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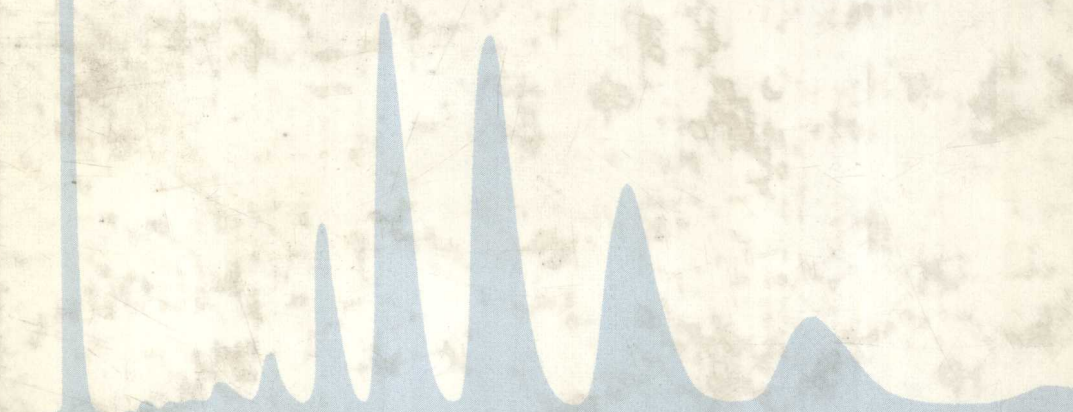
Biological/Biomedical Applications of Liquid Chromatography II



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Paul B. Champlin, Robert F. Hutton, Howard C. Jordi, and Chris Mol

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PREFACE

Published in this volume are selected papers presented at the second Liquid Chromatography Symposium: Biological/Biomedical Applications of LC which was held October 5 and 6, 1978 at the Boston Park Plaza Hotel in Boston, Massachusetts. Assembled here are twenty-four papers covering a range of topics of interest to those involved in the fractionation, characterization, and quantification of materials of biological interest.

We are indebted to the authors of these contributed papers for their efforts and cooperation. The editor also wishes to thank the associate editors: Paul Champlin, Robert Hutton, Howard Jordi, and Chris Mol, for reviewing the manuscripts and preparation of the subject index.

Also, a special note of thanks to the Waters Associates employees who gave of their time and talents in helping to make this second International LC Symposium: "Biological/Biomedical Applications of Liquid Chromatography" a success.

A special note of thanks to my secretary, Cecile Jennings, for her valuable assistance and patience at all stages of the preparation of this volume.

Lastly, thank you Kay Dimitri for your "last minute" heroics.

Gerald L. Hawk
Editor

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CONTENTS

CLINICAL ANTIBIOTIC ASSAYS BY HPLC

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I. INTRODUCTION

The microbiological assay is perhaps the most economical and widely used method to determine serum antibiotic levels in the clinical laboratory. The microbiological assay suffers from a lack of specificity and precision. Results from national surveys in Great Britain conducted by Reeves and Bywater (1,2) for gentamicin assays by microbiological methods were interpreted as showing that only a minority of laboratories produced values that were reliable enough for clinical purposes. A survey by the American College of Pathologists in 1975 of 129 laboratories performing gentamicin assays (3) obtained results that ranged from 0 to 32 µg/ml for a sample containing 6 µg/ml of gentamicin. A more recent survey using the same specimen (4) obtained results that were not much better. The range for bioassay procedures was 0.9 to 12.5 µg/ml, and the CV ranged from 30 to 39%, depending upon the indicator organism that was used. The therapeutic range for peak gentamicin levels is generally considered to be 4 to 8 µg/ml. Values greater than 12 µg/ml may be associated with toxicity, and values less than 4 µg/ml may be therapeutically inadequate. A need exists for more accurate standardization and more precise techniques to measure antibiotics with a narrow therapeutic range. This need has been filled in part by radioimmunoassay (RIA) pro-

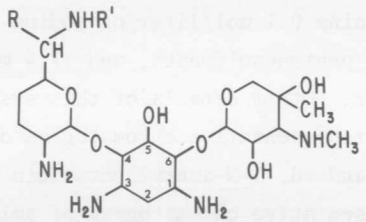
cedures. In the 1975 survey (3), only 11% of the laboratories used RIA. In the 1978 survey (4), over half (125 of 214) of the laboratories used RIA. The values reported by RIA ranged from 3.8 to 10.0 μg/ml with a CV of 12%. Numerous chemical assays that rely upon immunologic or enzymatic recognition of the analyte have been developed for aminoglycoside antibiotics (5,6), as well as for chloramphenicol (7,8), clindamycin (9), isoniazid (10), spectinomycin (11,12), and viomycin (13).

Chromatographic assays offer attractive alternatives to chemical assays. Chromatographic assays may be more economical when used for a small number of samples, because an internal standard may be incorporated with each specimen that may obviate the need to determine a calibration curve with each run. Chromatographic assays may be adapted with relative ease to a wide variety of antibiotics, and in unusual instances, the greater specificity may be advantageous. High-pressure liquid chromatography (HPLC) has been used to analyze relatively pure mixtures or pharmaceutical preparations of all of the antibiotic classes that are used clinically. The following review, however, will consider only applications in which HPLC has been used to assay clinical specimens. Assays for antibiotics that are used solely for cancer chemotherapy are omitted.

II. AMINOGLYCOSIDE ANTIBIOTICS

The aminoglycosides are the most frequently assayed antibiotics and constitute approximately 85% of our workload for antibiotic assays. In this group are included streptomycin, neomycin, kanamycin, gentamicin, tobramycin, and amikacin. Sisomicin and netilmicin are two newer members that are chemically related to gentamicin, but have not been as widely used. A major obstacle to widespread use of liquid chromatography has been the inability to measure these antibiotics at clinically significant concentrations with ultraviolet absorption detection. Significant chemical

and microbiological differences exist between the individual aminoglycosides; however, the problems encountered in development of assays sufficiently sensitive to be of clinical use can be described by reference to gentamicin as a model. Commercial preparations of gentamicin contain three major components (Fig. 1). Because of the lack of sufficient ultraviolet absorption, the approaches used to date for clinical assays have been based upon derivatization of the amino groups. Conceptually, derivatization may be performed either before or after chromatography. Derivatization before chromatography presents theoretical problems because of the multiplicity of reactive functional groups. In the absence of complete conversion of all reactive groups, product mixtures might be obtained that would be of variable composition or would lead to difficult chromatographic analysis. In addition, derivatized products may be unstable. Problems with variation in the product mixture are minimized in continuous-flow, post-column derivatization by the ease with which reaction conditions can be regulated, and product stability is not a problem. Post-column derivatization, however, requires that the chemical reactions occur rapidly in order to avoid unacceptable band-broadening. An additional pumping system is usually used for reagent, which adds



Gentamicin	C_{1a}	$R = R' = H$
Gentamicin	C_2	$R = CH_3$; $R' = H$
Gentamicin	C_1	$R = R' = CH_3$

Fig. 1. Chemical structures of the major components in gentamicin complex.