PESTICIDE ANALYTICAL MANUAL

VOLUME I: Multiresidue Methods

U.S. Department of Health and Human Services • Public Health Service
Food and Drug Administration

PESTICIDE ANALYTICAL MANUAL VOLUME I

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INTRODUCTION

The Food and Drug Administration (FDA) is responsible under the Federal Food, Drug, and Cosmetic Act for enforcing tolerances established by the Environmental Protection Agency (EPA) for amounts of pesticide residues that may legally remain on food (including animal feed). In meeting this responsibility, FDA collects and analyzes food from commercial channels of trade for determining compliance with EPA tolerances. The residue data gathered under this regulatory monitoring program are also used for evaluating the extent and significance of pesticide residues in the food supply.

The Pesticide Analytical Manual (PAM) is published by FDA as a repository of the analytical methods used in FDA laboratories to examine food for pesticide residues for regulatory purposes.[1] The manual is organized according to the scope of the analytical methods:

Volume I contains multiresidue methods (MRMs) that are used by FDA on a routine basis, because of their efficiency and broad applicability, especially for analyzing foods of unknown pesticide treatment history.

Volume II contains methods designed for the analysis of commodities for residues of only a single compound (although some methods are capable of determining several related compounds). These methods are most often used when the likely residue is known to the chemist and/or when the residue of interest cannot be determined by common MRMs.

PAM is designed to be used by analysts experienced in trace residue analysis. All of the techniques employed are subject to potential interferences from reagents, apparatus, containers, contaminated air supply, and handling by personnel. The experienced analyst is alert for these possibilities and recognizes the need to confirm results by other techniques that measure different chemical or physical properties of the analyte.

Experienced residue analysts are aware that no report of validation in another laboratory can substitute for verification that the method does indeed work in the analyst's own laboratory. The analyst should verify method performance in each particular application by a trial of the method that includes examination of reagent and sample blanks and measurement of the recovery of added analyte. The editors invite analysts to report results of their experiences with PAM methods.

Revisions

Starting with transmittal 96-1 (9/96), revisions of PAM I have been issued in two ways: (1) changes in most manual sections will be distributed as hard (paper) copies, with symbols ▶ or ◀ marking lines that have been changed, and (2) updates to the tables

[1] 40 CFR 180.101 (c)

in Chapters 3 and 4, Appendix I, and the indices to methods, names, and CAS Registry numbers will be issued only *via* Internet. No hard copies will be distributed for the latter updated sections, but updates will be available more frequently than in the past.

As chapter tables of contents are revised, they will include the date on which each section within the chapter was transmitted; dates associated with those sections distributed only electronically will reflect the most recent version at the time the table of contents issued.

Internet Access to PAM I Files

PAM I is now available *via* Internet as Adobe Acrobat "portable document format" (pdf) files. Pdf format permits the user to read and print the document from any computer using appropriate free software.

▶ To obtain a copy of PAM I files, go to the World Wide Web site at: http://vm.cfsan.fda.gov/~frf/pami1.html. The resulting page describes PAM and provides links to currently available files. Follow the instructions for downloading.

Adobe Acrobat Reader is required to view and print pdf files. Download a copy of this free software from Adobe's web site at http://www.adobe.com/acrobat/readstep.html. A link to that site is provided on the PAM I page. Choose the version of Acrobat Reader appropriate to your own computer system.

PREFACE TO PAM I 3RD EDITION

The third edition of PAM I follows by 26 years the publication of the second edition. During that period, 29 revisions were made, reflecting new or revised methods, new technologies, and periodic updates of tables describing the capabilities of PAM I methods. Preparation of PAM I 3rd edition was motivated by three major deficiencies in the oft-revised 2nd edition: outdated material, obsolete organization of methods, and lack of consistent style.

Changes in multiresidue methods (MRMs) over the past 26 years have been significant. Among the most notable changes are those related to instrumental determinative techniques. Capillary columns and improved detectors have enhanced GLC applications; HPLC, with its various operating modes, has extended multiresidue capabilities to pesticides not amenable to GLC determination; and mass spectrometry, in the form of compact, automated instruments readily combined with GLC, has replaced many cumbersome, less sensitive, and less definitive techniques. PAM I 3rd edition attempts to provide a more up-to-date picture of the status of instrumentation currently used in FDA pesticide laboratories.

