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Note

Rapid analysis of doxycycline from biological samples by high-performance liquid chromatography

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A high-performance liquid chromatographic (HPLC) method for the determination of tetracyclines, except for the more lipophilic compound doxycycline, in urine, plasma and animal tissues has been described by Sharma and co-workers[1,2]. Maximum resolution on reversed phase columns can be achieved with acidic eluents when their pH is below the isoelectric point of the tetracyclines (*ca.* pH 5)[3].

As the analysis of tetracyclines in biological samples, according to Sharma and co-workers[1,2], requires extraction into an organic solvent and reextraction into aqueous acids, I have developed a single-step extraction method with subsequent chromatographic analysis on RP-8 material that is applicable for tetracycline and pyrrolidinomethyltetracycline[4,5]. In principle, this method is also applicable to doxycycline since at pH 2.4 tetracycline and pyrrolidinomethyltetracycline are eluted prior to the more lipophilic doxycycline, as is also described by others[3]. However, the rate of recovery of doxycycline varies when different batches of column packing material are used. This difficulty can be overcome and the reproducibility of the results can be sufficiently enhanced by: (1) preequilibration of the columns with a compound more lipophilic than doxycycline or any other tetracycline to be analyzed; and (2) use of a final eluent less apolar than the equilibration eluent.

The improved procedure is described in this paper.

MATERIALS AND METHODS

All chemicals used were of analytical grade. Anhydrotetracycline was kindly provided by Hoechst (Frankfurt/M, G.F.R.). Doxycycline was a gift from Pfizer (Karlsruhe, G.F.R.).

The HPLC apparatus used in this study was essentially that described previously[4]. Columns (25 × 0.4 cm) packed with Nucleosil® 10 C_8 (Macherey, Nagel & Co., Düren, G.F.R.) were conditioned for 20 min with eluent I (0.01 *M* NaH_2PO_4 in water–acetonitrile, 70:30; adjusted to pH 2.4 with 1 *M* HNO_3) containing 0.1 mg anhydrotetracycline/100 ml, at a flow-rate of 1.9 ml/min. Complete equilibration was checked by UV analysis of the eluent before and after passing the column. The UV absorption was measured at 270 nm, *i.e.*, at the absorption maximum of anhydrotetracycline.

The chromatographic analysis of doxycycline was performed with eluent II (0.01 *M* NaH_2PO_4 in water–acetonitrile, 73:27; pH 2.4), the UV absorption being measured at 357 nm.

For the extraction of doxycycline from biological samples the procedure described for tetracycline and pyrrolidinomethyltetracycline[4] was slightly modified.

(a) Extraction from blood or serum. All steps were performed in the cold (*ca.* 0°). A 50- or 100-μl volume of blood or serum was mixed with 150 μl 0.03 *M* H_3PO_4; after 15 min 1 ml acetonitrile–buffer (0.01 *M* NaH_2PO_4, pH 2.4) (50:50) was added. 5 min later most of the precipitated proteins were spun down (2000 *g* for 5 min) and the supernatant taken for the HPLC analysis.

(b) Extraction from tissue. Organs were resected from the animals, rinsed in ice-cold 0.9% NaCl, dried on filter-paper and homogenized in ice-cold 0.03 *M* NaH_2PO_4 (1 part organ + 4 parts buffer) with an Ultra Turrax (Janke & Kunkel, Staufen, G.F.R.) at 15,000 rpm for 30 sec. After 15 min, 200 μl of the homogenate were mixed with 1 ml acetonitrile–buffer (0.01 *M* NaH_2PO_4, pH 2.4) (50:50) and centrifuged. For the chromatographic analysis, 50- or 100-μl aliquots of the supernatant were applied to the column.

For recovery experiments, doxycycline dissolved in 1.5 m*M* $MgSO_4$ was added to serum or organ homogenates (20% in 0.9% NaCl) to give a final concentra-

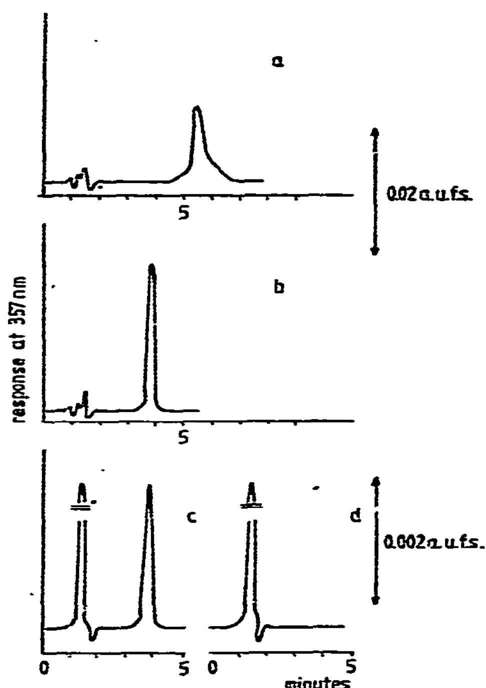

Fig. 1. Chromatographic analysis of doxycycline on RP C_8. Elution patterns: a, 208 ng doxycycline on an unconditioned column (tailing); b, 208 ng doxycycline on a column preequilibrated with anhydrotetracycline (no tailing, symmetric peak); c, 21.9 ng doxycycline, extracted from serum (10.5 μg/ml); doxycycline from tissue homogenates gave the same result; d, a control serum. The sensitivity of the photometer in c and d was ten times that in a and b.

tion of 0.5, 1, 5, 10, 50, 100 or 200 μg/ml. After incubation at 37° for 30 min, doxycycline was extracted as described above.

RESULTS AND DISCUSSION

High ionic strength of the eluent or an unconditioned column result in tailing of the eluted compound (Fig. 1)[3,4]. When the column is conditioned with the lipophilic anhydrotetracycline, an eluent with relatively low content of acetonitrile (27%) elutes doxycycline within 5 min (Fig. 1b). The anhydrotetracycline adsorbed to the column is not eluted as can be proven by UV analysis at 270 nm.

Doxycycline extracted from biological samples has the same retention time of 3.9 min as doxycycline standards dissolved either in eluent or in 1.5 m*M* $MgSO_4$.

The recovery of doxycycline from serum and organ homogenates is complete when the doxycycline concentrations are not higher than 100 μg/ml. At a concentration of 200 μg/ml the recovery from liver homogenates and from serum is only 80–85%. This may be due to a partial precipitation of doxycycline together with some proteins, as described for tetracycline[5]. In repeated experiments the standard deviation of the extraction and the chromatographic analysis was between 4 and 5%.

Pilot studies showed that in mice treated with doxycycline (50 μg/g body weight), i.v. doxycycline could be found in serum and liver 60 min after the injection of the drug. The serum concentration was 5.5 μg/ml; the liver contained 63 μg/g.

In conclusion, the main advantages of the method described are: (1) complete and simple extraction from serum and tissue samples; (2) rapid and highly reproducible analysis on different batches of the column packing material.

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