Table II—Results of Duplicate Determinations on a Series of Commercial Products

Product	Label, mg./Tablet	Found, mg./Tablet
A	250	234
B	325	319
C	150	151
D	300	310
E	325	327
F	120	131

Measure the absorbance in 1-cm. cells against a proper blank in a spectrophotometer set to 395 mμ. The absorbances obtained plot into a straight line.

Procedure—Weigh contents of 10 capsules. Weigh an aliquot of the powder to correspond to 120 mg. acetaminophen. Transfer it to a suitable container and add about 50 ml. 1 *N* hydrochloric acid. Agitate with mechanical shaker for 20 min. Filter through paper quantitatively into a 100-ml. volumetric flask and bring to the mark with 1 *N* hydrochloric acid. Mix. Transfer 1.0 ml. to a test tube. Add 5 ml. of 1 *N* hydrochloric acid and heat for 10 min. in boiling water. Treat a standard solution of acetaminophen in the same fashion. Allow tubes to cool. Transfer quantitatively to 50-ml. volumetric flasks. Add 10 ml. vanillin reagent to each. Bring to the mark with water and mix. Measure the absorbances in 1-cm. cells against a blank in a spectrophotometer set to 395 mμ. To calculate milligrams of acetaminophen in aliquot of powder weighed, divide absorbance of sample by absorbance of standard and multiply by 120.

Remarks—The satisfactory application of the procedure was shown by determination of known synthetic powder mix in Table I and a number of commercial pharmaceutical products, Table II.

SUMMARY

A rapid method for the determination of acetaminophen in typical cold capsule medications is outlined. Because of the specificity of the reaction involved, many tedious separations may be circumvented, and its application to routine control testing has been demonstrated.

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Application of Absorbance Ratios to Analysis of Pharmaceuticals V: Analysis of Tetracycline Hydrochloride in Presence of Anhydrotetracycline and Epianhydrotetracycline

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Abstract □ The principles inherent in the absorbance ratio technique are applied to the analysis of mixtures containing tetracycline HCl, anhydrotetracycline, and epianhydrotetracycline. The analysis is carried out without prior separation of the components of the mixture and is applicable to commercial preparations containing these substances.

Keyphrases □ Tetracycline HCl tablets—analysis □ Epi- and anhydrotetracycline presence—tetracycline HCl analysis □ Absorbance ratio technique—tetracycline HCl analysis □ UV spectrophotometry—analysis.

Titrimetric (1, 2), polarographic (3, 4), chromatographic (5–9), spectrophotometric (10–14), and microbiological (15, 16) methods of analysis have been used to determine the tetracyclines. The precision of the latter method is rarely more than 15% (17) and accuracy is limited by the presence of biologically active tetracycline-like substances in the samples being analyzed. Spectrophotometric procedures tend to be more accurate, but are based on a prior separation by TLC (5, 6, 17, 18) or on a conversion of the antibiotic to an anhydro compound (13, 19, 20).

An examination of the spectrophotometric characteristics of tetracycline (TC), anhydrotetracycline (ATC), and epianhydrotetracycline (EATC) in 0.1 *N* hydrochloric acid solution indicates that these substances may be analyzed by using the absorbance ratio method of analysis (21, 22). This method is based on the linear relationship between absorbance ratio values (Q values) and the fraction of one of the components in a mixture. Q values are calculated from absorbance values at a wavelength of maximum absorption and at an isosbestic point. Conversion of the parent substance to secondary compounds and prior isolation procedures are, therefore, unnecessary.

EXPERIMENTAL

Apparatus—UV Spectrophotometer.[1,2]

[1] Beckman Model DU

[2] Bausch & Lomb Spectronic 505.

Reagents—Methanol, reagent grade; hydrochloric acid, 0.1 *N* solution; tetracycline hydrochloride; the sample contained 93.2% TC (17) and 5.25% tetracycline-like substances (ATC and EATC); ATC and EATC may be prepared according to the method of Simmons (6).

