

THIN-LAYER CHROMATOGRAPHY OF BASIC WATER-SOLUBLE ANTIBIOTICS ON RESIN- COATED CHROMATOPLATES

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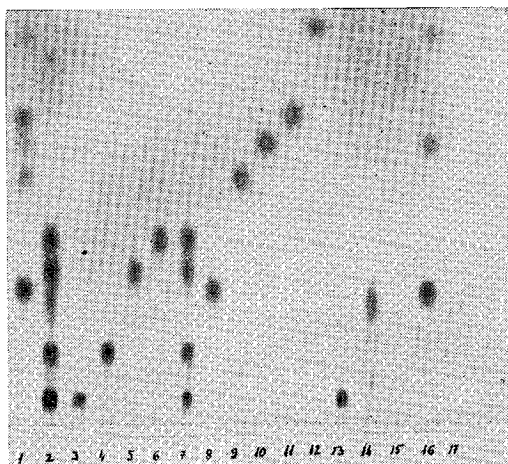
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Various methods for the identification of basic water-soluble antibiotics by the use of paper chromatography,^{1,9,12,16} thin-layer chromatography (cellulose⁸), silica gel,^{6,9,14,15} silica gel + Kieselguhr²) and electrophoresis^{5,10} have been reported. The separation of deoxystreptamine-containing antibiotics, having a similar structure is especially difficult, and can only be achieved by a combination of several procedures.

Ion-exchange resins are commonly used

Fig. 1.



Separation of basic water-soluble antibiotics and their degradation products on a Dowex 50×8 type resin-coated chromatoplate.

Eluting solution: 1.5 M sodium acetate (pH 8.5) containing 1.0 M sodium chloride and 10% *tert*-butanol, detected with ninhydrin-cadmium spray.

- 1) Neamine + paromamine + streptomine + deoxystreptamine + glucosamine
- 2) Gentamicin + neomycin + viomycin + paromomycin + kanamycin + cycloserine
- 3) Gentamicin 4) Neomycin
- 5) Paromomycin 6) Kanamycin
- 7) Gentamicin + neomycin + paromomycin + kanamycin
- 8) Neamine 9) Paromamine
- 10) Deoxystreptamine 11) Streptomine
- 12) Glucosamine 13) Capreomycin
- 14) Viomycin 15) Cycloserine
- 16) Neamine + deoxystreptamine

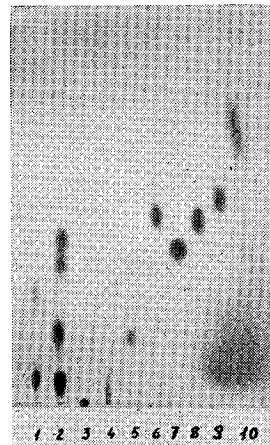
for the separation and purification of a number of antibiotics.^{3,7,11} The use of these materials, however, in analytical chemistry for the separation and identification of antibiotics has not been reported.

A simplified procedure was worked out on thin-layer chromatoplates coated with ion-exchange resins for the separation of deoxystreptamine-containing antibiotics as well as other water-soluble basic antibiotics of different structures.

Dowex 50×8 type resin-coated thin-layer chromatoplates (20×20 cm), developed for the separation of amino acids⁴) were applied in the sodium cycle (Ionex-25SA, Macherey, Nagel and Co., Düren, Germany). The best separation was obtained on plates which were run at 40°C with 1.5 M sodium acetate solution (adjusted to pH 8.5) containing 1.0 M sodium chloride and 10% *tert*-butanol. The spots were visualized by cadmium acetate containing ninhydrin spray reagent⁴). In the case of substances that do not react with ninhydrin, subsequent treatment with toluidine following chlorination was necessary.¹³)

Separations of antibiotics and their degradation products, respectively, are demonstrated in Figs. 1 and 2. The method is

Fig. 2.



Separation of antibiotics on a Dowex 50×8 type resin-coated chromatoplate.

Eluting solution as in Fig. 1, detection with the chlorine-toluidine reaction.

- 1) Capreomycin + viomycin
- 2) Gentamicin + neomycin + paromomycin + kanamycin
- 3) Polymyxin B 4) Bacitracin
- 5) Lincomycin 6) Hygromycin B
- 7) Streptidine 8) Dihydrostreptomycin
- 9) Streptomycin
- 10) Mannosidohydroxystreptomycin

suitable for the separation and identification of gentamicin, neomycin, paromomycin, kanamycin, capreomycin, viomycin, cycloserine as well as their degradation products: neamine, paromamine, deoxystreptamine, streptomycin and glucosamine. It will also separate polymyxin B, bacitracin, lincomycin, hygromycin B, streptidine, streptomycin, dihydrostreptomycin and mannosidohydroxystreptomycin. Compounds not separable by the solvent system described above, such as gentamicin and capreomycin, can be differentiated by the use of phosphate-containing solvent systems.

This method is particularly useful when screening for new antibiotics, for the separation and/or identification of known compounds, as well as for the identification of their respective hydrolytic degradation products. Moreover, it is a useful tool in the case of structure determination.

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Table 1. Rf Values of basic water-soluble antibiotics and their degradation products.

Solvent system: 1.5 M sodium acetate (pH 8.5) containing 1.0 M sodium chloride and 10% *tert*-butanol.

Substances	Rf
Gentamicin	0.05
Neomycin	0.15
Paromomycin	0.32
Kanamycin	0.39
Neamine	0.28
Paromamine	0.52
Streptomycin	0.65
Deoxystreptamine	0.59
Glucosamine	0.83
Capreomycin	0.05
Viomycin	0.25
Cycloserine	0.76
Polymyxin B	0.00
Bacitracin	0.01
Lincomycin	0.14
Hygromycin B	0.45
Dihydrostreptomycin	0.44
Streptomycin	0.49
Mannosidohydroxystreptomycin	0.66
Streptidine	0.37

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