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Note

Reversed-phase ion pair chromatography of oxytetracycline, epioxytetracycline and anhydrooxytetracycline

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The rapid separation of tetracycline from its degradation products by high-performance liquid chromatography (HPLC) has been recently reported[1]. Although the physicochemical properties of tetracycline and oxytetracycline (OTC) are closely related, no such method exists for OTC. Thus, it was necessary for this laboratory, concerned as it is with control analysis, to develop a rapid and efficient method for the separation of OTC from its degradation products: epioxytetracycline (EOTC) and anhydrooxytetracycline (AOTC). This paper describes the HPLC analysis of OTC, EOTC and AOTC in reversed-phase ion-pair mode.

EXPERIMENTAL

Chromatographic system

A Varian LC 8500 high-performance liquid chromatograph with a variable wavelength UV detector operating at 275 nm and a LiChrosorb RP-8 (Merck, Darmstadt, G.F.R.) column (10 cm × 4.7 mm I.D.), particle size 10 μm, was used.

Chemicals

Pure standard of oxytetracycline hydrochloride was purchased from Sigma (St. Louis, Mo., U.S.A.) and tetrabutylammonium hydrogen sulfate (TBA) was obtained from Aldrich Europe (Beerse, Belgium). The reference samples of EOTC (85% pure) and AOTC (55% pure) were a gift from Pfizer (Orsay, France). All solvents were of analytical reagent grade.

Analytical method

Isocratic elution was carried out with two different mixtures of TBA (0.5 g/l) in water (pH 2.6) and acetonitrile (92:8) and (80:20).

RESULTS AND DISCUSSION

Epimerization of OTC takes place with almost complete loss of bioactivity[2]. But OTC does this more slowly and to a lesser extent than tetracycline or chlortetracycline; this effect was attributed to hydrogen bonding between the hydroxyl and the amino groups of OTC[3]. This could perhaps explain the difficulty of separating OTC

from EOTC by HPLC in the reversed-phase mode. We have unsuccessfully tested numerous HPLC systems during this study. However, by using a mobile phase containing TBA as a counter ion (at pH 2.6), the baseline separation of OTC and EOTC was obtained (Fig. 1a).

Under the same experimental conditions AOTC was not eluted. So, another solvent composition was used (Fig. 1b). The AOTC peak was identified provisionally from the relative instability of AOTC. In fact, AOTC is quite unstable under the reaction conditions used for OTC dehydration and produces the isomeric phthalides: α and β apoterramycins. Thus, the AOTC isolation with a high degree of purity was quite difficult. When subsequently placed in dilute aqueous acid, AOTC goes rapidly to apoterramycins[4]. By HPLC analysis it was possible to note the enhancement of the first peak corresponding to apoterramycins (Fig. 1c). Final confirmation of the identity must await the availability of mass spectrometric identification of the eluted peaks.

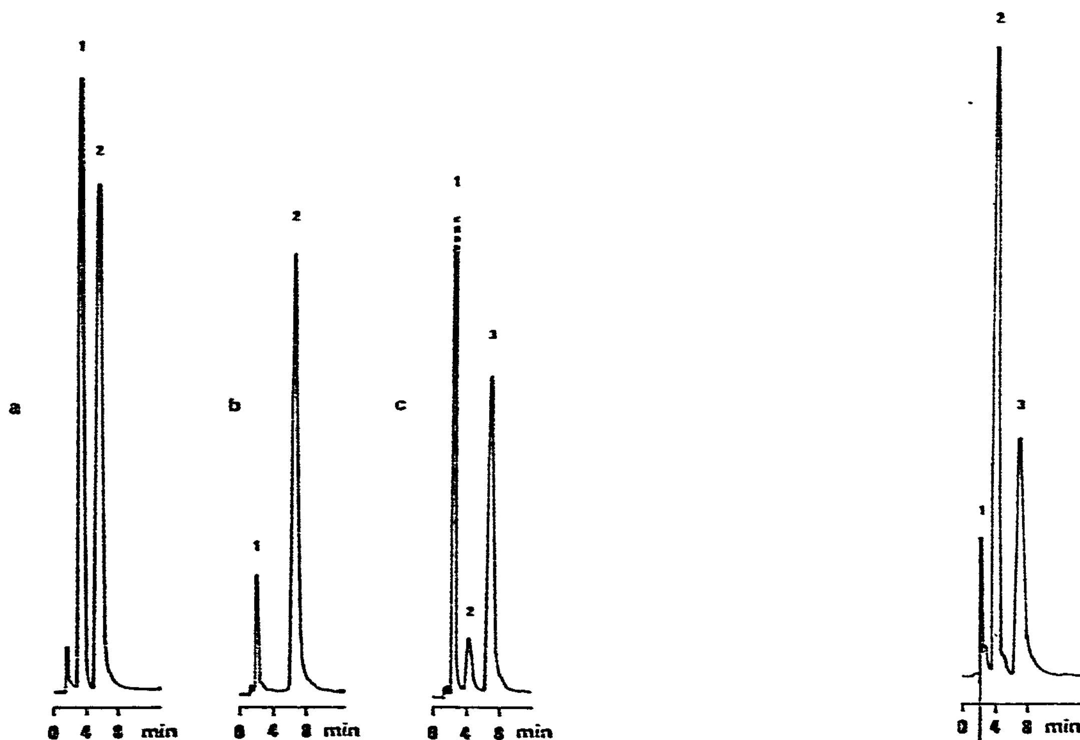

Fig. 1. HPLC of OTC, EOTC and AOTC on a LiChrosorb RP-8 column. (a) Mobile phase, TBA (0.5 g/l) in water–acetonitrile (92:8); flow-rate 60 ml/h; detector sensitivity 0.5 a.u.f.s. Peaks: 1 = EOTC (0.5 g/l in DMF); 2 = OTC (0.5 g/l in DMF). (b) Mobile phase, TBA (0.5 g/l) in water–acetonitrile (80:20), other conditions as in (a). Peaks: 1 = apoterramycins; 2 = AOTC (0.25 g/l in DMF). (c) Conditions as in (b). Peaks: 1 = apoterramycins; 2 = unknown; 3 = AOTC (0.25 g/l in HCl 1%).

Fig. 2. HPLC scan of OTC formulation containing prednisolone and chloramphenicol. Conditions as in Fig. 1a. Peaks: 1 = injection artefact; 2 = EOTC; 3 = OTC.

Moreover, it must be pointed out that reproducibility of the retention time was the most critical factor. It was necessary to achieve reequilibration after any mobile phase composition changes. For example, by using TBA as a counterion, equilibration times were 25–30 column volumes[5]. Under these conditions, the routine use of gradients would be greatly restricted.

CONCLUSION

Two ion-pair HPLC systems have been developed which are capable of separating oxytetracycline from its degradation products. This method would be useful in the quality control of raw materials and in the detection of EOTC and AOTC in commercially available veterinary preparations (Fig. 2).

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