Despite advances in instrumentation, the basic approach to determination of trace level residues has not departed dramatically from that used in the 1960s. Residues are still extracted from the food commodity, isolated from co-extracted materials, and determined by instrumental techniques that separate residues from one another. While these analytical steps continue to be part of any MRM, methods research, coupled with advances in analytical technologies, has produced MRMs capable of determining a greater number of widely different types of pesticide residues in a single extract, *i.e.*, "multiclass MRMs." Research has also produced other MRMs that determine multiple residues of chemically related pesticides, such as N-methylcarbamates; these types of methods are known as "selective MRMs."

PAM I 2nd edition organized methods according to the chemical class of the targeted residues, an organization that does not conform to modern methodology. A major change in the PAM I 3rd edition is its grouping of methods into multiclass MRMs (Chapter 3) and selective MRMs (Chapter 4).

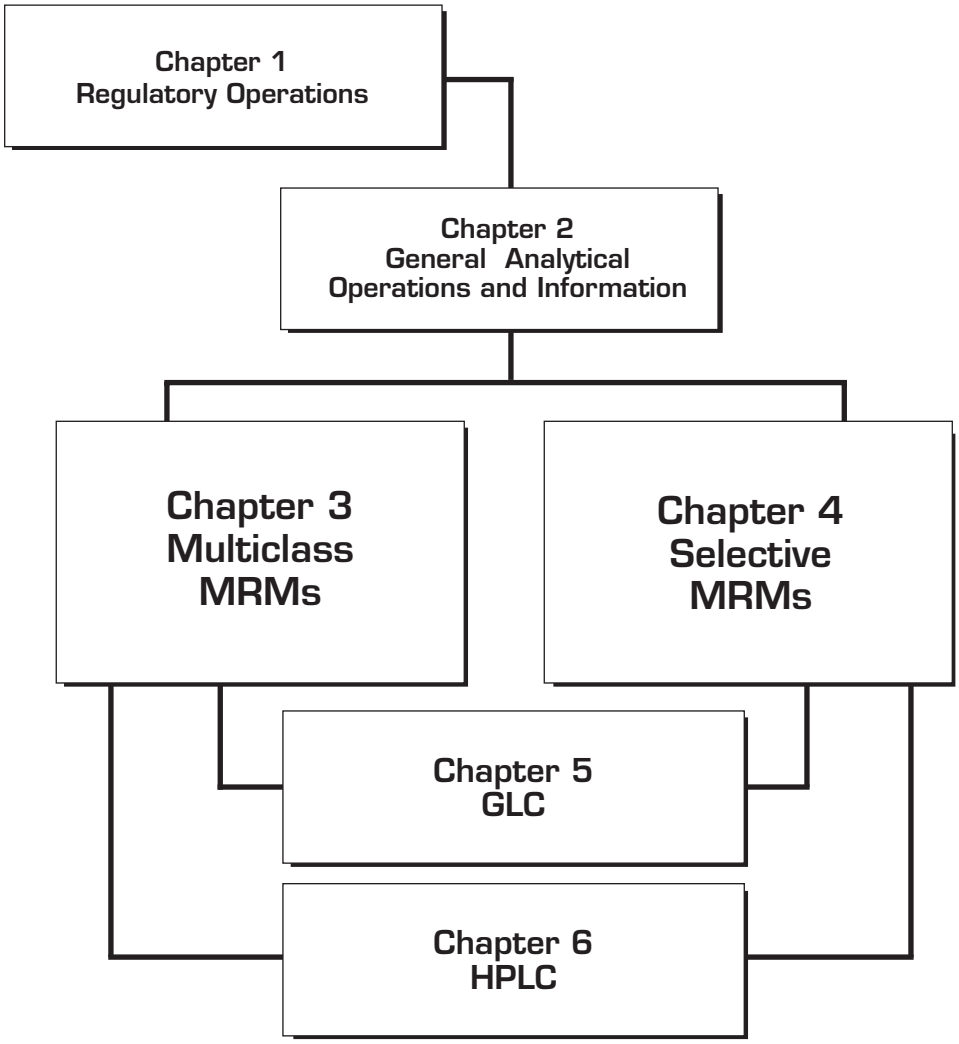
Another change in method descriptions accommodates the choices available to the experienced residue analyst. Typically, the residue laboratory chooses from among several validated options within basic methodology; choices are dictated by the particular commodity, available instrumentation, and targeted residues. Thus, PAM I 3rd edition method descriptions consist of individual extraction, cleanup, and determinative step modules, with indications of which combinations are validated. This organization permits easier reference to the precise combination of steps used in an analysis and facilitates sharing particular methods with colleagues. Future addition or revision of methods will be simplified by adding or replacing only the necessary sections or modules. The numbering system used in Chapters 3 and 4 is explained in the Guide to PAM I.

