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1. Lüderitz, O., Westphal, O., Staub, A. M. and Nikaido, H. In Ajl, S. J., Weinbaum, G. and Kadis, S., Eds., *Microbial Toxins, A Comprehensive Treatise*, Vol. 4, Academic, New York. *In the press.*
2. Hellerqvist, C. G., Lindberg, B., Svensson, S., Holme, T. and Lindberg, A. A. *Carbohyd. Res.* **8** (1968) 43.
3. Hellerqvist, C. G., Lindberg, B., Svensson, S., Holme T. and Lindberg, A. A. *Carbohyd. Res.* **9** (1969) 237.
4. De Belder, A. N. and Norrman, B. *Carbohyd. Res.* **8** (1968) 1.
5. Sticzay, T., Peciar, C. and Bauer, Š. *Tetrahedron* **25** (1969) 3521.
6. Kabat, E. A., Lloyd, K. O. and Beychok, S. *Biochemistry* **8** (1969) 747.
7. Beving, H. F. G., Borén, H. B. and Garegg, P. J. *Acta Chem. Scand.* **24** (1970) 919.
8. Dröge, W., Lüderitz, O. and Westphal, O. *European J. Biochem.* **4** (1968) 126.

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Thin Layer Chromatography (Bioautography) of Streptomycins

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A simple and rapid chromatographic method to separate and detect the components of the streptomycin group of antibiotics is of importance, as these may occur simultaneously.

Several chromatographic systems have been described,[1-4] but the resolution obtained is often poor, or the procedures are too complicated and time-consuming for routine work.

We have developed a thin layer chromatographic system, which gives a good separation of many streptomycins.

Silica gel plates were prepared by dipping glass plates measuring 5 by 20 cm in a 30 % (w/v) suspension of silica gel containing 13 % gypsum (Merck, Darmstadt, West Germany) in chloroform.

The gel layer on one side and the edge was removed. After drying in a current of air 2μ samples of solutions each containing 5 to 10 mg/ml of the compounds were applied.

The spots were dried in a current of air before development. The chromatograms were developed in distilled water for 1 h. After drying the chromatograms were either sprayed with the α-naphthol-diacetyl reagent, described by Halliday,[5] giving purple spots on a white background or bioautographed.

The bioautography was performed as follows:

Pieces of filter paper of the same size as the chromatograms were dipped in 0.1 N HCl and applied to the chromatograms. The wet papers were pressed to the silica gel layer by means of 1 cm thick glass plates for 20 min, and then allowed to dry while still placed on the chromatograms. To remove the last traces of HCl the papers were subsequently dried for 1 h in an oven at 40°C. The dried papers were applied to agar plates seeded with spores of *Bacillus subtilis* ATCC 6633 for 1—5 min, depending on the amounts and activities of the antibiotics. The agar plates were incubated for 16 h at 37°C yielding clear zones of inhibition. R_F values for some streptomycins are given in Table 1.

Table 1. R_F values relative to streptomycin.

Streptomycin	1.00
Streptidine	1.55
Dihydrostreptomycin	0.90
Dihydro-*N*-demethylstreptomycin	1.16
Hydroxystreptomycin	1.22
Streptomycylamin	0.53
Dihydrodesoxystreptomycin	0.80
Methylstreptomycin	0.66

The absolute R_F values varied considerably from run to run. The R_F value for streptomycin was in most cases between

0.45 and 0.55. Commercially available — ready to use — plates were found to be too activated giving R_F values from 0 to 0.05. Suitable deactivation was not possible.

1. Winsten, W. A. and Eigen, E. J. *J. Am. Chem. Soc.* **70** (1948) 3333.
2. Ito, Y., Namba, M., Nagahma, N., Yamaguchi, T. and Okuda, T. J. *Antibiotics, Ser.* **A 17** (1964) 218.
3. Nussbaumer, P. and Schorderet, M. *Pharm. Acta Helv.* **40** (1964) 205.
4. Heding, H. *Acta Chem. Scand.* **20** (1966) 1743.
5. Halliday, W. J. *Nature* **169** (9152) 335.

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