Thin-layer and column chromatography of erythromycins and some degradation products

In recent years the thin-layer technique has been used successfully for the chromatographic separation of erythromycins and their derivatives. ANDERSON[1] described their separation on layers of Kieselgel G in the system methylene chloride–methanol–benzene–formamide (80:20:20:2–5). The method makes it possible to distinguish erythromycins A and B, anhydroerythromycin and acetylerythromycins. However, tests in our laboratory have shown that the reproducibility of the method is unsatisfactory. Erythromycins A and B had similar R_F values and were practically undistinguishable. Another difficulty was connected with the formation of two solvent fronts on the plate: one from the volatile solvents, and the other from the slower moving formamide. As a consequence we occasionally observed spots of irregular shape.

RUCZAJ[2] used layers of phosphate-buffered silica gel and, as the mobile phase, *sec.*-butanol–nitromethane–ethyl acetate–water (60:30:20:20). Erythromycin A was readily distinguished from the anhydro compound, but erythromycins A and B had the same R_F values under these conditions.

The method described below is a modification of ANDERSON's method. Glass plates (10 × 20 cm) are coated as usual with a 1:1 w/w mixture of Kieselgel G and Kieselguhr G, dried for 1 h at 110°, and immersed to a depth of 1 cm in 15 % formamide in acetone for impregnation. After the front of the solution has reached the edge of the plate the layer is dried for a few minutes at room temperature and used directly for the chromatographic separation of erythromycins. We used two solvent systems, and their composition may vary over a fairly wide range, depending on the activity of the plate. With more active layers the system should contain more chloroform or methylene chloride and less *n*-hexane or benzene.

System I (ternary)

n-Hexane or benzene (40–60, v/v)
Chloroform or methylene chloride (55–35, v/v)
Methanol or ethanol (5, v/v).

System II (quaternary)

n-Hexane or benzene or carbon tetrachloride (15–45, v/v)
Chloroform or methylene chloride (40–20, v/v)
Ethyl acetate (40–30, v/v)
Methanol or ethanol (5, v/v).

The chloroform must be free of phosgene and hydrogen chloride. After the chromatogram has been run over a distance of about 11 cm, which takes some 30–50 min, the plate is kept at 110° in a drying oven for 10 min to remove formamide. The cooled chromatoplate is developed by conventional methods, *e.g.* sprayed with 50 % sulphuric acid. However, we have had much better results with a new and very sensitive developer composed of 1 % cerium sulphate and 2.5 % molybdic acid in 10 % sulphuric acid[3]. The chromatogram to be developed is sprayed liberally and kept a few minutes at 110°. The spots are dark blue against a white background, deeper after cooling, and stable without change for about 12 h.

