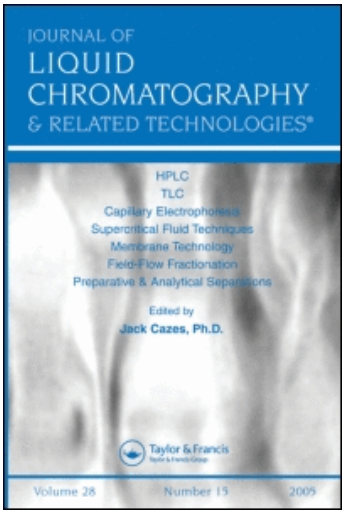

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Determination of Neomycin Components by Thin Layer Chromatography with Videodensitometry

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DETERMINATION OF NEOMYCIN COMPONENTS BY THIN LAYER CHROMATOGRAPHY WITH VIDEODENSITOMETRY

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ABSTRACT

A method was developed for determination of the three neomycin components, neomycin A, B and C using silica gel thin-layer chromatography with detection with p-dimethylamino benzaldehyde-ninhydrine reagent and videodensitometry. The method was used to monitor the composition in different stages of production. The results obrained were compared with that of biological determination.

INTRODUCTION

Neomycin, which is an aminoglycoside type antibiotic

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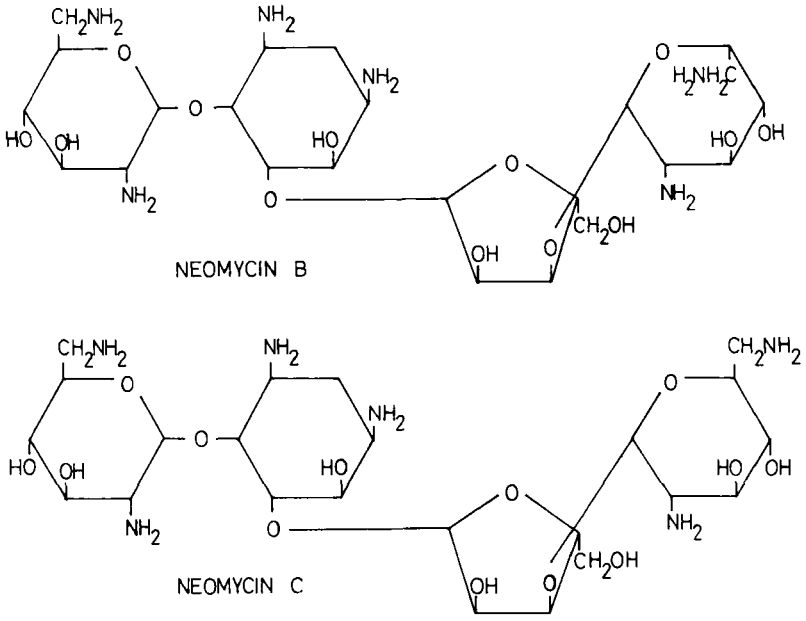

Figure 1.: Structure of neomycins

produced by Streptomyces fradiae, consists of three main components, neomycin A, B and C having very similar structure (Fig. 1.)

The neomycin, as antibiotic, is used for treatment of infections caused by Gram positive and Gram negative bacteria. This antibiotic inhibits the protein synthesis /2./. The neomycin components are not equivalent from therapeutical point of view, having different toxicity. Because of it the knowledge of the exact composition is essential. It is particularly important to determine the neomycin

B and C content, because the C component has twice ototoxicity than does the B component. Because of its biological and commercial interest, methods have been devised for the determination of the composition most often based on gas-liquid chromatography /3,4,5/.

Quantitativ determination of the neomycin components by GLC is difficult, because derivatization procedures are required for the analyses. /6/ This paper describes a simple procedure employing thin layer chromatography with videodensitometry for the direct determination of the components. The method is shown to be accurate and sensitive to μg amounts of neomycin B and C.

EXPERIMENTAL

Reagents and Materials :

Whatman K5 silica gel thin layer plates (20 x 20 cm) were used Neomycin A (neamine), B and C components, as standards were prepared by column chromatographic method in BIOGAL, and Neomycin B was from WHO. p-Dimethylaminobenzaldehyde (DABA), ninhydrine, cyclohexane, ethanol and pyridin were purchased from

REANAL (Hungary). For detection of spots the chromatogram was air dried and sprayed using a solution of p-DABA (500 mg) and ninhydrin (200 mg) in a mixture of cyclohexane : ethanol : pyridine /3 : 7 : 1/.

Apparatus :

The spots were quantitated with a TELECHROM OE 976 (CHINOIN, BUDAPEST) type videodensitometer in reflexion mode. Rectangular, heavy-wall glass tanks 30 cm x 10 cm x 28 cm were used as developing chambers.

Thin-layer chromatography :

Stock solution of Neomycin B (WHO) standard was prepared at the 6 mg/ml level in water and 0,5 - 2,5 µl aliquots were applied to layers with Hamilton microsyringe. Stock solutions of samples were prepared at the 50 mg/ml level in water and 2 µl aliquots for determination of Neomycin B and 10 µl aliquots for determination of Neomycin C were applied to layers with Hamilton microsyringe. Plates were developed at room temperature for a distance of 10 - 15 cm beyond the origin line, which was located 2 cm above the bottom of the plate

TABLE 1.