Finally, PAM I 3rd edition incorporates a new and consistent design. A new numbering system is used, in which chapter and subsection numbers avoid the restrictive 2nd edition decimal system. Pages are numbered within major subsections. Four indices are included: (1) to methods applicable for individual residues, (2) to preferred names for pesticides, (3) to Chemical Abstracts Service (CAS) Registry Numbers for the chemicals, and (4) to subjects by key word. An introductory Guide to PAM I, on the following pages, explains the organization of chapters

and the most useful path for finding pertinent information within the volume. The user is urged to take advantage of these tools and to offer comments or improvements that would make them more useful. PAM I remains, as always, a loose-leaf volume, designed for continuing update.

The Editors acknowledge the continuing cooperation and support of the pesticide chemists in FDA District and Regional Laboratories, District Research Centers, and Division of Pesticides and Industrial Chemicals; those who contributed substantially to 3rd edition preparation are included as technical advisors on the title page. Many of these advisors drafted or reviewed individual sections of the 3rd edition, and all FDA chemists responded repeatedly to requests for information about the applications of analytical methodology for pesticide residues. The editors also acknowledge the preparation of specialized sections by Mark Wirtz (QA/QC and GLC Quantitation by Electronic Integration) and Ann Stack (Safety), numerous editorial reviews performed by Norma Yess, secretarial assistance provided by Joan Duy, and comments on portions of the chapters on GLC and HPLC by Dr. Colin Poole. Without the assistance of all these individuals, the 3rd edition would not have been possible, and we are grateful to them.

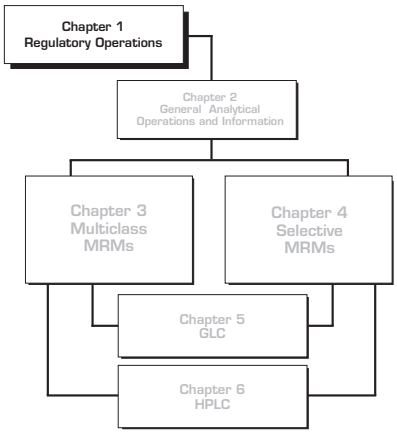
December, 1993



GUIDE TO PAM I

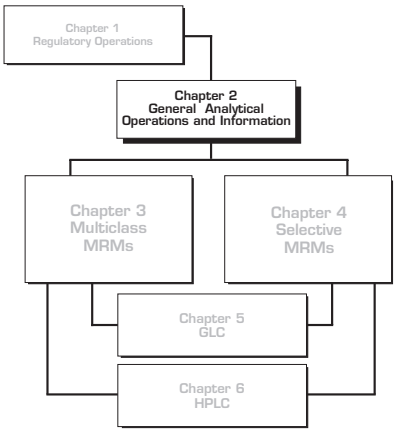
Each chapter in Volume I covers a different topic. Effective use of PAM I requires an understanding of the reasons that specific information is included in the chapter in which it appears.

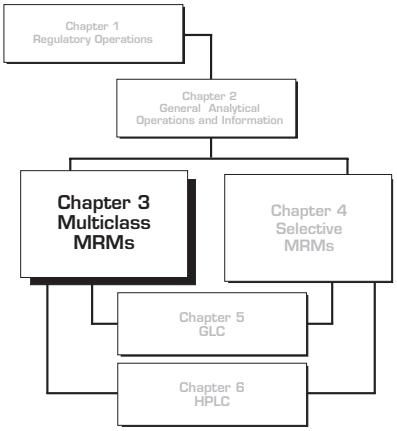
The user is advised to: (1) become familiar with the information in this manual and where it is located, as explained on pages x–xi; (2) understand how to choose and find appropriate methods, as detailed on pages xii–xiii; (3) review pages xiv–xvi for other information about Volume I and comparison to the 2nd edition; and (4) learn to use the indices provided.



Chapter 1 provides information and directions that reflect FDA regulatory policy. PAM I is not the authoritative source for publication of FDA policy, but policy decisions that directly affect the application of pesticide analytical methodology are included here as a service to the manual user.

