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Letter to the Editor

Automated high-performance liquid chromatographic analysis of tetracycline in urine

Sir,

In humans, the antibiotic tetracycline is largely excreted unchanged in urine [1]. Thus, pharmacokinetic or bioavailability studies of this drug may be performed by monitoring urinary excretion of tetracycline. Although several methods have been reported for the analysis of tetracycline by high-performance liquid chromatrography (HPLC) [2–6], some require complex extraction procedures, and none of the published procedures employ an internal standard. We report a method which is simple, inexpensive, involves minimal sample handling, and includes the use of an internal standard so as to permit automated injection.

EXPERIMENTAL

Materials

Samples of tetracycline hydrochloride and the internal standard, demeclocycline hydrochloride, were kindly provided by Upjohn (Kalamazoo, MI, U.S.A.) and Lederle Labs. Division, American Cyanamid Company (Pearl River, NY, U.S.A.), respectively. Stock solutions in 0.1 *M* hydrochloric acid were prepared weekly. All other reagents, analytical-reagent grade or better, were purchased from commercial sources and used without further purification. Mobile phase components were filtered and degassed after mixing.

Apparatus and chromatographic conditions

Analyses were performed using a Waters chromatography system consisting of an M-6000 pump, a Lambda-Max Model 480 variable-wavelength spectrophotometric detector set at 355 nm, and a WISP 710 automatic injector (Waters Assoc., Milford, MA, U.S.A.). Isocratic separation of analytes was achieved on

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a 15 cm×4.6 mm I.D. PLRP-S 5-μm column with the recommended guard cartridge (Polymer Labs. via Rainin Instruments, Woburn, MA, U.S.A.) using a mobile phase composed of 7.5 m*M* phosphoric acid–acetonitrile–methanol (20:3:3, v/v) at a flow-rate of 0.8–0.9 ml/min and at ambient temperature. The mixture of organic modifiers is consistent with the manufacturer's recommendation for optimum column performance.

Preparation of samples

All samples were diluted at least 1:2 as follows: 0.5 ml of urine and 25 μl of internal standard solution (demeclocycline 1.0 mg/ml as the free base in 0.1 *M* hydrochloric acid) were placed in automatic injector vials. After the sample was brought to a total volume of 1.0 ml with 0.1 *M* hydrochloric acid, the vial was capped tightly and inverted several times to assure complete mixing, and placed directly into the injector tray.

Calibration standards were prepared by adding internal standard solution (25 μl) and 1, 2.5, 5, 10, 20, 50 and 75 μg of tetracycline as the free base (in 0.1 *M* hydrochloric acid) to autoinjector vials containing 0.5 ml tetracycline-free human urine and sufficient 0.1 *M* hydrochloric acid to yield a total volume of 1.0 ml. Calibration standards were prepared in duplicate and quantitated at the beginning and end of each analytical run.

Clinical study

A healthy male volunteer ingested a single 500-mg oral dose of tetracycline hydrochloride, equivalent to 462 mg of tetracycline base (Panmycin, Upjohn) with 180 ml of water. All urine was collected for the next 48 h, in intervals divided as follows: 0–2, 2–4, 4–8, 8–12, 12–24, 24–36, and 36–48 h. The volume of each sample was measured, and aliquots were separated and frozen until assay as described above.

RESULTS AND DISCUSSION

Tetracycline and the internal standard demeclocycline produced resolved chromatographic peaks having approximate retention times of 6.5 and 10.2 min, respectively (Fig. 1). Standard curves were always linear from 1.0 to 75 μg/ml tetracycline, with correlation coefficients of 0.99 or greater. Within-day coefficients of variation for identical samples ($n=6$ at each concentration) were 10.2% at 1 μg/ml, 12.8% at 2.5 μg/ml, 11.5% at 5 μg/ml, 5.3% at 10 μg/ml, 3.2% at 20 μg/ml, 1.0% at 50 μg/ml, and 1.9% at 75 μg/ml. Between-day variation was assessed by analysis of previously prepared and frozen quality control samples (containing 7.1 and 73.6 μg/ml tetracycline, respectively) which were analyzed with each set of unknowns. The overall mean value for the 7.1 μg/ml quality control sample across nine analytic runs was 7.4 μg/ml,

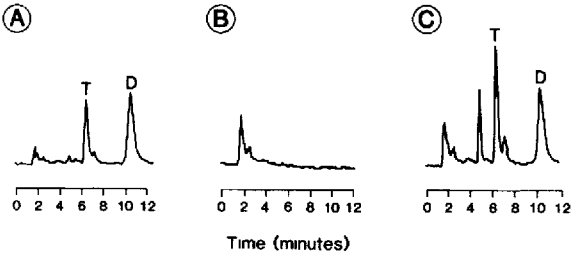

Fig. 1. (A) Chromatogram of a calibration standard containing tetracycline (T), 20 μg/ml, and demeclocycline (D), 25 μg/ml (0.05 a.u.f.s.). (B) Chromatogram of a urine sample from a subject prior to drug administration. (C) Chromatogram of a urine sample from a subject collected from 36 to 48 h after tetracycline administration.

TABLE I

URINARY EXCRETION OF TETRACYCLINE IN THE VOLUNTEER SUBJECT

Collection interval (h)	Urine volume (ml)	Tetracycline concentration (μg/ml)	Tetracycline excretion (mg)
0-2	140	151.4	21.2
2-4	106	313.9	33.3
4-8	139	280.7	39.0
8-12	318	119.9	38.1
12-24	740	94.9	70.2
24-36	700	50.3	35.2
36-48	850	16.7	14.2
			Total 251.2

with a coefficient of variation of 9.7%; for the 73.6 μg/ml sample, the overall mean was 69.0 μg/ml, with a coefficient of variation of 9.3%.

Table I shows data on urinary tetracycline excretion in the volunteer subject. A total of 251.2 mg of tetracycline (54.4% of the administered dose) was excreted during the 48-h period.

The described method allows rapid, direct automated analysis of tetracycline in human urine samples. An internal standard is utilized so that the accuracy of the analysis will not be influenced by variations in injection volume. Use of a polyvinylstyrene column minimizes peak tailing without the use of amine modifiers.

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