Spectrophotometric Determination of Chloramphenicol and Tetracycline Hydrochloride in Mixtures

By R. C. SHAH, P. V. RAMAN, and B. M. SHAH

A rapid method for the spectrophotometric determination of chloramphenicol and tetracycline hydrochloride in binary mixtures without prior separation is described. Chloramphenicol is determined by the measurement of difference in (extinction) values (ΔE) at 282 mμ and 233 mμ, and tetracycline hydrochloride is determined by measurement of absorbance at 380 mμ, using 0.1 N ammonium hydroxide as the solvent.

WITH THE extensive use of antibiotics, and the increased prevalence of mixed antibiotic therapy, the pharmaceutical analyst is faced with the problem of developing quick and reliable methods for the assay of individual components in mixtures. Spectrophotometric and other physicochemical methods are now finding increased acceptance over the bioassay methods (1, 2). Marzys (3) has described the application of spectrophotometric procedures for the determination of individual components in mixtures where the results are computed by solving simultaneous equations. Pernarowski, et al. (4), have described the application of spectrophotometric assay for the components of binary mixtures based on the location of isoabsorptive points. Elvidge and Peutrell (5) have described a (ΔE) spectrophotometric method which is based on a single wavelength and two solvents at different pH. The present paper describes a (ΔE) spectrophotometric method based on two wavelengths and one solvent for the determination, without prior separation, of chloramphenicol and tetracycline hydrochloride present in binary mixtures.

EXPERIMENTAL

Materials and Equipment.—Chloramphenicol U.S.P. (99.5%) from Carlo Erba, S.p.A., Milan, Italy, tetracycline hydrochloride U.S.P. (99.6%) from Messrs. Eigenmann & Veronelli, S.p.A., Milano, 0.1 N ammonium hydroxide, a Beckman spectrophotometer model G 2400 with DU photomultiplier combination G 4700 using hydrogen discharge tube, and 1-cm. silica cells were the materials and equipment employed in this study.

From a study of the spectral characteristic curves of chloramphenicol and tetracycline hydrochloride using water as the solvent, it was observed that by determining the absorptivities ($E_{1\,cm.}^{1\%}$) at 278 mμ and 360 mμ and using simultaneous equations, the composition of the binary mixture could be determined. However, this procedure involves relatively long calculations.

Experiments were performed to determine the isoabsorptive wavelength using various solvents. Water was found unsuitable as a solvent for this purpose, and the use of 0.1 N acetic acid gave two isoabsorptive wavelengths, viz., 282.5 mμ and 297.5 mμ. The slopes of curves at the former wavelength were more steep than those at the latter wavelength. Various mixtures of chloramphenicol and tetracycline hydrochloride were assayed by this method by measuring absorbances at 278 mμ, 297.5 mμ, and 360 mμ but the method lacked precision. The use of 0.1 N sodium hydroxide gave some interesting results. Chloramphenicol gave almost the same spectral characteristics as with 0.1 N acetic acid except for a slight depression of absorbance at the maxima. Tetracycline hydrochloride gave almost constant values of absorptivity in the spectral range of 230 mμ to 280 mμ. In this range, $E_{1\,cm.}^{1\%}$ for chloramphenicol registered a steep continuous increase up to 275 mμ, whereas those for tetracycline hydrochloride remained almost the same.

It was thus evident that chloramphenicol could be determined in the presence of tetracycline hydrochloride by making use of the "difference in absorptivity values" (ΔE) at two suitable wavelengths. The use of 0.1 N sodium hydroxide was found to be critical as regards time for ΔE measurements. Higher concentrations of alkali, such as 0.25 N
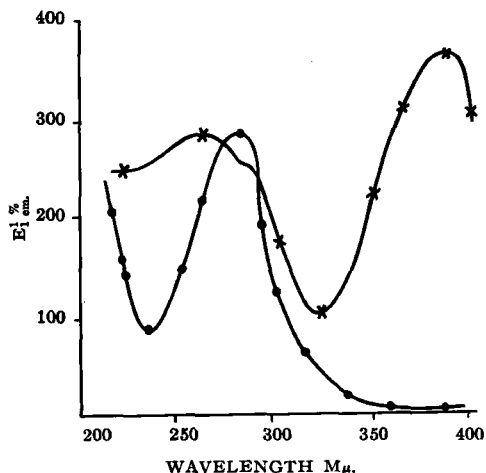

Fig. 1.—$E_{1\,cm.}^{1\%}$ curve for chloramphenicol ($\bullet$—$\bullet$), $E_{1\,cm.}^{1\%}$ curve for tetracycline hydrochloride (X—X).

