- PN-ADC-535 -

Supporting Egypt's Processed Foods Export Industry

Pesticide Safeguards on Foods Imported into the USA

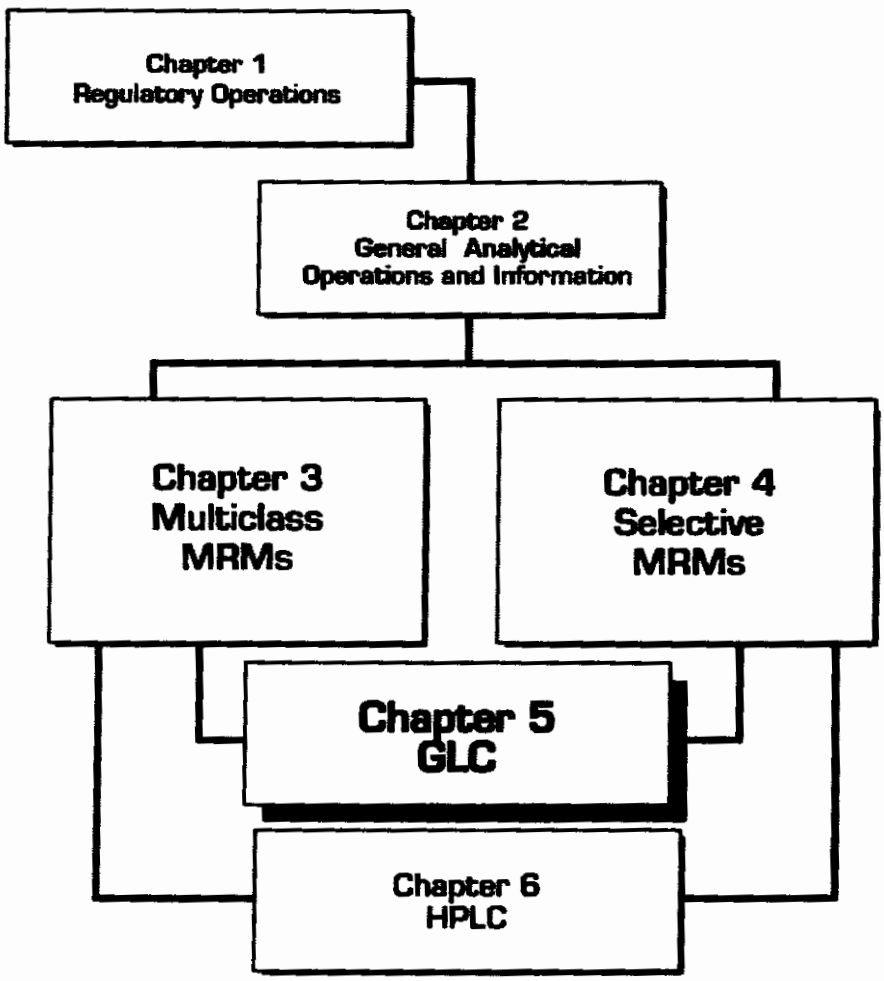
Chapter 5
Gas Liquid Chromatography

Volume VIII of XI

Prepared By
Agriculture-Led Export Businesses (ALEB)

February 2000

Funded by
U.S. Agency for International Development
USAID/Egypt
RP 0264
Under Contract No:
263-C-00-99-00011-00
USAID Cognizant Technical Office:
USAID/Egypt/EG/ED
CTO: Mr. Tarek Shata



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501: GENERAL INFORMATION

Multiresidue methodology by definition requires determinative steps capable of separating analytes from one another so each can be detected and measured individually. Both gas-liquid chromatography (GLC) and high performance liquid chromatography (HPLC) provide these capabilities, and both are used in modern laboratories.

GLC has been the predominant determinative step in pesticide multiresidue methodology for over 30 years. Because GLC involves interaction between a vapor phase and liquid phase, its application is restricted to analytes that can be vaporized without degradation. For heat-labile chemicals, HPLC offers a variety of alternative schemes for separating analytes according to chemical or physical characteristics, but GLC's relative simplicity and ruggedness cause it to remain the determinative step of choice for residues to which it is applicable.