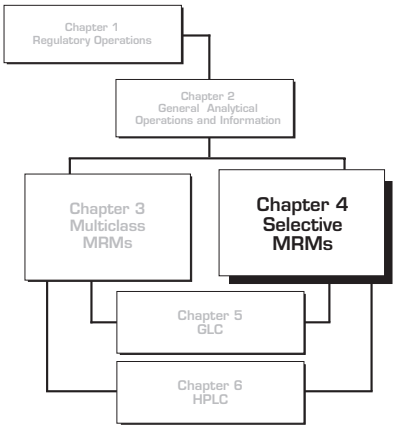
Chapter 2 is a collection of data and directions on a variety of unrelated topics, each of which provides background information needed to perform methods of Chapters 3 and 4. Where information in Chapters 1 and 2 appears to overlap (*e.g.*, Sections 102, Preparation of Analytical Samples; and 203, Equipment and Procedures for Comminuting), the material in Chapter 1 reflects the agency policy that must be followed for enforcement of regulations, and Chapter 2 provides information and hints found useful by FDA pesticide chemists.





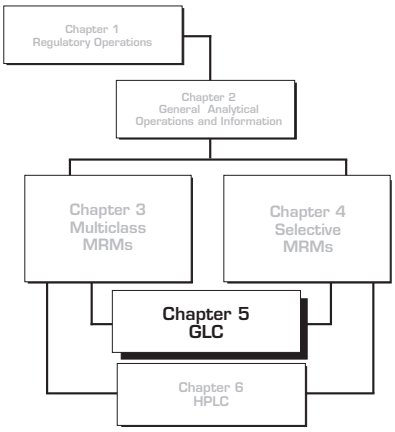
Chapter 3 includes multiclass multiresidue methods (MRMs), those that are capable of determining pesticide residues of many chemical types. The actual number and identity of the residues determinable by the methods are usually dependent on the number and variety of different determinative steps used to examine the extract. Each method in this chapter is presented as a series of modules for the extraction, cleanup, and determinative steps; a complete method is defined as a combination of one or more modules from each step. Complete methods that have been validated in interlaboratory studies, including collaborative studies performed under the auspices of AOAC International, are listed; these are sanctioned for use in regulatory analyses. Other combinations of modules must be treated as experimental methodology; additional supporting data for the validity of the analysis are required when such combinations are used in regulatory analyses.

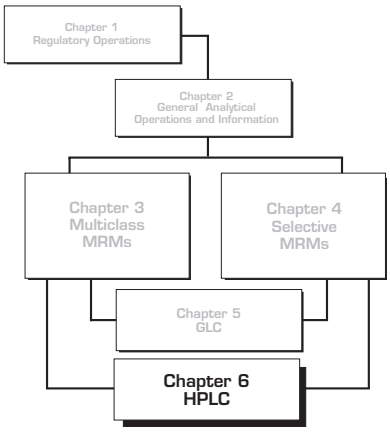
Chapter 4 includes descriptions of selective MRMs, *i.e.*, methods designed to determine a limited number of residues related by chemical structure. Selective MRMs target residues that are not amenable to determination by the multiclass MRMs of Chapter 3. In actual practice, FDA laboratories often examine an extract from a multiclass MRM with determinative step(s) from selective MRMs to broaden the scope of the analysis. When such uses have been validated in interlaboratory studies, the determinative steps are included as modules in Chapter 3 methodology. When no interlaboratory validation has occurred, such combinations are treated as experimental methodology, with the same requirements noted above for use in regulatory analyses.



Tables of data are provided for each method in Chapters 3 and 4 to describe the analytical behavior of each chemical tested through the method. Data are available only for methods sanctioned for use by virtue of previous interlaboratory studies.

Chapter 5, GLC, and Chapter 6, HPLC, provide background information about the two major determinative steps used in MRMs. Basic information about the techniques is included, as well as specific directions for implementing use of the instruments based on experiences in FDA laboratories. Manual users attempting to employ the determinative steps defined in Chapters 3 and 4 may need to refer to Chapters 5 and 6 for additional information and advice.





Appendix I combines information on GLC behavior of particular chemicals with recoveries of the chemicals through Chapter 3 methods. Appendix II defines the steps of protocols that were used to develop such method behavior data, for use in continuing testing.

