PESTICIDE ANALYSIS

edited by

K. G. Das

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FOREWORD

I began my career in pesticide research, specifically, the analysis of pesticides, twenty-five years ago. At that time, the only available text on pesticide analysis was Gunther and Blinn's *Analysis of Insecticides and Acaricides*, Interscience (1955). The analytical methods in vogue in those days were mostly colorimetric techniques, bioassays, total chloride, and a few scattered spectrophotometric techniques. These were the days prior to the revolution wrought by chromatography.

Even when I edited the first four volumes of *Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives*, Academic Press, I did not suspect that the field would grow so rapidly that less than two decades later an additional seven volumes would have appeared. Even now, Dr. Joe Sherma and I are planning additional volumes to cover advances in analytical chemistry that are being adapted to pesticide analysis.

In 1977, I made a plea in my Wiley Award Address for advances in confirmational methods and a lesser emphasis on greater sensitivity. K. G. Das of the National Chemical Laboratory at Poona, India, has recognized this fact and also the need to publish a single-volume text on pesticide analysis. The present book is the fruition of his efforts and is simply titled *Pesticide Analysis*. Its audience will be pesticide chemists, mainly those analytical chemists or biologists who are just entering the field of pesticide research and analysis. For those scientists, the present volume should be a welcome addition to the bookshelf or a constant companion on the laboratory bench.

Dr. Das has ably succeeded in assembling a group of knowledgeable experts in their respective fields of analytical chemistry and put together a very useful and comprehensive volume on pesticide analysis. I think that this book might be considered a worthy successor to Gunther and Blinn's classic as a basic primer in the English language on the subject of pesticide analysis.

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PREFACE

Among agrochemicals, pesticides constitute one of the largest and most important groups of compounds. Analytical methods are widely used in pesticide technology for the analysis of formulations, residues, and their degradation products in the environment. Although there have been numerous reviews as well as several books on pesticide analysis, we feel it is desirable to have a collection of articles on some of the recent spectroscopic and chromatographic methods.

The introduction is followed by three chapters on chromatographic methods. The polarographic method, which is the chief electrochemical method, is sandwiched between the chromatographic methods and the spectral methods discussed in Chapters 5 through 9. The last chapter deals with confirmatory methods. Determination by biological methods is conspicuous by its absence. Attempts are made to compare the various nonbiological methods and to highlight their advantages and limitations.

The authors have given comprehensive and authoritative coverage while avoiding lengthy discussions on instrumentation and experimental techniques. The chapters are by no means complete compilations. They are present-status reports on the various techniques with selected applications. It is hoped that the chapters will help practicing pesticide analytical chemists working in government laboratories, agricultural research organizations, and industry in using some of these techniques effectively.

The editor is most thankful to the authors and to the publisher for their cooperation.

K. G. Das

INTRODUCTION

The worldwide, steady expansion of population demands increases in food production, attention to public health, and protection of the environment. Pest control is an integral part of the development of every country. Pests harm crops and transmit diseases. Since chemical control of pests is so successful, there has been an explosive expansion in the development of synthetic organic pesticides. More than a thousand pesticides are in common use. The popular ones include about a hundred insecticides, fifty herbicides, fifty fungicides, many acaricides, nematocides, and other chemicals. Except for the organochlorine compounds, most of these chemicals persist for only a few weeks or months in the environment. However, as a result of the continued use of pesticides, appreciable quantities of pesticide residues and their degradation products accumulate in the biota. The monitoring of residues in the environment has become a very important aspect of pesticide technology and application.

Since a wide range of compounds is used as pesticides, a variety of methods is used in their analysis. Pesticide residue analysis comprises four steps: (1) extraction from the sample matrix, (2) removal of interfering co-extractives, (3) identification and estimation, and (4) confirmation of presence and identity.

The various stages of the analytical procedure are dictated both by the chemical type of the pesticide and the nature of the substrate. The sensitivity and versatility of modern instruments are remarkable. Gas-liquid chromatographic analysis with electron capture and phosphorus-sensitive flame ionization detection made it possible to detect nanogram and picogram levels of pesticide. Nuclear magnetic resonance and mass spectrometry are useful in identifying pesticides, their metabolites, and degradation products.

