Separation and Determination of Anhydrotetracycline, 4-Epianhydrotetracycline, Tetracycline, and 4-Epitetracycline in a Tetracycline Mixture

PETER P. ASCIONE and GEORGE P. CHREKIAN

Abstract □ A column chromatographic method for the determination of anhydrotetracycline, 4-epianhydrotetracycline, tetracycline, and 4-epitetracycline has been developed. The chromatogram involves the separation of these tetracyclines on a column of acid-washed diatomaceous earth treated with buffer consisting of 0.1 *M* ethylenediaminetetraacetic acid disodium salt at pH 7.0, glycerin, and polyethylene glycol 400, followed by a modified spectral determination of column eluates.

Keyphrases □ Tetracycline mixture—anhydrotetracycline, 4-epianhydrotetracycline, tetracycline, 4-epitetracycline determination □ Column chromatography—separation □ Colorimetric analysis—spectrophotometry

The method described in this paper was modified and adapted from previous studies (1, 2). Known tetracycline mixtures were determined with recoveries of 98.0 to 102.0% for anhydrotetracycline (ATC), 4-epianhydrotetracycline (EATC), tetracycline (TC), and 4-epitetracycline (ETC).

In the course of work in these laboratories on the stability of TC, it was necessary to find a specific and accurate method for the determination of degraded and nondegraded TC in crystalline and pharmaceutical preparations. The methods described by various workers (3–7) for separation of degraded TC from TC have been found workable and reproducible here, but they had the disadvantage of not determining all the components of a TC mixture on a single chromatogram.

TLC and column chromatographic methods were developed in these laboratories for the separation, examination, and determination of TC (1, 2). With modification of both the column chromatographic and spectrophotometric method, a specific and accurate method of assay for ATC, EATC, TC, and ETC, in TC mixtures, has been developed. This method allows a separate analysis for all components, thus providing a scheme for determining the column recovery quantitatively.

EXPERIMENTAL

Materials and Methods—Reagents, the preparation of diatomaceous earth[1] and chromatographic columns, and the apparatus have been described previously (2). A pH of 7.0 was maintained.

Determination of ATC, EATC, TC, and ETC—The general procedure, column preparation, and sample preparation for crystalline TC HCl or capsules and tablets were as described for demethylchlortetracycline HCl (2).

Sample Preparation—*TC Neutral and Syrups*—This was the same as described for demethylchlortetracycline neutral (2), except that 20 mg. of TC neutral or 1.0 ml. of syrup equivalent to 20–25 mg. of TC was used.

[1] Celite 545, Johns-Manville Corp., New York, NY 10016

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

No.	Standard Mixtures	Amount Present, mg.	Amount Found, mg.	Recovered, %
1	ATC HCl	2.58	2.55	98.8
	EATC HCl	2.32	2.31	99.6
	TC HCl	19.01	18.98	99.8
	ETC HCl	0.51	0.50	98.0
2	ATC HCl	2.32	2.27	97.8
	EATC HCl	3.29	3.30	100.3
	TC HCl	18.91	18.98	100.4
	ETC HCl	0.50	0.51	102.0

Development of Column—Use a 25-ml. glass-stoppered graduate as the primary receiver under the column. Add 20 ml. of benzene to the column. When the solvent level reaches the top of the column packing, add 60 ml. of chloroform. After collecting 10 ml. (Cut 1), remove the primary receiver and replace with a 10-ml. graduate. Collect 5 ml. in this graduate and discard. Replace the 10-ml. graduate with another 25-ml. glass-stoppered graduate. After collecting 15 ml. (Cut 2), remove the second graduate and replace with a 50-ml. volumetric flask. Collect the eluate in this flask until the level of the solvent in the column again reaches the top of the packing. Replace the 50-ml. volumetric flask (Cut 3) with a 10-ml. graduate, and add 40 ml. of 50% *n*-butanol in chloroform to the column. When the volume of eluate in the graduate reaches 8 ml., replace the 10-ml. graduate (Cut 4) with a 50-ml. glass-stoppered graduate and collect all of the last solvent added to the column (Cut 5).

Assay of Column Cuts—Determine the absorbance of Cut 1 (ATC) and Cut 2 (EATC) in a 1-cm. cell against chloroform at a wavelength of 438 mμ on a suitable spectrophotometer.