Spectral Characteristics of ATC, EATC and TC—Solutions of ATC, EATC, and TC were prepared by dissolving 100-mg. samples in methanol and diluting with 0.1 *N* hydrochloric acid solution to a concentration of 20 mg./l. ATC and EATC showed absorption maxima at 272 and 434 mμ. Absorption maxima for TC occur at 272 and 357 mμ. Solutions of TC were stable over a 5-hr. period.

Location of the Isosbestic Points—The isosbestic points for TC, ATC, and EATC were located by a spectrophotometric comparison of 0.002% solutions. On the basis of this data, such points occur at 298 and 391 mμ. The isosbestic point at 391 mμ (see Fig. 1) was also located by determining absorptivity values for TC, ATC, and EATC at this wavelength. This value was found to be 8.27 ± 0.10 on the basis of 37 determinations. The details associated with the location of isosbestic points are given elsewhere (21).

Procedure—Weigh and powder 20 tablets or remove and weigh the contents of 20 capsules. Accurately weigh an amount of powder containing the equivalent of 250 mg. of TC. Transfer the powder to a 250-ml. volumetric flask with the aid of about 30 ml. of methanol. Dilute to volume with water. Shake well and filter through Whatman No. 1 filter paper. Transfer a 20-ml. aliquot of the filtered solution to a 1,000-ml. volumetric flask and dilute to volume with 0.1*N* hydrochloric acid solution. Measure the absorbance of this solution at 357 and 391 mμ using 0.1 *N* hydrochloric acid solution as a blank. Calculate the relative concentration of TC in the sample by substituting the observed values into the following equation.

$$\% \text{ TC} = \frac{\text{Q:357:391} - 0.23}{0.0367} \qquad \text{(Eq. 1)}$$

Q:357:391 is the absorbance ratio value for the solution and is obtained by dividing the observed absorbance at 357 mμ by that observed at 391 mμ. The numerical values of the intercept and the slope of the Q curve are 0.23 and 0.0367, respectively. Absolute concentrations of TC may be determined using the method previously reported (22).

$$\text{mg. TC} = \% \text{ TC} \times \frac{A_{391}}{8.27}$$

A_{391} is the absorbance of the solution at 391 mμ and 8.27 is the absorptivity value at this wavelength.

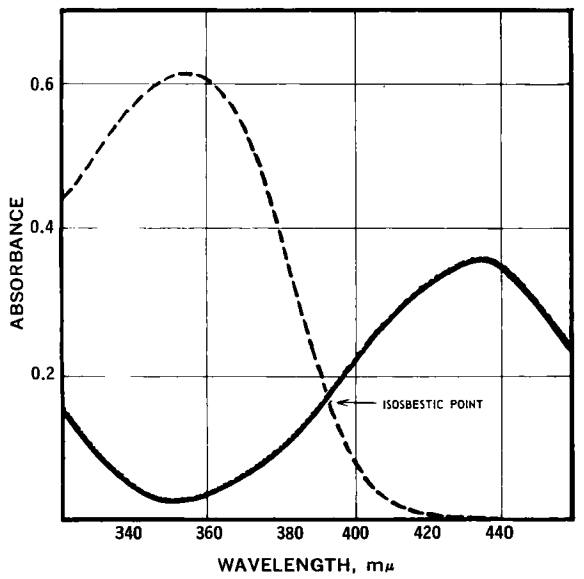

Figure 1—*Spectrophotometric curves for tetracycline* (---) *anhydrotetracycline* (——), *and epianhydrotetracycline* (——). *Each solution contains 20 mg. drug/l. of 0.1*N *HCl.*

Table I—Results of Analysis of Synthetic Mixtures Containing TC, ATC, and EATC

Mixture, mg.			Tetracycline,		
TC	ATC	EATC	Expected, mg.[a]	Found, mg.[b]	TC in Mix, %
247.0	10.2	0.0	230.2	230.0	91.7
252.2	5.1	4.8	235.1	232.7	90.9
247.4	5.1	9.6	230.6	230.7	90.4
235.8	10.2	9.6	219.8	218.1	86.5
247.6	0.0	9.6	230.8	229.8	91.7

[a] Based on an assay value of 93.2% for the TC used in the investigation. [b] Average of two determinations for each reported value. Mean recovery = 99.6 ± 0.6%.