The solvent extraction step should be more than 80% efficient and should be sufficiently selective to require only minimum cleanup. The solvents must be purified by distillation, adsorption, or chemical treatment, and contamination by contact with rubber, plastics, grease, or similar materials must be avoided. Pesticides in air are extracted by adsorption on packed columns, absorption in a liquid phase, or freezing out in traps filled with glass helices. Water samples containing pesticides are extracted with solvent or passed through columns filled with carbon. The adsorbed pesticides are eluted from carbon.

The amounts and types of co-extractives that can be tolerated differ with the different method of estimation. The electron capture detector and gas chromatography-mass spectrometry are extremely intolerant of impurities. Fatty materials and other co-extractives affect thin-layer chromatography. Hence, before estimation the extract must be cleaned up by an appropriate procedure such as adsorption, solvent partition, sweep co-distillation, or gel chromatography. The nature of the co-extractives determines the type of cleanup method to be adopted. A common procedure used is the Florisil column cleanup. The sweep co-distillation technique and gel chromatography offer potential cleanup techniques.

The estimation methods commonly used are biological, spectrophotometric, and chromatographic. (Electrochemical and radiochemical methods are of only limited application.) Determinations based on bioassay do not come within the scope of this book. Among spectrophotometric methods, fluorescence and phosphorescence methods, and among chromatographic methods, paper chromatography, are not discussed.

Thin-layer chromatography is a quick, cheap, and efficient technique for qualitative and quantitative analysis and for cleanup. Single-dimensional chromatography using multiple development and two-dimensional development of chromatograms are some of the innovations. Carbamates that are not amenable to analysis by gas chromatographic methods are identified and estimated by thin-layer chromatography. The thin-layer chromatographic enzyme-inhibition method was developed

by Mendoza for carbamates and organophosphorus pesticides. Thin-layer chromatography has been combined with other techniques.

Gas-liquid chromatography is undoubtedly the most versatile and sensitive method. It is desirable to examine all samples on at least two different types of columns. The electron capture detector is best suited for organochlorine compounds. The detectors used for phosphorus compounds are flame ionization and flame photometric. Some of the commonly used stationary phases are the organosilicones: SE-30, QF-1, DC-200, 07-17, and so on. Derivatization of pesticides helps in gas-liquid chromatographic analysis.

Liquid-liquid chromatography is now finding applications in pesticide analysis. Since there are no limitations set by volatility, a wide range of compounds can be separated. The detectors used are refractometers, electrolytic conductivity cells, spectrophotometers, and flame ionization detectors.

Several pesticides containing oxidizable or reducible groups have been detected and estimated by polarography. Derivatives are used in the case of pesticides with no such group. Cathode stripping analysis, ac polarography, cyclic voltammetry, and oscillographic polarography are finding applications in pesticide analysis. A polarographic microdetector has been coupled with a liquid chromatograph to obtain detection limits of 10^{-8} M.

Visible and ultraviolet spectrophotometry are useful when the pesticides are not amenable to chromatographic analysis. A chromophoric structure has to be generated in the pesticide for analysis by spectrophotometry in the visible region. For reliable results the solvent used and the co-extractives should not absorb light in the region of the spectrum that is characteristic of the pesticide.

In the absence of interfering materials, pesticide residues in microgram quantities can be unequivocally characterized and estimated by infrared techniques. It is desirable to prepare the sample for analysis free from water. Multiple residues are analyzed by initial separation by thin-layer or preparative gas-liquid chromatography followed by microinfrared analysis.

The low sensitivity of nuclear magnetic resonance (NMR) spectroscopy limits its applications in pesticide residue analysis, even though it is one of the most useful structural tools. This technique finds application in the identification of pesticide metabolites and degradation products. As a confirmation method, NMR has great importance. Semiquantitative analysis of each isomer present in a mixture of pesticides is possible.

Mass spectrometry is a very sensitive and specific method of analysis. Pesticides and their metabolites, after separation by thin-layer chromatography, gas-liquid chromatography, or high-pressure liquid chromatography, can be identified by mass spectrometry. High-resolution mass spectrometry makes it possible to determine the elemental compositions of pesticides. The gas-liquid chromatography-mass spectrometry combination unit performs prior separation and positive characterization. The newer ionization techniques, such as positive and negative chemical ionization, field ionization, field desorption, and ion kinetic energy spectroscopy, have added new dimensions to mass spectral pesticide analysis.

It is necessary to carry out suitable confirmatory tests for the unequivocal identification of pesticide residues.

