of CEX or AB–PC in pharmaceutical preparations.

Keywords——spectrophotometry; cephalexin; ampicillin; *o*-hydroxyhydroquinonephthalein; cetyltrimethylammonium chloride

Various methods for the determination of penicillins and cephalosporins commonly referred to as the β-lactam antibiotics have been reported and there have been many related reviews and commentaries.[2–11] There are several spectrophotometric methods for the assay[12] of these penicillins and cephalosporins by using metal ions such as iron, mercury, copper and vanadium. Since they are based on the reaction between the degradation product of penicillins or cephalosporins and metal ions, these spectrophotometric methods are not sensitive, rapid, simple or reproducible. However, these defects of the methods may be overcome by determining residual free metal ions in direct reactions between penicillins or cephalosporins and metal ions.

On the other hand, the spectrophotometric determination of palladium (II) [Pd(II)]using *o*-hydroxyhydroquinonephthalein (Qn. Ph.) was interfered with severely by micro amounts of sulfur compounds such as sulfide and thiourea, and the decrease in absorbance of Qn. Ph.–Pd(II) was proportional to the concentration of sulfur compounds.

In this paper, a new sensitive, rapid and simple spectrophotometric assay of cephalexin (CEX), a cephalosporins, and ampicillin (AB–PC), a penicillins, which are widely used in apclinical chemotherapy, is described. The proposed method using Qn. Ph. and Pd(II) was plied to the determination of CEX or AB–PC in pharmaceutical preparations.

Experimental

Materials and Reagents——Pd(II) Solution: A 1.0×10^{-2} M aqueous solution of Pd(II) was prepared by dissolving palladium chloride in water, and the titer of this solution was corrected by chelatometric titration. The working solution 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.[13] This solution was stable for at least six months when stored in a dark, cool place.

Standard CEX and AB–PC Solutions:[14] CEX and AB–PC solutions were prepared by dissolving cephalexin monohydrate (potency: 969 μg/mg) and anhydrous ampicillin (potency: 1003 μg/mg) in fresh water. The working solutions were prepared by suitable dilution of these solutions as required.

Cetyltrimethylammonium chloride (CTAC) Solution: A 1.0×10^{-2} M aqueous solution of CTAC was prepared by dissolving CTAC in water.

Buffer Solution: Walpole (sodium acetate–acetic acid, pH 5.0) buffer solution was used.

All other materials and reagents used were of analytical reagent grade. All solutions were prepared with deionized water.

Apparatus——Absorption spectra and spectrophotometric measurements were made with Shimadzu Model 200, 202 and 240 automatic recording spectrophotometers, with 1.0 cm matched quartz cells. The pH measurements were made with a Hitachi-Horiba Model M-5 pH meter.

Standard Procedure——Up to 17 μg of CEX or 14 μg of AB-PC was placed in a 10.0 ml volumetric flask and 1.0 ml of 2.0×10^{-4} M Pd(II) solution, 0.3 ml of 1.0×10^{-2} M CTAC solution, 2.0 ml of Walpole buffer solution (pH 5.0) and 0.5 ml of 1.0×10^{-3} M Qn.Ph. solution were added. Then the mixture was diluted to 10.0 ml with water and kept at 60°C for 20 min. After the solution had cooled to room temperature, the difference of absorbance ($=\Delta A$) between the Qn.Ph.–Pd(II)–CEX (or AB–PC) solution and the Qn.Ph.–Pd(II) solution was measured at 630 nm.

Results and Discussion

Absorption Spectra

Absorption spectra of Qn.Ph.–Pd(II) solution in the presence of CEX at pH 5.0 are shown in Fig. 1.

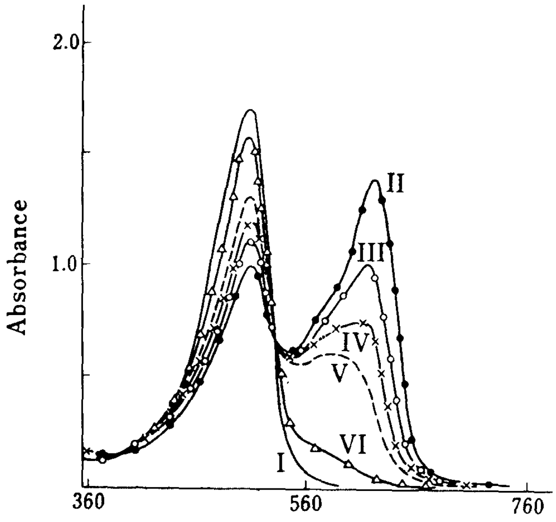

Fig. 1. Absorption Spectra of Qn.Ph. Solution and Qn.Ph.–Pd (II) Solution in the Presence of CEX at pH 5.0

Pd(II): 1.5×10^{-5} M; Qn.Ph.: 5.0×10^{-5} M; CTAC: 3.0×10^{-4} M; Reference: water; curve I: Qn.Ph. solution; curve II, III, IV, V and VI: Qn.Ph.-Pd (II) solutions (CEX concentration: curve II, O; curve III, 2.0×10^{-6} M; curve IV, 4.0×10^{-6} M; curve V, 6.0×10^{-6} M; curve VI, 1.5×10^{-5} M).

