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Note

Separation and semi-quantitative determination of tetracycline degradation products in tetracycline hydrochloride powders and capsules by thin-layer chromatography

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The British Pharmacopoeia[1] (BP) and International Pharmacopoeia[2] have set limits for anhydrotetracycline hydrochloride (0.5%), 4-epianhydrotetracycline hydrochloride (0.5%), chlortetracycline hydrochloride (2.0%) and 4-epitetracycline hydrochloride (4.0%) in tetracycline hydrochloride powders using a thin-layer chromatographic (TLC) method. For tetracycline hydrochloride capsules, spectrophotometric measurements at 430 nm were used by the BP to measure the two anhydro derivatives. 4-Epianhydrotetracycline hydrochloride has been identified as a cause of Fanconi-type syndrome[3–6]. Chlortetracycline hydrochloride is a more active antibiotic than tetracycline hydrochloride, and therefore reliance on microbiological potency alone for tetracycline hydrochloride may give rise to misleading results if a significant amount of chlortetracycline hydrochloride is present in samples of tetracycline hydrochloride. 4-Epitetracycline hydrochloride is virtually inactive as an antibiotic. Anhydrotetracycline hydrochloride is inactive and it might undergo epimerization, under storage conditions, leading to the formation of 4-epianhydrotetracycline hydrochloride.

Several methods for the separation of tetracycline hydrochloride from its major degradation products using TLC have been published[7–12], and most of them suffer from an excessive time for preparation or for development and/or poor resolution. We have developed a TLC method that is simple, rapid and has an excellent resolving power for tetracycline hydrochloride and its major degradation products and for chlortetracycline hydrochloride.

EXPERIMENTAL

Materials

Tetracycline hydrochloride powder and capsules, chlortetracycline hydrochloride, 4-epianhydrotetracycline hydrochloride, anhydrotetracycline hydrochloride and 4-epitetracycline hydrochloride were provided by Lederle Laboratories (American Cyanamid, Pearl River, NY, U.S.A.). All other chemicals and reagents were of analytical-reagent grade.

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Preparation of plates

A 30-g amount of Whatman cellulose powder CC41 for TLC (W. & R. Balston, Maidstone, Great Britain) was mixed with 65 ml 0.1 *M* EDTA, disodium salt and a 0.3-mm layer of the homogeneous slurry was applied to five plates (20 × 20 cm) using a TLC spreader. The plates were air dried at room temperature for 1 h and then heated at 90°C for 20 min.

Preparation of standard solutions

The following standard solutions were prepared by dissolving the appropriate weights in methanol: (a) 1.0% tetracycline hydrochloride; (b) 0.005% 4-epianhydrotetracycline hydrochloride; (c) 0.005% anhydrotetracycline hydrochloride; (d) 0.04% 4-epitetracycline hydrochloride; (e) 0.02% chlortetracycline hydrochloride.

Preparation of test solutions

A 250-mg amount of the tetracycline hydrochloride powder to be tested was dissolved and made up to volume with methanol in a 25-ml volumetric flask. For tetracycline hydrochloride capsules, the contents of ten capsules each containing 250 mg of tetracycline hydrochloride were dissolved and made up to volume with methanol in a 250-ml volumetric flask.

Application of solutions to the plates

For tetracycline hydrochloride powder, 5 μl of each of the test and standard solutions (a, b, c, d and e) were applied separately to the plate. A mixture of the standard solutions (a, b, c, d and e) (5 μl of each) was also applied to the plate as a single spot. For tetracycline hydrochloride capsules, the same procedure as for tetracycline hydrochloride powder was used. In addition, 10 μl each of two standard solutions (b and c) were applied separately and as a mixture.

Chromatographic procedure

The plate was sprayed uniformly with 4 ml of water and placed immediately in a filter-paper-lined chromatographic chamber that had been tightly closed and equilibrated with chloroform saturated with 0.1 *M* EDTA, disodium salt, solution for 1 h. The plates were developed to a height of 15 cm in about 25 min. After development the plates were dried at room temperature and placed for 2 min in a chamber saturated with ammonia. The plate was then examined under a UV lamp having maximum output at about 366 nm. The intensities of the zones of 4-epianhydrotetracycline hydrochloride, anhydrotetracycline hydrochloride, 4-epitetracycline hydrochloride and chlortetracycline hydrochloride in the sample were then compared visually with the intensities of the zones of the standards. The contents of the four components in the sample were then deduced. The R_F values are given in Table I.

RESULTS

Three samples of tetracycline hydrochloride powders were examined, and chlortetracycline hydrochloride could not be detected in any of them. The contents of the other three components were found to comply with the BP requirements. The

TABLE I

R_F VALUES OF TETRACYCLINE HYDROCHLORIDE AND ITS DERIVATIVES

The R_F values are average values from five plates.

Compound	*R_F value*	*Colour of spot*
4-Epitetracycline hydrochloride	0.035	Yellowish green
Tetracycline hydrochloride	0.06–0.23 (band)	Yellow
Chlortetracycline hydrochloride	0.32	Yellow
4-Epianhydrotetracycline hydrochloride	0.52	Yellow-brown
Anhydrotetracycline hydrochloride	0.92	Yellow-brown

procedure was also applied to ten samples of proprietary tetracycline hydrochloride capsules and the results are given in Table II.

