

Stability of rolitetracycline in aqueous solution

Rolitetracycline (RTC) (2-*N*-pyrrolidinomethyltetracycline) is a Mannich base formed by *N*-aminomethylation of the carboxamide function of tetracycline (TC) which enhances water solubility. RTC is formulated either as the base or nitrate salt for intravenous or intramuscular use. Formulations are intended to be reconstituted with water at the time of use and are claimed by the manufacturers to be stable for up to 24 h. There is some doubt of the stability of RTC. Siedel, Söder & Lindner (1958) claimed solutions of RTC in organic acid solutions at pH 5 to be stable for several hours, whilst Brunzell (1962) has claimed that RTC is rapidly hydrolysed in aqueous solution with the formation of TC and 4-epitetracycline (ETC). The official method of assay of RTC in Canada (NF XIII, 1970) is microbiological, and gives no information of the extent of its hydrolysis. Brunzell (1962) found that RTC was hydrolysed on paper chromatograms. In thin-layer chromatographic (t.l.c.) studies of RTC (Levorato, 1971; Ochab & Borowiecka, 1969), R_f values close to those of TC are often reported, indicating that, here too, hydrolysis has probably occurred. In other t.l.c. systems (e.g. those of Simmons, Ranz & others, 1969; Fernandez, Noceda & Carrera, 1969; Ascione, Zager & Chrekian, 1967) freshly prepared solutions of RTC give spots at or near the origin, but always together with spots or streaks of R_f near to that of TC (Hughes & Wilson, unpublished). We have examined the stability of RTC in aqueous solution using high speed liquid chromatography (HSLC) and a system described for the resolution of tetracyclines (Butterfield, Hughes & others, 1973).

Typical recorder traces for a freshly prepared aqueous solution of RTC (10 mg ml⁻¹) and a similar solution aged for 2 h at 25° are presented in Fig. 1A. They illustrate the simultaneous detection of both RTC and TC in such samples by the

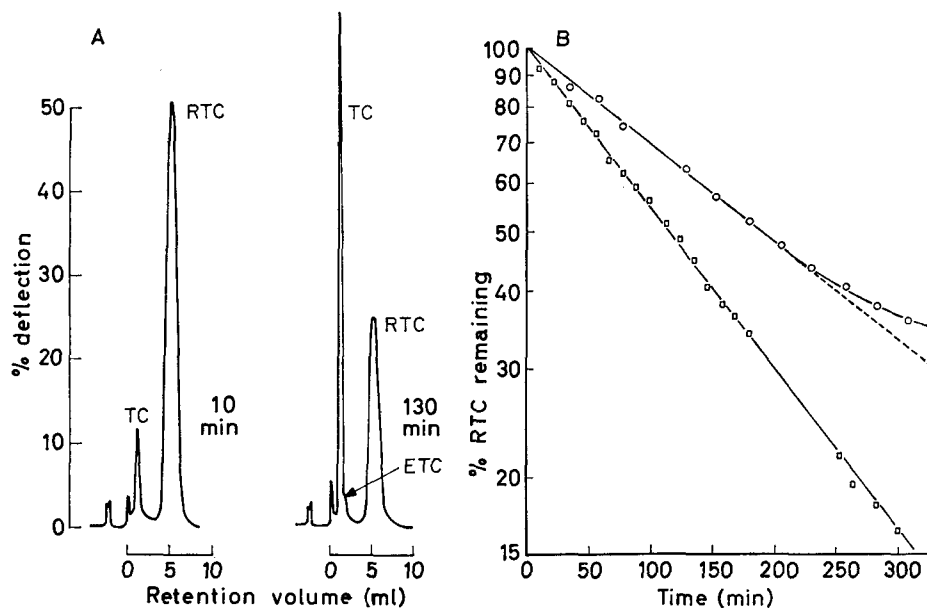


FIG. 1A. HSLC curves for aqueous solutions of RTC at 25° (times indicated). Peak at 0 ml is solvent. Chromatographic conditions: column-Pellionex CP-128 (Northgate Laboratories, Inc. Hamden, Conn., U.S.A.); mobile phase-40.0% v/v ethanol, 0.1 M Na⁺, 0.003 M EDTA²⁻, pH 4.4, 25°, 60 ml h⁻¹.

B. Stability of RTC base (□) and nitrate (○) in aqueous solution (10 mg ml⁻¹ at 25°)

method and the increase in TC content and decrease in RTC content with time. Small amounts of ETC were also observed in aged solutions.

In Fig. 1B the stabilities of RTC and RTC nitrate in aqueous solution at 25° are compared. The rate of hydrolysis was monitored by measuring the decreasing height of the peak attributed to RTC with repeated injections from the aqueous solutions. The percentages of RTC remaining in the solutions are plotted on a logarithmic scale against the time interval measured from preparation of the solution to elution of RTC from the column. Peak heights are proportional to RTC concentration over the range used.

As seen from Fig. 1B both RTC and its nitrate salt are rapidly hydrolysed in dilute aqueous solution, each being more than 50% destroyed within 3 h at 25°. The base was found to be consistently more rapidly hydrolysed than was the nitrate in solutions of comparable concentration. The degradation curve for the base (Fig. 1B) suggests a first order reaction; however, the non-linearity of the nitrate curve (after 50% hydrolysis) indicates that a more complex mechanism may be operating perhaps involving oxidation or epimerization before hydrolysis.

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August 16, 1973

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