Journal of Chromatography, 151 (1978) 256–258

CHROM. 10,679

Note

Thin-layer chromatographic assay of tetracyclines

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(Received August 22nd, 1977)

In the last few years, several methods have been evolved for the thin-layer chromatography of tetracyclines[1–5]. In our laboratory, further attempts have been made to develop high-performance procedures for the separation, detection and assay of tetracyclines. A method using cellulose pre-treated with a stationary phase consisting of a buffer and an organic solvent proved to be advantageous. Of the impregnating agents examined, ethylene glycol exhibited the highest efficiency.

For the detection of tetracyclines, the possibility of fluorogen formation was studied. Detection in a strong ammonia atmosphere gives poor results in a quantitative assay, owing to the short duration of the colour obtained. Significantly better results can be achieved with the amines and metal salts proposed by Ragazzi and Veronese[4], but both the delayed formation of the fluorogen and the poor sensitivity of the compounds investigated are disadvantages. During studies with similar fluorogen-forming agents, it was found that by carrying out detection first with metal salts and then with organic solvents, fluorogens with relatively high intensities are formed. The procedure is described in this paper.

EXPERIMENTAL

Cellulose (mikrokristallin, E. Merck, Darmstadt, G.F.R.) (30 g) was suspended in a mixture of 0.2 *M* disodium hydrogen orthophosphate (70 ml) and 0.1 *M* citric acid (84 ml) in a mixer, coated as 0.3-mm layers on glass plates (20 × 20 cm) with the help of a Camag automatic TLC coater and left to dry at room temperature for 24 h. Prior to use, the plates were submerged in a methanolic ethylene glycol solution (20%, v/v) and the superfluous solvent was sucked off by placing the plates on filter-paper. Samples (0.1 μg) of tetracycline dissolved in 1 *N* hydrochloric acid–methanol (1:99) (200 μg/ml) were applied on to the plate, which was divided into three bands (in our experiments, the samples were working standards purified in the laboratory). Development was carried out twice in glass chambers containing a saturated atmosphere of ethyl acetate saturated with water. After development, the first band (A) of the chromatogram was sprayed with a 0.2 *M* magnesium chloride solution–95% ethanol (1:1), the second (B) with methanolic triethanolamine (10%, v/v), and the third (C) first with the reagent used for band A and then after 5 min with the reagent used for band B. Evaluation was carried out with an Opton PMQ-2 spectrophotometer coupled to a Camag-Z-Scanner (excitation, mercury-lamp, 366-nm filter; absorption, 540 nm).

RESULTS

The R_F values obtained (chlorotetracycline, 0.72; α-doxycycline, 0.66; oxytetracycline, 0.47; tetracycline, 0.35; methacycline, 0.20; β-doxycycline, 0.11; and apoterramycin, 0.07) show a satisfactory degree of separation.

Of the above substances, oxytetracycline (OTC), its degradation product apoterramycin (AT) and methacycline (MC) or α-doxycycline (DC) obtainable from it, were studied in detail. A chromatogram obtained by applying equal amounts (0.2 μg) of the compounds and developing them according to this method (Fig. 1) shows compact spots that are easy to evaluate. The increase in intensity achieved by the combined procedure (C) in comparison with those obtained with method A or B is striking.

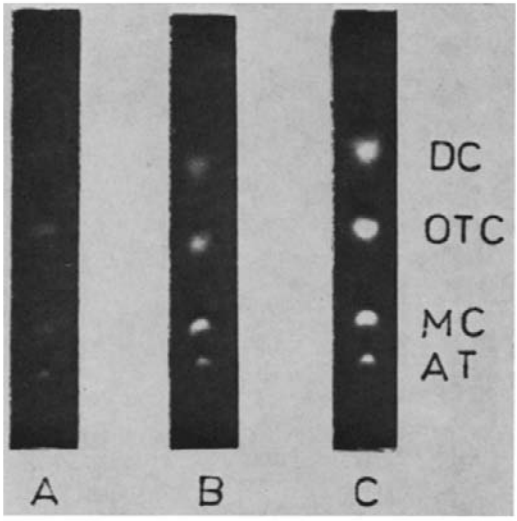

Fig. 1. Chromatograms of tetracyclines. Detection: (A) 0.1 *M* magnesium chloride solution–95% ethanol (1:1); (B) methanolic solution of triethanolamine (10%, v/v); (C) reagent A followed by reagent B.

The fluorograms prepared from the above chromatograms (Fig. 2) permit a quantitative evaluation of these intensity differences with the help of surface integrals (*I*).

In Fig. 3, the fluorescence intensity values procedure C obtained with are plotted as a function of time. With DC and OTC there is virtually no change in intensity between 30 min and 20 h. However, with MC, there is a gradual increase in intensity, but the rate of increase is so low after 2 h that it still permits a satisfactory determination. Under these conditions, for amounts in the range 0.05–1.00 μg, there is a linear correlation between the concentration of tetracyclines and the fluorescence intensities measured.

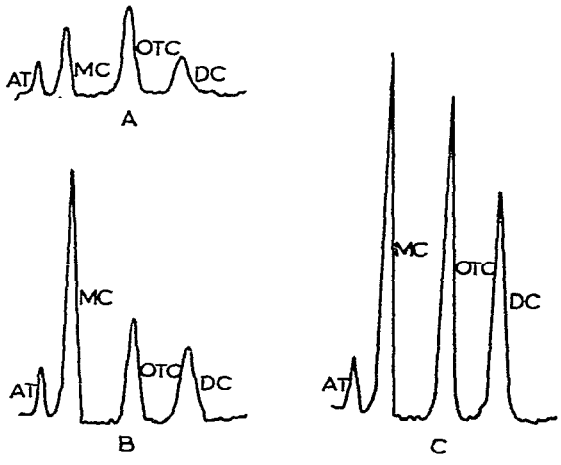

Fig. 2 Fluorograms of the chromatograms shown in Fig. 1.

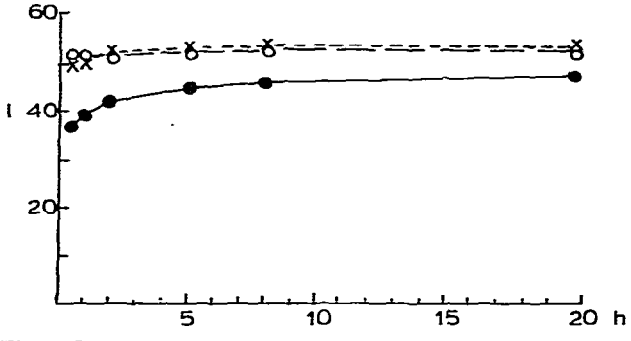

Fig. 3. Intensity (I) changes plotted as a function of time for chromatograms made visible according to method C. ×, DC; ○, OTC; ●, MC.

ACKNOWLEDGEMENT

The authors express their thanks to Mrs. Ilona Balogh for technical assistance.

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