On addition of CEX to the Qn.Ph.–Pd (II) solution, the absorption peak at 630 nm was lowered significantly, and the magnitude of the decrease in absorbance was proportional to the concentration of CEX. Consequently, ΔA at 630 nm was used for the determination of CEX or AB–PC.

Effect of pH and Surface-active Agent

The effect of pH on the reaction was examined by measuring ΔA at 630 nm. The maximum and constant ΔA was obtained between pH 4.4 to 5.6. Walpole (sodium acetate-acetic acid, pH 5.0) solution was found to be satisfactory for this purpose.

Of various surface-active agents, CTAC was the most effective dispersion agent, and the maximum ΔA could be obtained over the range of 0.2—0.4 ml of 1.0×10^{-2} M CTAC solution.

Influence of Qn.Ph. and Pd(II)

The influence of the amount of Qn.Ph. on ΔA was examined by varying the molar ratio of Qn.Ph. to Pd(II), the amounts of Pd(II) and AB–PC being kept constant. The results indicated that the molar ratio of Qn.Ph. *vs.* Pd(II) should be more than 1.5.

The effect of metal ions in the reaction mixture was examined by measuring the difference of absorbance between Qn.Ph.–metal ion–AB–PC and Qn.Ph.–metal ion solutions. The maximum difference of absorbance was obtained with Pd(II) among various metal ions tested, such as Pd(II), Cu(II), Fe(III), Bi(III) and Pt(IV). In a similar manner, the optimum volume of Pd(II) was found to be 1.0 ml of 2.0×10^{-4} M Pd(II) solution in relation to the determination limit of AB–PC or CEX.

Accordingly, all further work was carried out with 2.0×10^{-5} M Pd(II) and 5.0×10^{-5} M Qn.Ph. solutions in a volume of 10.0 ml.

Stability

Experiments on the effect of time and temperature proved that on heating at 60°C the maximum and constant ΔA was obtained at 20—30 min. The value of ΔA remained unchanged for at least 2 h after the solution was cooled to room temperature.

Calibration Curve

The calibration curve for the determination of CEX and AB–PC was prepared by the standard procedure. It obeyed Beer's law up to —17 μg of CEX and —14 μg AB–PC in 10 ml of the solution. The spectrophotometric sensitivities were estimated to be 0.0015 μg/cm^2 CEX and 0.0012 μg/cm^2 AB–PC for an absorbance unit of 0.001 at 630 nm.

Interference

Under the standard conditions, various substances were examined for interference. Several metal ions such as Fe(III), Cu(II), Al(III) and Mo(VI) interfered because of the formation of colored substances with Qn.Ph. in weakly acidic media. Other metal ions such as K(I), Na(I), Ca(II), Mg(II), Zn(II), Co(II) and Hg(II) did not interfere when present in 10—50 fold excess. Anions such as chloride, bromide, fluoride, phosphate, carbonate, citrate, nitrate and sulfate did not interfere in 50—100 fold excess. As shown in Table I, though amino acids such as tryptophan and glycine interfered in micro amounts, other substances such as urea, glucose, lactose, starch, antipyrine and salicylic acid did not interfere.

Table I. Effect of Other Substances

Substances	Added as	Added (μg/10 ml)	Diff. of absorbance at 630 nm
—	—	—	0.475
Fe(III)	Sulfate	0.6	0.399
Cu(II)	Nitrate	0.6	0.388
Mo(VI)	Sodium	4.8	0.549
Zn(II)	Chloride	26.2	0.475
Ca(II)	Chloride	40.1	0.475
I^-	Potassium	2.5	0.595
S^{2-}	Sodium	1.6	0.556
CN^-	Potassium	0.5	0.666
$C_6H_5O_7^{3-}$	Acid	37.8	0.476
Tryptophan	—	2.0	0.555
Glycine	—	3.0	0.570
Creatinine	—	2.3	0.643
Urea	—	120.1	0.477
Hippuric acid	—	35.8	0.476
Glucosamine	Hydrochloride	17.9	0.555
Glucose	—	360.3	0.475
Caffeine	—	38.3	0.475
Uric acid	—	3.4	0.562
Pyridoxine	Hydrochloride	8.4	0.524

CEX taken: 7.0 μg/10 ml; Pd(II): 2.0×10^{-5} M; Qn.Ph.: 5.0×10^{-5} M; CTAC: 3.0×10^{-4} M; pH: 5.0; Reference: water.