STEP-BY-STEP SAMPLE ANALYSIS

PAM I provides information and/or directions to facilitate performance of each step in sample analysis. Use these sections for information needed at each step:

REVIEW PROCEDURES	Secs. 101, 103	Appropriate procedures for regulatory analyses
PREPARE SAMPLE	Sec. 102	Portion of commodity to include in analytical sample, regulatory requirements for subsamples
	Sec. 203	How to comminute or homogenize various commodities
CHOOSE METHOD		Based on commodity type: _____ Based on targeted residues: _____
REVIEW BACKGROUND INFORMATION (as needed)	Sec. 201	Percentage fat, water, and sugars in commodity
	Sec. 202	Detailed directions for column chromatography, solvent concentration
	Sec. 204	Directions for preparing frequently used reagents
	Sec. 205	Reference standards
	Sec. 206	Quality assurance/quality control
	Sec. 207	Safety
	Sec. 208	Hazardous waste disposal
	Chap. 5	Operation of GLC systems
	Chap. 6	Operation of HPLC systems
PERFORM ANALYSIS	Chap. 3 Chap. 4	Method directions
IDENTIFY RESIDUES	Tables, Appendix I	Details of behavior of chemicals tested through methods, for tentative identification
QUANTITATE RESIDUES	Secs. 504, 606	Quantitation for GLC, HPLC
CONFIRM RESIDUES	Sec. 103	Approach to confirmation of residue identity
REPORT RESIDUES	Secs. 104, 105	FDA procedures

CHOICE OF METHOD BASED ON COMMODITY TYPE

PRODUCTS >2% FAT (dairy, meat, fish, oilseeds, *etc.*)	Sec. 304	Appropriate extraction for product; relatively nonpolar residues; actual residues covered depends on determinative step(s) included
	Sec. 401	N-methylcarbamate residues
	Sec. 402	Acidic and phenolic residues
NONFATTY PRODUCTS (<2% fat) (fruits and vegetables, grains, *etc.*)	Sec. 302	Nonpolar and polar residues, if no cleanup is used; actual residues covered depends on determinative step(s) included
	Sec. 303	Relatively nonpolar residues; actual residues covered depends on determinative step(s) included
	Sec. 401	N-methylcarbamate residues
	Sec. 402	Acidic and phenolic residues
	Sec. 403	Phenylurea herbicide residues
	Sec. 404	Benomyl (as MBC), thiophanate-methyl, allophanate, and thiabendazole
EGGS, EGG WHITES	Sec. 303	E2; relatively nonpolar residues
DRIED EGG WHITES	Sec. 303	E3; relatively nonpolar residues

CHOICE OF METHOD BASED ON TARGETED RESIDUES

SPECIFIC RESIDUE(S)		Use Index to Methods to find method(s) applicable to targeted residue(s).
N-METHYL-CARBAMATES	Sec. 302	Use appropriate extraction + C3 or C4 + DL1. Confirm residues with Sec. 401.
ACIDS, PHENOLS	Sec. 402	Confirm residues by use of additional appropriate GLC systems.
PHENYLUREAS	Sec. 403	Confirm residues as directed in method.
BENZIMIDAZOLES	Sec. 404	Confirm residues as directed in method.
NO TARGET	Sec. 301	Use scheme for multiclass MRM, Figure 301-a.

NOTES ON TERMINOLOGY

Within PAM I, abbreviations are explained the first time they are used within any major subsection of a chapter (Section 101, 204, *etc.*). Subsequent use within that major subsection is not explained.

Certain common abbreviations are used without explanation throughout the volume; these include units of length, weight, volume, time, and concentration.

PAM I alphabetized tables of data use the following sequence: [space] ! " # $ % & ' ( ) * + , - . / 0 1 2 3 4 5 6 7 8 9 : ; < = > ? @ A B C D E F G H I J K L M N O P Q R S T U V W X Y Z [\]. Because commas precede hyphens in this sequence, chemical names that start with combinations of numbers, hyphens, and commas may not appear where expected, *e.g.*, 2,4,5,-T precedes 2-chloroethyl caprate.

"LIB" references refer to FDA's in-house Laboratory Information Bulletins, issued by Office of Regulatory Affairs, Division of Field Science, 5600 Fishers Lane, Rockville, MD 20857. LIB references are used only for material that was not subsequently published in the scientific literature; the latter reference is used whenever available.

NUMBERING OF METHODS MODULES

Methods described in Chapters 3 and 4 of this volume are presented as a series of extraction, cleanup, and determination modules. This organization offers flexible combination of modules as appropriate to the commodity being analyzed and/or the residues being targeted. The analyst and laboratory are responsible for assuring that the combination is valid.