To Cut 3 (TC), add Cut 4 and mix well. Add 2.0 ml. of alkaline methanol solution and dilute to volume with chloroform. Determine the absorbance of this solution in a 1-cm. cell against chloroform, at a wavelength of 366 mμ, on a suitable spectrophotometer within 10 min. after making the solution alkaline. To Cut 5 (ETC), add 2.0 ml. of alkaline methanol; adjust the volume to the nearest milliliter graduation with chloroform, mix, and record the volume. Determine the absorbance of this solution in a 1-cm. cell against chloroform, at a wavelength of 366 mμ, within 10 min. after making the solution alkaline.

RESULTS AND DISCUSSION

In earlier work (1, 2), the separation and determination of TC by TLC and column chromatography suggested the development of this column chromatographic technique. The effectiveness of the column for the separation of ATC, EATC, TC, and ETC was demonstrated by the use of TLC (1). The column eluates were collected in fractions of 5 ml. and examined by TLC. Figure 1 shows the chromatography of column eluates. According to the results of TLC, the TC was separated on a column of acid-washed diatomaceous earth treated with EDTA, polyethylene glycol 400 (PEG 400), and glycerin at pH 7.0, using benzene, chloroform, and butanol as the developing solvents. The ATC and EATC are visible yellow bands which can be seen during chromatography. The TLC shows the distribution of the TC components isolated on