The BP uses a spectrophotometric method to measure the total amounts of anhydrotetracycline hydrochloride and 4-epianhydrotetracycline hydrochloride in tetracycline hydrochloride capsules. The absorbance limit set by the BP is twice that which has been set for tetracycline hydrochloride powder. Therefore, in our method, volumes of standard solutions of anhydrotetracycline hydrochloride and 4-epianhydrotetracycline were doubled to allow for such an increase in the limit for these two degradation products in tetracycline hydrochloride capsules.

Samples of tetracycline hydrochloride capsules that had been found by TLC to contain more anhydrotetracycline hydrochloride and 4-epianhydrotetracycline hydrochloride than in the standards were also examined by measuring the absorbance at 430 nm according to the BP method. The absorbance was found to be much more than the permitted limit, confirming our TLC findings.

TABLE II

CONTENT OF ANHYDROTETRACYCLINE HYDROCHLORIDE, 4-EPIANHYDROTETRACYCLINE HYDROCHLORIDE, 4-EPITETRACYCLINE HYDROCHLORIDE AND CHLORTETRACYCLINE HYDROCHLORIDE IN TETRACYCLINE HYDROCHLORIDE CAPSULES

Sample	*Anhydrotetracycline·HCl (%)*	*4-Epianhydrotetracycline·HCl (%)*	*4-Epitetracycline·HCl (%)*	*Chlortetracycline·HCl (%)*
A	0.5	0.5	<4.0	0
B	>1.0	>1.0	>4.0	0
C	0.5–0.9	0.5	<4.0	0
D	<0.5	0.5	<4.0	0
E	<0.5	<0.5	<4.0	0
F	Trace	0	Trace	0
G	>1.0	>1.0	<4.0	0
H	0.5–0.9	0.5–0.9	<4.0	0
I	<0.5	<0.5	<4.0	0
J	0.5–0.9	0.5–0.9	<4.0	0

DISCUSSION

The BP method, which is based principally on the method used by Ascione *et al.*[9], proved to be tedious, the results were variable and the R_F values were very

close. There is an overlap between anhydrotetracycline hydrochloride and 4-epianhydrotetracycline hydrochloride in the method of Simmons *et al.*[7]. To solve this problem, Simmons *et al.*[8] used two-dimensional TLC, which in our opinion is not easily used as a routine control method. Simmons *et al.*[10] used a spectrophotometric assay for the determination of the anhydrotetracycline hydrochloride and 4-epianhydrotetracycline hydrochloride after TLC separation. The method described by Fernandez *et al.*[11] for the separation and determination of tetracycline hydrochloride and its degradation products is very difficult to apply to tetracycline hydrochloride powders and capsules as the content of tetracycline hydrochloride is very large compared with that of the impurities and overlapping could occur between the anhydrotetracycline and the tetracycline hydrochloride.

Lloyd and Cornford[12] used methanol–1.0 *N* hydrochloric acid (95:5) as the solvent to dissolve samples of tetracycline hydrochloride. We have found that this acidic solvent decomposes tetracycline hydrochloride rapidly to anhydrotetracycline hydrochloride. However, when we omitted the 1.0 *N* hydrochloric acid very poor resolution was obtained.

We consider that our method is simpler, saves much time and makes possible the conclusive separation and semi-quantitative determination of the degradation products of tetracycline hydrochloride and chlortetracycline hydrochloride. The method can be adopted for the routine quality control of tetracycline hydrochloride powders and capsules. The method could easily be made quantitative by applying to the plate different concentrations of the standard solutions and comparing the intensities of the zones of the test solution with those of the standard solutions.

REFERENCES

1 *British Pharmacopoeia 1973*, Her Majesty's Stationery Office, London, p. 468.
2 *International Pharmacopoeia*, Vol. 1, World Health Organization, Geneva, 3rd ed., 1979, p. 80.
3 G. W. Flimpter, *J. Amer. Med. Ass.*, 184 (1963) iii.
4 M. Fulop and A. Drapkin, *N. Engl. J. Med.*, 19 (1965) 986.
5 L. I. Ehrlich and H. S. Stein, *Pediatrics*, 31 (1963) 339.
6 S. R. Sulkowski and J. R. Haserick, *J. Amer. Med. Ass.*, 189 (1964) 178.
7 D. L. Simmons, C. K. Koorengenel, R. Kubelka and P. Seers, *J. Pharm. Sci.*, 55 (1966) 219.
8 D. L. Simmons, C. K. Koorengenel, R. Kubelka and P. Seers, *J. Pharm. Sci.*, 55 (1966) 1313.
9 P. P. Ascione, J. B. Azgar and G. P. Chrekian, *J. Pharm. Sci.*, 56 (1967) 1393.
10 D. L. Simmons, R. J. Ranz, H. S. L. Woo and P. Picotte, *J. Chromatogr.*, 43 (1969) 141.
11 A. Al Varez Fernandez, V. Torre Noceda and E. Sanchez Carrera, *J. Pharm. Sci.*, 58 (1969) 443.
12 P. B. Lloyd and C. C. Cornford, *J. Chromatogr.*, 53 (1970) 403.