Table II. Analytical Results for CEX or AB–PC Pharmaceuticals

Sample	Calcd (%)	Found (%)	Recovery of proposed method (%)
CEX dry syrup	10.0	10.34	97.8—102.6
AB–PC dry syrup	10.0	10.24	95.3—104.3

Taken: CEX (3.5—7.0) μg/10 ml; AB–PC (3.5—7.0) μg/10 ml.

Application of the Proposed Method

The procedure was applied to the determination of CEX or AB–PC pharmaceuticals (10% dry syrup). The results are given in Table II.

In the procedure described above, the difference of absorbance between the Qn.Ph.–Pd(II) and Qn.Ph.–Pd(II)–sulfur-containing material solution was measured at 630 nm. The results are shown in Table III.

Table III. Application of This Method to Various Sulfur Atom-containing Substances

Substance	Added		Diff. of absorbance at 630 nm
	M	μg/10 ml	
CEX	2.0×10^{-6}	7.0	0.475
AB–PC	2.0×10^{-6}	7.0	0.586
CEC[a]	2.0×10^{-6}	7.2	0.567
SB–PC[a]	2.0×10^{-6}	9.1	0.693
AM–PC[a]	2.0×10^{-6}	8.4	0.657
AC–PC[a]	2.0×10^{-6}	6.8	0.502
6-APA[a]	2.0×10^{-6}	4.3	0.420
Thiourea	2.0×10^{-6}	1.5	0.576
2-Thiouracil	2.0×10^{-6}	2.6	0.384
Methiocil	2.0×10^{-6}	2.8	0.395
Thioglycolic acid	2.0×10^{-6}	1.8	0.507
6-Mercaptopurine	2.0×10^{-6}	3.0	0.637
2-Mercaptobenzimidazole	2.0×10^{-6}	3.0	0.336
Cysteine	2.0×10^{-6}	2.4	0.578
Chlothiazepam[b]	2.0×10^{-6}	6.7	0.245
Methionine	2.0×10^{-6}	3.0	0.385
Thiamine·HCl	5.0×10^{-5}	168.6	0.350
Biotin	5.0×10^{-5}	122.2	0.382
Sulfanilamide	$5.0\times1.^{-5}$	86.1	0.058
Chlorpromazine·HCl[b]	5.0×10^{-5}	177.7	0.060
Saccharin·Na	5.0×10^{-5}	120.6	0.090
Sulpyrine[c]	2.0×10^{-5}	70.3	0.575
Sulfanilic acid	5.0×10^{-5}	86.9	0.010

Pd(II): 2.0×10^{-5} M; Qn.Ph.: 5.0×10^{-5} M; CTAC: 3.0×10^{-4} M; pH: 5.0; Reference: water; *b*) Yoshitomi Pharmaceutical Co., Ltd., Osaka. *c*) Sumitomo Ind. Co., Ltd., Osaka, *a*) Takeda Pharmaceutical Ind. Co., Ltd., Osaka, Japan: CEC (sodium cephacetril, potency: 912 μg/mg), SB-PC (disodium sulbenicillin, potency: 904 μg/mg), AM-PC (amoxycillin, potency: 854 μg/mg), AC-PC (cyclacillin, potency: 990 μg/mg), 6-APA (6-aminopenicillanic acid, assay: 100.7%).

Conclusion

A new simple, rapid and highly sensitive procedure for the spectrophotometric assay of CEX and AB–PC utilizing the decrease in absorbance of Qn.Ph.–Pd(II) complex was developed. Beer's law was obeyed up to —17 μg of CEX and —14 μg of AB–PC in 10 ml. The effective molar "desorptivities" in this reaction were 2.37×10^5 l mol^{-1} cm^{-1} for CEX and 2.93×10^5 l mol^{-1} cm^{-1} for AB–PC at 630 nm. This method is about ten times more sensitive than the method using imidazole-mercury.[12)] The precisions were estimated for 7.0 μg of CEX and 7.0 μg of AB–PC, and the coefficients of variation for 8 determinations were 0.66% and 0.87%, respectively. This procedure was applied to a few commercial preparations (10% dry syrup) containing CEX or AB–PC. Application of the proposed method in biological and clinical fields was not studied because various substances interfered with the determination of CEX and AB–PC. However, this method should be useful for the determination of various medicinal sulfur-atom-containing drugs.

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