DISCUSSION

The spectral characteristics of TC, ATC, and EATC have been reported and are illustrated in Fig. 1. These characteristics indicate that the principles of absorbance ratio method of analysis may be applied to a system containing these components. Isosbestic points were found at 298 and 391 mμ. However, only the point at 391 mμ and the wavelength at which TC exhibited maximum absorption, 357 mμ, were chosen for the analysis. This choice of wavelength results in optimum conditions and minimizes the possibility of interference by other substances which usually absorb radiant energy at shorter wavelengths. The proposed method of analysis results, therefore, in a direct measure of TC actually present in the sample. The combined amounts of ATC and EATC in the sample may be similarly determined by measuring absorbance values at 391 and 434 mμ. The latter procedure does not, however, differentiate between these substances.

A plot of Q:357:391 values versus the TC concentration in various synthetic mixtures indicates that a linear relationship exists between the two variables. On the basis of nine solutions containing TC, ATC, EATC, the equation for this straight line was found to be

$$\text{Q:357:391} = 0.0367 \text{ Ft.} - 0.23 \qquad \text{(Eq. 2)}$$

where Ft. is the fraction of TC present in the mixture. This equation yields only relative values. However, absolute values may be obtained by the method previously reported (22).

To test the overall accuracy and precision of the proposed method of analysis, several synthetic mixtures of known composition were prepared and analyzed. The results of these analyses are shown in Table I and indicate that the TC can be determined with a high degree of accuracy and precision. The expected quantity of TC in the mixture was calculated on the basis of a 93.2% assay value for the TC powder used. Recovery values are given in Table I. These values can be obtained by accurately diluting stock solutions. However, relative values (that is, the percent purity TC in the sample) can be obtained by preparing solutions in graduated cylinders and without an accurate weighing of the drug sample.

Six samples of commercial tablets and capsules were assayed by the proposed method. The results are summarized in Table II. These results would indicate that the proposed method is capable of yielding the same level of precision as that observed for synthetic mixtures. Product excipients do not seem to interfere with the determination.

The results show that all products complied with the pharmacopeial limits of not less than 85% of the amount of TC claimed on the label. However, all samples contained significant quantities (7–10%) of ATC and EATC. The clinical effects of these degradation products are not clearly established. However, it has been reported (6, 23) that a reversible renal dysfunction (Fanconi-type syndrome) occurs when degraded TC products are ingested.

The above preparations were also examined chromatographically and the drug in the product quantitatively determined by using a direct densitometric reading of the developed TLC plates. The precision of this technique was less than satisfactory (ap-

Table II—Results of the Analysis of Commercial Preparations Containing Tetracycline Hydrochloride[a]

Brand	Tetracycline HCl Found[b]	
	% purity (TC)	mg. TC/Capsule or Tablet
A	93.2 ± 0.9	247.5 ± 1.7
B	93.1 ± 0.5	236.2 ± 1.0
C	90.4 ± 1.1	259.3 ± 3.8
D	94.9 ± 0.7	240.0 ± 2.0
E	93.2 ± 0.8	237.5 ± 2.8
F	93.7 ± 0.8	223.8 ± 2.0

[a] Label claim = 250 mg./capsule or tablet. [b] Average of 10 determinations for each reported value.

proximately ±15%) but the results did show that the only degradation products in these preparations were ATC and EATC.

SUMMARY

The proposed method of analysis is based on a linear relationship between absorbance ratio values and the relative concentration of TC in mixtures containing TC, ATC, and EATC. It involves no prior separation of the components of the mixture, the manipulative techniques are simple, and the accuracy and precision of the method is equal to that reported in the literature for other methods of analysis. The method is particularly applicable to the routine analysis of products containing TC in degradation studies where it is necessary to determine relative TC concentrations. However, the method cannot be used if an identification or a quantitation of ATC and EATC is an analytical requirement.

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