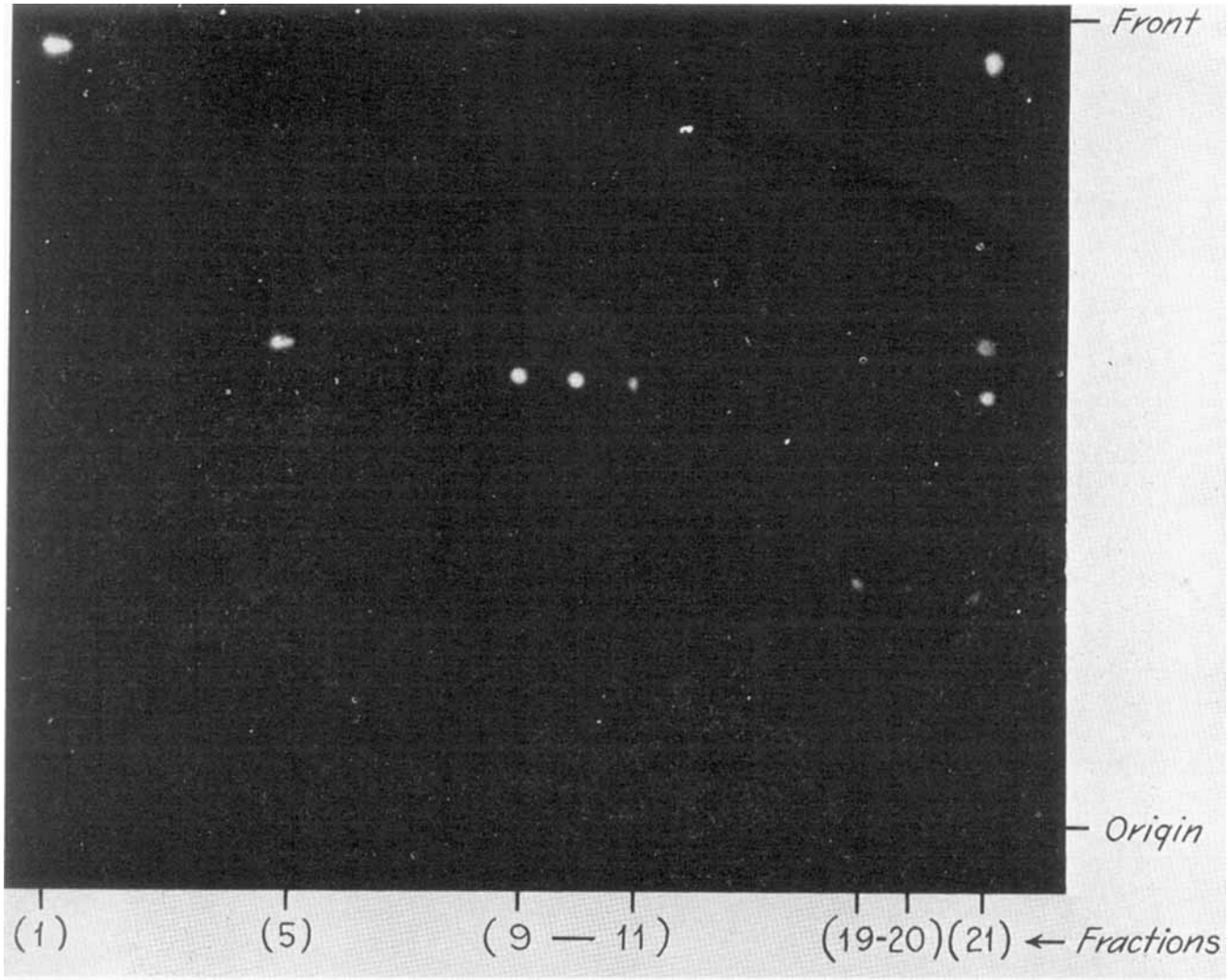

Figure 1—*TLC of TC mixture column fractions: (1) ATC; (5) EATC; (9-11) TC; (19-20) ETC; and (21) reference standard mixture.*

the column and having R_f values similar to those of standard test references.

Absorption Spectra—Absorption spectra in the region of 350–550 mμ were determined on a Cary model II recording spectrophotometer. Standard solutions of ATC and EATC, containing 0.25 mg./ml., were prepared by dissolving 25 mg. of standard in 10 ml. of methanol and diluting to 100 ml. with chloroform. Six milliliters of the chloroform solution was transferred to a 25-ml. volumetric flask and then diluted to 25 ml. with chloroform. Figures 2 and 3 show the spectra of ATC and EATC with both maxima at 438 mμ.

Through examination of the anhydro standards in these laboratories, the absorptivity for both ATC and EATC at 438 mμ was found to be 1.85×10^{-2}. The absorptivity is defined as the optical

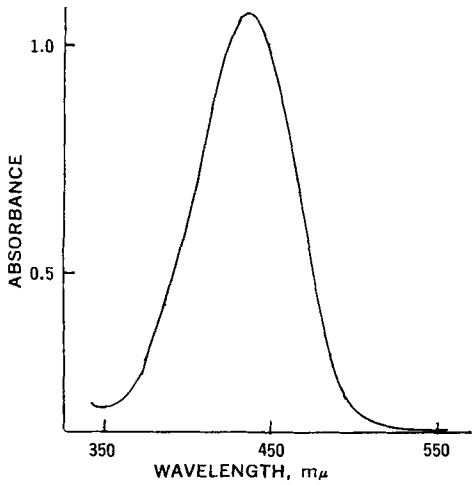

Figure 2—*Absorption spectrum of ATC.*

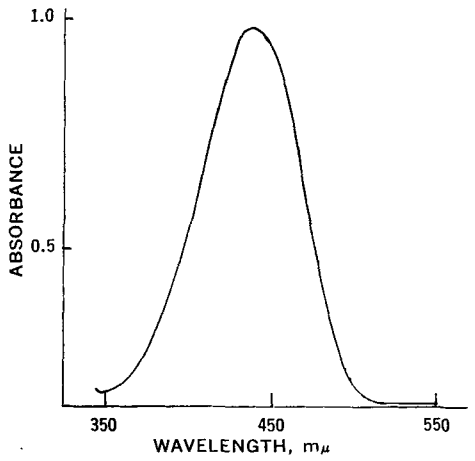

Figure 3—*Absorption spectrum of EATC.*

density at the wavelength of each particular TC for a solution containing 1 mcg. of TC per milliliter, using a 1-cm. cell.

A standard calibration curve of ATC HCl was prepared in chloroform solutions. In the analysis of ATC HCl, a linear relationship over the range of 0.025–1.5 mg./50 ml. is obtained. The absorptivity for TC and ETC was determined in alkaline methanol at 366 mμ and found to be 3.29×10^{-2} as previously described (2). The determination of absorbances of the column chromatography eluates provided a quantitative spectrophotometric method for assaying ATC, EATC, TC, and ETC in solvent solutions when compared with standards treated in a like manner.

To test the accuracy of this column chromatographic procedure for TC mixtures, a known mixture was made of standards of ATC, EATC, TC, and ETC, which was analyzed by the column procedure with the recoveries shown in Table I.