Each method in Chapters 3 and 4 is treated as a major subsection, *i.e.*, it is numbered consecutively with a whole number: 301, 302, *etc.*, 401, 402, *etc.* Within those subsections, modules are numbered according to the following scheme:

Extraction	**E**, followed by a number. "E" module numbers are repeated in different methods (*i.e.*, E1 of Section 302 is not the same as E1 of Section 303) so both the section number and the module number must be included in a reference. For example, 302 E1 defines an extraction step, but E1 does not.
Cleanup	**C**, followed by a number. As above, "C" module numbers are repeated in different methods and both the section number and the module number must be referenced.
Determination	**DG**, followed by a number, for GLC determinative steps. **DL**, followed by a number, for HPLC determinative steps. Unlike E and C numbers, there is no repetition of DG or DL numbers, because the same determinative steps are used to examine cleaned-up extracts from many different methods. DG1 always refers to the same GLC system, no matter what section of Chapter 3 (or 4) it is combined with.

COMPARISON TO SECOND EDITION

Where PAM I 2nd edition material appears in the 3rd edition, section numbers are different. The following list of equivalent references applies:

2nd Edition	Description	3rd Edition
Methods		
211.13a-k/231.1	(Mills) method for fatty foods extractions	304 E1-E5
211.14a	Petroleum ether-acetonitrile partitioning	304 C1-C4
211.14b	Petroleum ether-acetonitrile "backwash"	not in 3rd ed
211.14c	Partition chromatography	not in 3rd ed
211.14d	Florisil cleanup with ethyl ether/petr ether	304 C1, C3
211.15a-c	Supplemental cleanups	not in 3rd ed
211.15d	Alkaline hydrolysis supplemental cleanup	part of 304 C7
212.13a-d/232.1	(MOG) method for nonfatty foods extractions	303 E1-E5
212.14	Cross-reference to Florisil with ethers	303 C1
212.2	(Luke) method for nonfatty foods with Florisil cleanup	302 E1+C5
221.1	(Hopper) method for chlorophenoxy acids, phenols	402
232.2	Sweep co-distillation method for OPs	not in 3rd ed
232.3	(Storherr) method for OPs	not in 3rd ed
232.4/242.1	(Luke) method for nonfatty foods, no cleanup	302 E1
242.2	(Krause) method for N-methylcarbamates	401
242.3	(PICRC) method for benzimidazoles	404
242.4	(Luchtefeld) method for substituted ureas	403
251.1	Silicic acid separation of PCBs and pesticides	not in 3rd ed
251.2	Derivatization and separation of PCBs, pesticides	not in 3rd ed
252.1	Florisil elution with methylene chloride	303 C2, 304 C2, C4
253	Exhaustive extraction of organochlorine residues	not in 3rd ed

Gas Chromatography (Chapter on GLC has been revised extensively; references to equivalent sections reflect the same topic but not necessarily the same information.)

300	Application of GLC to pesticide residue analysis	501, 504
301	Columns	502
310	Detectors	503
311	Electron capture detector	503 B
312	Microcoulometric detector	not in 3rd ed
313	Potassium chloride thermionic detector	not in 3rd ed
314	Flame photometric detector	503 C
315	Electrolytic conductivity detector	503 D
316	N/P detector	503 E
320	Multiple detectors	not in 3rd ed
330	GLC parameters and data tables	302 DG1-DG23, Appendix I

HPLC (Chapter on HPLC has undergone only minor revision, but chapter and sections are renumbered.)

500	General information	601, 608
510	HPLC columns	602
520	Mobile phase selection, preparation, delivery	603

530	Injection systems	604
540	Detectors	605
550	Data handling	606
560	Quality assurance and troubleshooting	607

Tables

201	Chemicals tested through specific methods	tables follow method sections
202	Percentage Fat, Water, and Sugar in Foods	201
Appendix I	PESTDATA lists of GLC data and recoveries	Appendix I

Other Information

110, 120	[Combined] lists of apparatus and reagents	not in 3rd ed
130	Standards	205
141-142	Preparation of analytical portion, compositing	102, 203
143.1	Reporting analytical results	104 B
143.2	Analytical limit of quantitation	105
230	Organophosphate residues	not in 3rd ed
Chapter 4	TLC	not in 3rd ed
Chapter 6	Confirmation	not in 3rd ed, except 103 E