CONTENTS

Chapter 1

THIN-LAYER CHROMATOGRAPHY

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I. INTRODUCTION

Thin-layer chromatography (TLC) has been widely used since its development in the late 1930s [1]. It has gained a prominent niche in the field of chemistry. The method is easily adaptable in any

chemical laboratory. TLC is an integral and important method in modern pesticide residue analysis. The procedure is simple, sensitive, precise, versatile, rapid, and inexpensive.

The procedure is simple since it requires only a layer of adsorbent and a solid support such as silica gel and a glass plate, respectively. With some chemical compounds, the detection limits are in the nanogram or even picogram range. The degree of precision can be high, so that reliable analysis of chemical compounds can be attainable. Various solvents or solvent combinations (mobile phase), adsorbents (stationary phase), thickness of the adsorbents, detection system, and conditions during separation can be used for qualitative and quantitative analysis of many compounds. Various amounts of test compounds can be applied on TLC plates for qualitative, quantitative, and preparative procedures. The method is rapid enough that results can be obtained in a few minutes. The low cost involved makes the procedure readily adaptable in many chemical laboratories.

The TLC method has been utilized with unequivocal success in different areas of chemistry, for example, biological, organic, inorganic, and forensic chemistry. The method has also been used in instances where other methods failed. It has been used for purification of chemicals prior to identification and quantitation steps.

The object of this chapter is to give an overview of the TLC methods as applied to pesticide analyses. Representative methods are discussed in order that analysts can use them without going through an extensive literature survey. Details of individual TLC methods as well as the basic principle of individual detection reactions are considered beyond the scope of this chapter.

Among references available, the following deal specifically with pesticide analysis. Conklin [2], Abbott and Thomson [3], Wise [4], Getz [5], and Mendoza [6] review the application of TLC to the analysis of pesticides. Gets [5] also reviews some aspects of quantitation. Macek et al. [7-9] gives bibliographies of TLC and paper chromatography of pesticides. The lists are classified according to types of pesticides studied (carbamates, organochlorine,

fungicide, etc.); reviews and general techniques for pesticides are listed separately. Mendoza [10,11] reviews the application of TLC-enzyme inhibition (TLC-EI) technique for determination of pesticides.

II. BASIC PRINCIPLES

The principles involved in TLC are the adsorption of the test compound on a thin layer of adsorbent or stationary phase and the solubility of the compound in the solvent system or mobile phase. The mobile phase is allowed to migrate from the origin to a predetermined front. The origin is the point where the compound was spotted or applied. The front is the demarcation line indicating where solvent migration stops (Figure 1). The ratio of the distance

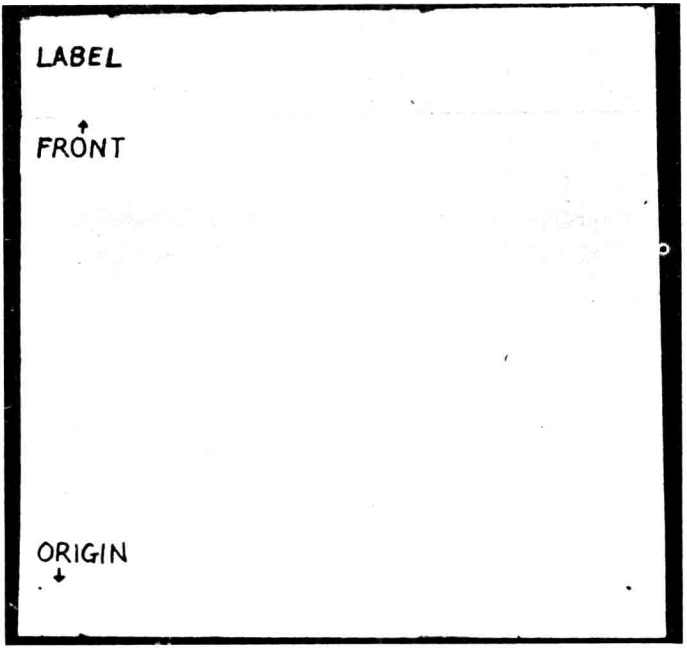

Fig. 1 A TLC plate with a line scored to indicate the front and dots on the left and right margins of the plate to indicate the origin. Labels to identify the experiment should be scored on the top of the plate. It is advisable to scrape off a section of the area above the front for handling in order to avoid contamination of the plate from crumbled gel.