R_f values and spot colors of Neomycin components on Whatman K5 layers developed with water : ethanol (7:3v/v) containing 2,5 M NH_4Cl.

Component	R_f	Color
Neomycin A	0,42	purple
Neomycin B	0,32	purple
Neomycin C	0,16	purple

using water : ethanol = 7 : 3 containing 2,5 mol NH_4Cl as solvent. For detection the plates sprayed with DABA reagent were placed in a 110° oven for 15 minutes.

RESULTS AND DISCUSSION

Among the studied mobil phases the water : ethanol /96 %/ = 7 : 3 containing 2,5 M NH_4Cl proved the best for separation of Neomycin components. R_f values and spot colors are shown in Table 1. Differences among R_f values would allow this method to detect and quantitate the components. As seen in Table 1. the components were detected as purple spots, which color is the best for videodensitometric determination with Telechrom

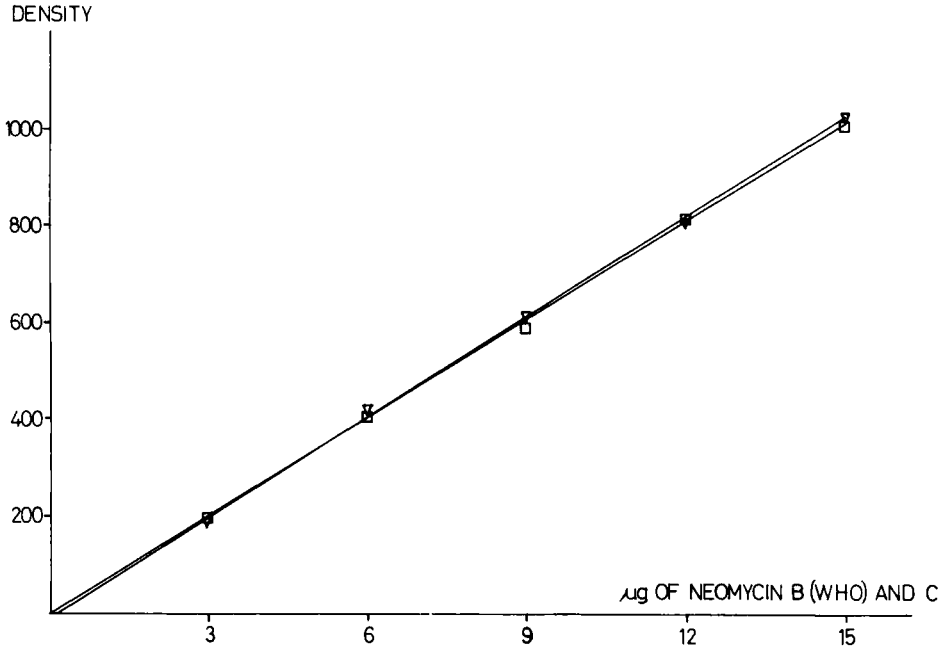

Figure 2.: Calibration curves of neomycin B and C components

neomycin B-ninhydrin-complex: a=401,8 b=1,5r = 1.00
neomycin C-ninhydrin-complex: a=402,2 b=4,7r = 1.00

OE 976. DABA reagent was the best for this purpose. The calibration plot for neomycin B and C was linear in 0-15 µg range (Fig.2.). Calibration curves of B and C components were identical having the same slope. This makes possible to determine the content of C component using neomycin B /WHO/ as standard (Fig.2.).

Calibration curves were quite reproducible in terms of slope and linarity from plate to plate, but standards should always be run on the same plate with samples to obviate the effects of any variations when using the method.

To check reproducibility, eleven 10 µg spots of Neomycin B /WHO/ were spotted across separate K 15 plates, the plates were developed, and the spots were detected and videodensitometrically measured. The relative standard deviation was ± 5,7.

On the basis of the result of thin-layer chromatography of numerous factory samples, the relative amounts of neomycin C was estimated in the percentage of the sum of Neomycin B and C.

The neomycin B and C display different activity against Bacillus pumilis, used in routin test, the activity of neomycin B is twice as much as that of neomycin C. The quantitative results of thin-layer chromatography-videodensitometry give preliminary informations about the probable biological activity.

This method was also used for the control of the steps and modifications of the technology and the results were compared to the biological activity.

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TABLE 2.

Thin-layer chromatography - videodensitometric determination (v.d.) - biological activity (b.a.)

N° of sample	T L C - v.d. neomycin B %	neomycin C %	neoC x 100 / neoB + Neo C	b.a. NU/mg
1.	12	10	45	160
2.	29	13	31	350
3.	38	12	24	420
4.	52	15	22	570
5.	58	14	19	590
6.	62	12	16	650
7.	65	11	14	650
8.	68	9	12	710
9.	69	9	12	710
10.	69	10	13	710

steps and modifications of the technology and the results were compared to the biological activity. Neomycin B and C content of the samples, the relativ amount of neomycin C and the biological activity of each sample (NU/mg) are summarized in Table 2.

SUMMARY

Thin-layer chromatography with videodensitometry provides rapid preliminary information on the quality of the product. Advantages of this method include simplicity, high sample through put, and the ability to analyse multiple samples at the same time under indentical conditons and to process

standards in parallel.

Precision and accuracy are shown to be satisfying from analytical point of view.

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