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Note

Method for rapid determination of urinary tetracycline by high-performance liquid chromatography

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Measurement of the amount of tetracycline excreted unchanged in the urine offers a convenient means of investigating the pharmacokinetics of this antibiotic in man [1, 2]. Several analytical techniques have been developed for the separation and quantitation of tetracycline by high-performance liquid chromatography (HPLC) [3—7]. However, most of these methods are suitable only for the quality control of tetracycline in pharmaceutical preparations and are subject to interference from UV-absorbing components of biological fluids [8]. Sharma et al. [8] have developed a sensitive and precise method to measure tetracycline in plasma and urine; however the procedure is time-consuming and requires the formation of a tetracycline—calcium complex followed by two extraction steps. Similarly, gradient elution methods [9, 10] which have been developed for tetracycline are unsuitable for processing of large numbers of samples necessary for pharmacokinetic studies. Mack and Ashworth [7] have investigated the utility of reversed-phase chromatography of tetracyclines for quality control purposes. We report a rapid, sensitive and precise method for measuring urinary concentrations of tetracycline for pharmacokinetic studies.

EXPERIMENTAL

Materials

Isopropanol and diethanolamine were supplied by Ajax Chemicals (Sydney, Australia). Both compounds were used without further purification. Tetra-ammonium EDTA was synthesized [7] from ammonia solution (BDH Chem-

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icals, Port Fairy, Australia). Tetracycline hydrochloride reference compound was kindly supplied by Commonwealth Serum Labs. (Melbourne, Australia).

Chromatographic system

All HPLC analyses were carried out using an M-6000A solvent delivery system connected to a Model 440 absorbance detector (Waters Assoc., Milford, MA, U.S.A.) set at 365 nm. Chromatographic separations were achieved on a 25 cm × 4.6 mm I.D. Brownlee RP-10A column maintained at 40°C in a water bath. A 3 cm × 4.6 mm I.D. Brownlee guard column (RP-GU) was used to protect the analytical column. The sorbent in the analytical column and the guard column consisted of 10-μm totally-porous particles of LiChrosorb RP-8 (E. Merck, Darmstadt, G.F.R.). The mobile phase consisted of isopropanol—diethanolamine buffer—tetraammonium EDTA—distilled water (11:5:1:83). The diethanolamine buffer was prepared by adjusting a 1 *M* aqueous solution of diethanolamine to pH 7.3 with orthophosphoric acid [7]. The flow-rate of the mobile phase was maintained at 2 ml/min. The samples were centrifuged (Microfuge B; Beckman, Fullerton, CA, U.S.A.) and 20 μl of the supernatant phase were injected onto the column through a syringe loading sample injector (Rheodyne, Berkeley, CA, U.S.A.). Chromatograms were obtained on a pen recorder having a 10-mV input and run at a chart speed of 0.25 cm/min.

Calibration graphs

A master stock solution (5 mg/ml) of tetracycline hydrochloride was prepared in 0.03 *N* hydrochloric acid and used for the preparation of primary stock solutions containing 0.1, 0.5, 1, 2, 3 and 4 mg/ml in 0.03 *N* hydrochloric acid. The favourable stability of tetracycline in 0.03 *N* hydrochloric acid had been established previously [11]. Working standards of tetracycline hydrochloride were prepared by appropriate dilution of the stock solutions with tetracycline-free urine to give antibiotic concentrations of 1, 5, 10, 20, 30, 40 and 50 μg/ml. These solutions were submitted to HPLC analysis and acted as external standards in the assay. Calibration graphs were constructed by plotting the peak height of tetracycline against concentration of the antibiotic. Tetracycline standards were chromatographed routinely at the beginning, middle and end of the day's assays and the results averaged.

RESULTS AND DISCUSSION

Fig. 1 shows liquid chromatograms obtained from the assay of blank urine, tetracycline hydrochloride (30 μg/ml) in urine and urine from a subject who had taken 500 mg of tetracycline hydrochloride orally, 2 h previously. Tetracycline had an average retention time of 6 min when 11% (v/v) isopropanol in diethanolamine buffer was used as the mobile phase. Between 10 and 12% of isopropanol could be used with this buffer depending on the age and condition of the column. This solvent system gave minimal tailing of the tetracycline peak on the RP-8 column. Various combinations of methanol, acetonitrile and inorganic buffers were tried and found to be unsatisfactory in this regard.

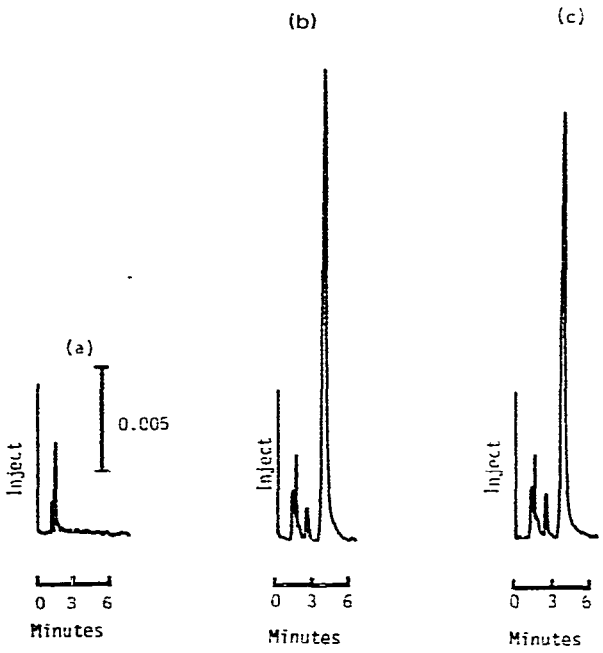

Fig. 1. Representative chromatograms of (a) tetracycline-free urine, (b) urine containing 30 μg/ml of tetracycline hydrochloride and (c) urine from a patient taking a 500-mg dose of tetracycline hydrochloride. In each case samples were diluted 1:10 in 0.03 *N* hydrochloric acid before assay. A detector response of 0.005 a.u. is indicated by the range bar.

The choice of 365 nm as the detector wavelength was important as this offered high sensitivity with minimal interference from other UV-absorbing constituents of urine. Calibration graphs of tetracycline hydrochloride concentration versus peak height were linear from 1 to at least 50 μg/ml ($r > 0.99$) and passed through the origin. The assay can detect 0.4 μg/ml of tetracycline hydrochloride in urine although 0.1 μg/ml of the antibiotic could be detected using an injection volume of 100 μl. To determine the between-day precision of the assay, solutions of tetracycline hydrochloride in urine containing 1, 5, 20 and 50 μg/ml were assayed on ten different days. The results

TABLE I

BETWEEN-DAY PRECISION OF HPLC ASSAY FOR URINARY TETRACYCLINE

The data were obtained from 10 repeat assays of tetracycline·HCl in urine

Tetracycline·HCl concentration (μg/ml)	Mean peak height (mm)	Standard deviation	Coefficient of variation (%)
1	18.1	0.70	3.9
5	79.5	3.88	4.9
20	345.5	15.27	4.4
50	903.8	28.96	3.2

in Table I indicate the precision of the assay over this range of tetracycline concentrations.

The minimal pre-treatment of the urine samples does not demand the use of an internal standard for the assay provided that a constant volume of sample is injected onto the column. This simplifies the procedure and permits at least 50 to 60 samples to be assayed during a normal working day.

The HPLC method described is convenient, rapid and precise and would be valuable for measuring urinary concentrations of tetracycline when the bioavailability and pharmacokinetics of the antibiotic are under investigation.

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