CHROM. 5002

A thin-layer chromatographic limit test for the detection of anhydrotetracycline and 4-*epi*-anhydrotetracycline in tetracycline

Following the publication of reports[1–5] in the literature of the Fanconi-type syndrome associated with the ingestion of degraded tetracycline, which principally implicated the degradation products anhydrotetracycline and 4-*epi*-anhydrotetracycline, a large number of chromatographic procedures concerned with the separation and identification of tetracyclines has appeared. Recently the *British Pharmacopoeia*[6] pulished a thin-layer chromatographic (TLC) limit test for the detection of anhydrotetracycline, 4-*epi*-anhydrotetracycline and 4-*epi*-tetracycline in tetracycline which appears to be based principally on the procedure reported by ASCIONE *et al.*[7]. Such a system is particularly useful both for quality control of raw material and for the stability evaluation of aged tetracycline proprietary products. In our hands, however, the B.P. procedure proved tedious, the results variable and, in particular, the limits of detection appeared to be optimistic.

SIMMONS *et al.*[8] described a quantitative method involving TLC separation, elution and spectrophotometric assay of the separated tetracycline compounds. Using the TLC separation procedure described by these workers as a basis, we have been able to develop a simple and reproducible limit test for the detection of anhydrotetracycline and 4-*epi*-anhydrotetracycline in tetracycline hydrochloride raw materials and in proprietary products containing tetracycline hydrochloride.

Experimental

Preparation of plates. Rapidly mix 30 g of microgranular cellulose (Whatman)* with 75 ml distilled water in a pestle and mortar. Apply a 0.5-mm layer of homogenous slurry to five clean glass plates (20 × 20 cm) using a suitable TLC spreader. Air-dry the plates at room temperature for 10 min and then heat in an oven at 90° for 30 min.

Preparation of standard solutions. Weigh accurately 15.0 mg of anhydrotetracycline hydrochloride A.S.** and 15.0 mg of 4-*epi*-anhydrotetracycline hydrochloride A.S.** into a 25-ml volumetric flask, dissolve in and dilute to volume with a solvent mixture consisting of absolute methanol (analytical grade)–1.0 *N* hydrochloric acid (analytical grade) (95:5).

Into each of five labelled 10-ml volumetric flasks weigh accurately 100 mg of tetracycline hydrochloride U.S.P. reference standard, into these flasks pipette aliquots of the anhydrotetracycline hydrochloride–4-*epi*-anhydrotetracycline hydrochloride solution as indicated in Table I. Then dissolve in and dilute to volume with methanol–1.0 *N* hydrochloric acid (95:5) solvent mixture.

Preparation of test solutions. 100 mg of the sample of tetracycline hydrochloride to be tested is accurately weighed into a 10-ml volumetric flask and dissolved in and diluted to volume with the methanol–1.0 *N* hydrochloric acid (95:5) solvent mixture.

Application of solution aliquots to the chromatoplate. Manually apply 20-μl aliquots of test solution and standard solutions to the chromatoplate as indicated in Table II.

* W. & R. Balston Ltd., Great Britain.

** Authentic specimens available from the British Pharmacopoeia Commission, 8 Bulstrode Street, London, W.1, Great Britain.

TABLE I

VOLUMES OF ANHYDROTETRACYCLINE HYDROCHLORIDE–4-*epi*-ANHYDROTETRACYCLINE HYDROCHLORIDE SOLUTION REQUIRED IN THE PREPARATION OF THE STANDARD SOLUTION

Flask No.	*Mixture (ml)*
1	0.25
2	0.50
3	1.00
4	2.00
5	4.00

Chromatography. Spray the plate uniformly with 10 ml of buffer solution (0.1 *M* disodium EDTA–0.1% ammonium chloride). Immediately develop the chromatoplate in a chromatographic chamber containing 100 ml of buffer-saturated chloroform (analytical grade). After development for a distance of 16 cm (*ca.* 45-min duration), the chromatoplate is removed and air-dried in a stream of cool air. The chromatoplate is then exposed for 2 min in a second chamber to an atmosphere saturated with ammonia.

The chromatoplate is then examined under an enclosed short-wave UV lamp. The intensities of the zones of anhydrotetracycline hydrochloride and 4-*epi*-anhydrotetracycline hydrochloride separated in the sample being tested are then visually compared with the intensities of the zones of the standards and the anhydrotetracycline

TABLE II

SEQUENCE OF APPLICATION OF STANDARD AND SAMPLE SOLUTIONS TO THE CHROMATOPLATE

Solution	*Equivalent % contents of anhydrotetracycline hydrochloride and of 4-epi-anhydrotetracycline hydrochloride in the tetracycline hydrochloride sample being tested*
Standard solution 1	0.15
Standard solution 2	0.30
Sample solution	—
Standard solution 3	0.6
Sample solution	—
Standard solution 4	1.2
Standard solution 5	2.4

hydrochloride and 4-*epi*-anhydrotetracycline hydrochloride contents of the sample thus deduced.

R_F values. The R_F ranges of the three compounds are as follows: tetracycline hydrochloride (band), 0 to 0.25; anhydrotetracycline hydrochloride, 0.93; 4-*epi*-anhydrotetracycline hydrochloride, 0.48.

Results

Using the procedure described, recent and 18-month-aged samples of tetra-

TABLE III

ANHYDROTETRACYCLINE HYDROCHLORIDE CONTENT OF SAMPLES OF PROPRIETARY TETRACYCLINE HYDROCHLORIDE PRODUCTS EXAMINED

Product	*Age (months)*	*% AT content in sample*
A	48	0.5
A	26	0.5
A	23	0.5
A	13	0.5
A	3	0.25
B	13	3.0
B	12	2.0
C	12	0.25–0.50
D	12	0.25–0.50
E	—	0.25
F	12	0.25

cycline hydrochloride were examined; 4-*epi*-anhydrotetracycline hydrochloride (4-EAT) could not be detected in any of the samples, 0.25% of anhydrotetracycline hydrochloride (AT) was detected in the 18-month samples.

The procedure was also applied to samples of proprietary tetracycline hydrochloride products. No 4-EAT could be detected. The AT content is given in Table III.

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