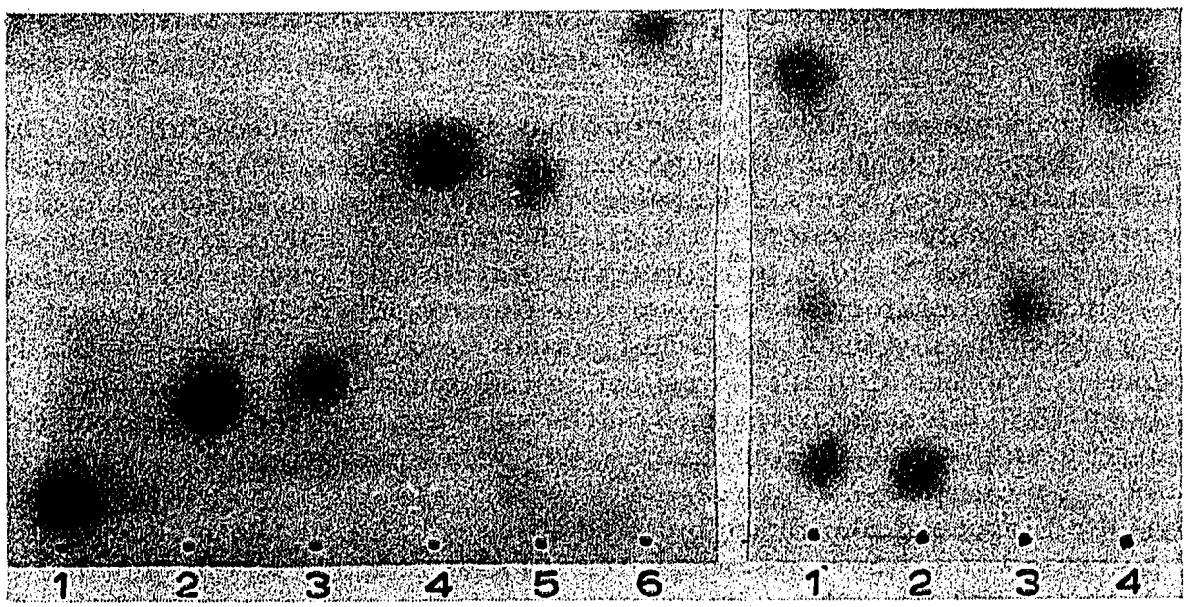

Fig. 1. Kieselgel G and Kieselguhr G (1:1), impregnated with formamide. Solvent: methylene chloride–*n*-hexane–ethanol (60:35:5). Lower part of the chromatogram run to a height of 11 cm; natural size. 1 = Erythromycin C (4 μg); 2 = erythromycin A (4 μg); 3 = anhydroerythromycin C (4 μg); 4 = erythromycin B (4 μg); 5 = anhydroerythromycin A (4 μg); 6 = erythralosamine (4 μg).

Fig. 2. Kieselgel G and Kieselguhr G (1:1), impregnated with formamide. Solvent: methylene chloride–*n*-hexane–ethanol (60:35:5). Lower part of the chromatogram run to a height of 11 cm; natural size. 1 = Mixture of erythromycins A, B, and C; 2 = erythromycin C; 3 = erythromycin A; 4 = erythromycin B.

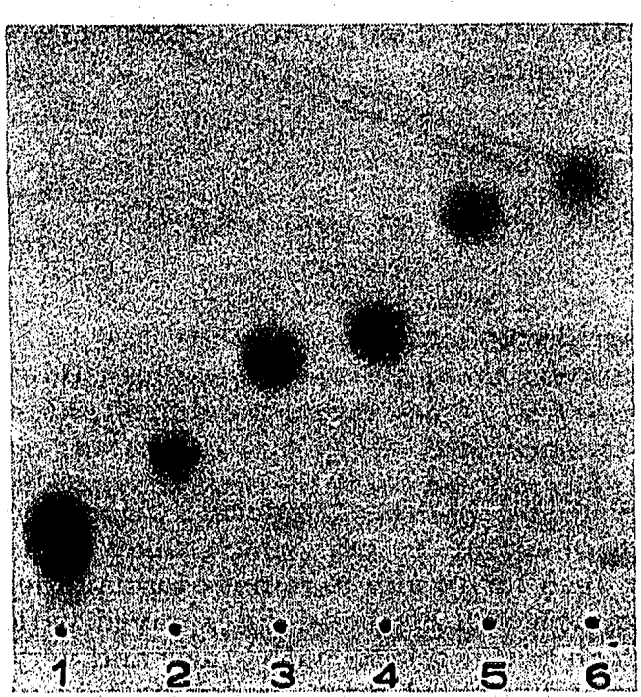

Fig. 3. Kieselgel G and Kieselguhr G (1:1), impregnated with formamide. Solvent: methylene chloride–ethyl acetate–*n*-hexane–ethanol (40:40:15:5). Lower part of the chromatogram run to a height of 11 cm; natural size. 1 = Erythromycin C; 2 = erythromycin A; 3 = anhydroerythromycin C; 4 = erythromycin B; 5 = anhydroerythromycin A; 6 = erythralosamine.

Figs. 1–3 show the results. It is evident that the ternary solvent system readily separates erythromycins A, B, and C; on the other hand, anhydroerythromycins A and C have similar R_F values to erythromycins B and A respectively, but are well separated by the quaternary solvent system. The method described makes it possible to detect 1% of erythromycin B in the presence of 99% of erythromycin A, and about 5% erythromycin C in the presence of 95% of compound A. With lower contents of compounds B and C, the spot of erythromycin A makes it impossible to detect them.

Erythromycins can also be separated on plates coated with aluminium oxide and impregnated with formamide as described. In this case the solvent system is *n*-hexane–chloroform (or methylene chloride)–ethanol, 75:20:5. The reproducibility of the results is however poor.

The principle of the method described can be suitably adapted to column separation of erythromycins on a preparative scale. For this purpose silica gel (we used Kieselgel für Chromatographie, unter 0.08 mm, Merck), 1 part by wt., is treated with 0.5 part by wt. of formamide in 1.5 parts of acetone. Acetone is removed in a rotatory evaporator, and the gel is suspended in benzene and introduced onto the column. The substance to be separated, preferably in benzene solution, is put on the column in the proportion of 1:100 with reference to the gel. The following solutions were used as eluent:

(I) *n*-Hexane or benzene (35–40, v/v)
Chloroform or methylene chloride (60–50, v/v)
Ethanol (5, v/v).

(II) *n*-Hexane (35, v/v)
Chloroform or methylene chloride (30, v/v)
Ethyl acetate (30, v/v)
Ethanol (5, v/v).

When the column is filled with similarly impregnated aluminium oxide, the eluent is *n*-hexane–chloroform (or methylene chloride)–ethanol, 75:20:5. The small quantities of formamide in the fractions from the column are removed by washing with water.

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