In the two experiments performed, recoveries of 98.0–102.0% were obtained on TC mixtures. Quantitative analysis of synthetic mixtures of TC was achieved with high degree of accuracy (Table I) on the column of acid-washed diatomaceous earth treated with buffer consisting of 0.1 *M* EDTA, glycerin, and PEG 400 at pH 7.0. Therefore, this method has the advantage of determining the entire content of a TC mixture on a single chromatogram.

REFERENCES

(1) P. P. Ascione, J. B. Zagar, and G. P. Chrekian, *J. Pharm. Sci.*, **56,** 1393(1967).

(2) *Ibid.*, **56,** 1396(1967).

(3) R. G. Kelly, *J. Pharm. Sci.*, **53,** 353(1964).

(4) B. W. Griffiths, *ibid.*, **55,** 353(1966).

(5) D. L. Simmons, C. K. Koorengevel, R. Kubelka, and P. Seers, *ibid.*, **55,** 219(1966).

(6) A. A. Fernandez, V. T. Noceda, and E. S. Carrerd, *ibid.*, **58,** 443(1969).

(7) D. L. Simmons, R. J. Ranz, H. S. L. Woo, and P. Picotte, *J. Chromatogr.*, **43,** 141(1969).

ACKNOWLEDGMENTS AND ADDRESSES

Received January 20, 1970, from the *Department of Analytical Development, Quality Control Section, Lederle Laboratories, Pearl River, NY 10965*

Accepted for publication April 30, 1970.

The authors thank John B. Zager of these laboratories and Mrs. D. Budd of the Literature Service Department for their valuable assistance with this report.

Spectrophotometric Assay of Potassium Permanganate Tablets (USP XVII)

A. MANCOTT and J. TIETJEN

Abstract □ Assay of potassium permanganate tablets (USP XVII) was accomplished by a spectrophotometric procedure. This method is comparable in accuracy to the USP XVII titrimetric procedure.

Keyphrases □ Potassium permanganate tablets—analysis □ Colorimetric analysis—spectrophotometer

The quantitative determination of potassium permanganate by a spectrophotometric method has been reported by Bastian *et al.* (1). The application of this method to the assay of USP XVII potassium permanganate tablets, as compared to the standard USP XVII titrimetric assay for potassium permanganate tablets (2), is reported in this article.

The spectrophotometric method reported here is comparable in accuracy to the USP XVII assay, but it is considerably simpler to do and results in an appreciable saving of time, labor, and materials.

EXPERIMENTAL

Apparatus—Spectra and absorbance measurements were made with a spectrophotometer[1] (slit width 5 Å). Matched cells with a 1-cm. optical path were used.

Reagents and Chemicals—Potassium permanganate solution (0.1 *N*)[2] was standardized against 0.1 *N* oxalic acid solution.[3] Potassium permanganate tablets USP XVII (300 mg.)[4] were assayed. All other reagents used where of the highest commercial grade available.

[1] Bausch and Lomb, model 505.
[2] Fisher Certified reagent.
[3] Fisher Certified reagent.
[4] Eli Lilly and Co., Indianapolis, Ind.

Table I—Absorbance of Known Potassium Permanganate Solutions

Concentration of $KMnO_4$, mg./l.	Absorbance
44.354	0.693
47.515	0.743
50.676	0.792
53.737	0.841
56.898	0.891
60.059	0.939
63.220	0.988

Procedure—Standardized solutions of potassium permanganate were prepared and their absorbances measured. Twenty 300-mg. potassium permanganate tablets USP XVII were weighed and finely powdered. An accurately weighed portion of the powder, 50–55 mg., was dissolved in water and diluted to 1 l. The absorbance of the solution was measured at 526 mμ and compared with the standards to determine its concentration. The same samples were also assayed according to the USP XVII titrimetric procedure.

RESULTS AND DISCUSSION

Absorbance readings for the standardized potassium permanganate solutions in the concentration range of 44–64 mg./l. were obtained (Table I). A graph of absorbance *versus* concentration was linear with a slope of 0.0156.

The percent potassium permanganate in the sample used is found from:

$$\% KMnO_4 = \frac{A}{0.0156 \times W} \times 100 \qquad \text{(Eq. 1)}$$

where A = absorbance, and W = weight in milligrams of the $KMnO_4$ sample.

USP XVII standards for potassium permanganate tablets contain not less than 95% and not more than 105% of the labeled amount of $KMnO_4$ for tablets of 300 mg. or more, and not less