TABLE I.—$E_{1cm.}^{1\%}$ OF CHLORAMPHENICOL AND TETRACYCLINE HYDROCHLORIDE IN SUITABLE RANGES OF WAVELENGTHS USING 0.1 N AMMONIUM HYDROXIDE

Wavelength, mμ	231	232	233	234	235	280	281	282	283	284
Chloramphenicol	110.5	107.5	102.5	97.5	97.5	290	290	292.5	290	285
Tetracycline hydrochloride	257.5	260	262.5	265	267.5	267.5	265	262.5	262.5	260

TABLE II.—PERCENTAGE RECOVERY OF CHLORAMPHENICOL AND TETRACYCLINE HYDROCHLORIDE FROM SYNTHETIC MIXTURES

Synthetic Mixtures		Found by the Method Described		Percentage Recovery	
Chloramphenicol, mcg./ml.	Tetracycline Hydrochloride, mcg./ml.	Chloramphenicol, mcg./ml.	Tetracycline Hydrochloride, mcg./ml.	Chloramphenicol	Tetracycline Hydrochloride
2	18	2.3	17.5	115	96.9
4	16	4.2	15.9	105	99.4
6	14	5.9	14	98.3	100
8	12	8.1	12	101	100
10	10	9.9	10.1	99	101
12	8	12.1	8.1	100.1	100.1
14	6	13.8	5.9	98.6	98.3
16	4	15.9	4	99.4	100
18	2	17.6	2.25	112.5	97.7

sodium hydroxide, were not critical in the determination of ΔE values for chloramphenicol. However, with this strength of sodium hydroxide the determination of tetracycline hydrochloride spectrophotometrically at 380 mμ requires strict adherence to time (6, 7). The use of 0.1 N ammonium hydroxide satisfied all requirements. The spectral characteristics of both chloramphenicol and tetracycline hydrochloride were almost the same as with 0.1 N sodium hydroxide. Furthermore, with ammonia, the normality of the solution and time for taking readings were not critical. The absorbance values remained almost the same for 3 hours.

Determination of ($\Delta E_{1cm.}^{1\%}$) for Chloramphenicol and $E_{1cm.}^{1\%}$ for Tetracycline Hydrochloride.—Solutions containing 20 mcg./ml. of tetracycline hydrochloride and chloramphenicol were prepared in 0.1 N ammonium hydroxide and spectrophotometric readings were taken in the ultraviolet range of 220 mμ to 400 mμ using 0.1 N ammonium hydroxide as blank (Fig. 1). For the determination of chloramphenicol, the most suitable wavelengths were 233 mμ and 282 mμ, and $\Delta E_{1cm.}^{1\%}$ was found to be 190 (Table I). For tetracycline hydrochloride, the most suitable wavelength was 380 mμ and $E_{1cm.}^{1\%}$ was 355.

Recovery Experiments.—Various mixtures containing a total of 20 mcg./ml. of chloramphenicol and tetracycline hydrochloride were prepared in 0.1 N ammonium hydroxide. Absorbances were measured at 233 mμ, 282 mμ, and 380 mμ using 0.1 N ammonium hydroxide as blank. Chloramphenicol was determined from the difference in absorptivities at 282 mμ and 233 mμ taking $\Delta E_{1cm.}^{1\%}$ as 190. Tetracycline hydrochloride was determined from the absorptivity at 380 mμ taking $E_{1cm.}^{1\%}$ as 355. The results are tabulated in Table II and show that the method gives very satisfactory results except at the two extremes.

Results.—The method was satisfactorily applied for the assay of commercial capsules of chloramphenicol 200 mg. and tetracycline hydrochloride

100 mg.[1] The usual excipients like lactose, amylum, and talcum were not found to interfere in the assay. The results compared very well with those of the usual method of determining chloramphenicol and tetracycline hydrochloride, after resolving the mixture into its components, by selective solvent extraction with ethyl acetate.

DISCUSSION

From Table II, it is evident that the method can safely be applied to the mixtures containing 20% and 80%. In practice, the majority of preparations will contain these two antibiotics within this range and hence the method is of great practical value. Again the method offers an advantage over the official spectrophotometric method for the determination of tetracycline hydrochloride (7) because strict adherence to time is not necessary when 0.1 N ammonium hydroxide is used as a solvent. The normality of ammonium hydroxide is not critical as it may range from 0.07 N to 0.13 N without affecting the results. Finally the ΔE value for chloramphenicol and $E_{1cm.}^{1\%}$ at 380 mμ value for tetracycline hydrochloride may vary slightly from instrument to instrument; hence these values must be ascertained on the individual instrument.

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[1] Marketed as Kemicycline capsules by Carlo Erba S.p.A., Italy.