501 A: PRINCIPLES

Separation in GLC is achieved by differences in distribution of analytes between mobile and stationary phases, causing them to move through the column at different rates and from it at different times [1]. A measured aliquot of solution is injected into a gas chromatographic column through an inlet heated to a sufficiently high temperature that analytes are vaporized. In this state, the flow of inert gas that forms the mobile phase sweeps analytes through the column; retarding this movement is the analyte's solubilization in the liquid phase. During passage through the column, analytes that were injected in the same solution separate from one another because of their different vapor pressures and selective interactions with the liquid phase [2]. When analytes elute from the column and enter a detector, the detector responds to the presence of a specific element or functional group within the molecule. The detector's response causes a change in electronic signal, which is proportional to the amount of residue; the signal is amplified and recorded as a chromatogram.

Analytes are identified by the time it takes them to pass through a column of specific liquid phase (retention time), at a specified temperature and gas flow. Quantities are calculated from the detector response. Both retention time and response are compared to values obtained for a reference standard solution injected into the same system.

501 B: EQUIPMENT FOR GLC

Gas Chromatographic Components

The basic gas chromatograph consists of an inlet system, column, detector, electronic equipment to amplify the detector signal, and a recorder or other data-handling device. Carrier gas(es), with appropriate pneumatic system(s), are also integral to the GLC system. The inlet system, column, and detector are maintained in temperature-controlled environments.

The following are desirable features in GLC hardware:

1) Inlet, column oven, and detector should be individually heated and temperature-controlled. Temperature should be maintained to ±0.1° C.

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Control of detector temperature usually is not as critical but should be well controlled, constant, and not affected by such things as line voltage fluctuations.

2) Temperature readout should be available for column, detector, and inlet. (Check accuracy of instrument temperature indicators with accurate pyrometer.)

3) Instrument design should be simple enough to facilitate troubleshooting and repairs. Design should permit easy removal or inspection of either column or detector without affecting the temperature of the other.

4) System should be designed to prevent or minimize contact between sample injection and any metal parts; system should be all-glass (or as near as possible).

   Several sizes of packed and open tubular capillary columns are used in residue analysis, and hardware for inlet and column must accommodate configurations that will be needed. Section 502, Columns, includes directions for adapting equipment.

5) Certain detectors may require multiple heated zones, including combustion furnaces. For flexibility, designs that permit ready access for servicing and maintenance are preferred. Section 503 provides details on various detectors used in pesticide residue determination.

6) Electrical signal monitoring equipment is usually one of two designs: (1) amplifier with 1 or 10 mV output, compatible with strip chart recorder, and (2) amplifier with 1 or 10 V output, compatible with data processing by either electronic integrator or computer. Other remote devices such as autosamplers can be easily adapted to any of these systems.

Other Apparatus

Gas Regulators. Two-stage gas pressure regulators with stainless steel diaphragms are required for all GLC determinations of trace residues. Regulators with a secondary stage maximum pressure of 80 psi are acceptable, but those with 200 psi offer more flexibility. If a hydrogen purifier is used (below), the latter type of regulator is required, because higher pressure is needed.

Gas lines that connect gas tanks to the chromatograph must be clean and free of components that contain oil or gas-purgeable elastomers; "refrigeration grade" copper (*i.e.*, cleaned of all oil) is preferred. Tubing (even refrigeration grade) should be sequentially rinsed with methylene chloride and acetone before use. Plastic and nylon lines must be avoided to reduce the likelihood of air contaminating the gas.

Syringes. The most common syringes for injection of food extracts into a chromatograph are 5 and 10 µL fixed needle syringes with 22° bevel points; some other sizes may be needed for special purposes. Hamilton syringes or equivalent are available from all chromatography suppliers. Plunger "guides" are available as options to minimize bending the plunger during injection.

Some specialty products exist to facilitate injection and minimize aggravation, and each has found favor with some analysts. For example, syringes with removable needles permit replacement of needles on which "burrs" have formed that destroy septa; removable needles with a "side port point" do not shred the septa as do standard bevel point needles; and syringes with plungers and needles made of a titanium alloy cannot be bent.

Reagents and Gases

Reagents associated with GLC include column liquid phases and solid supports, gases used for mobile phase and for detector reactions, and certain other reagents relevant to detector operation. Most of these reagents are discussed further in pertinent sections of this chapter; only gases, including filters used to remove contaminants from gas flow, are included in this introductory section.

Helium, hydrogen, and nitrogen are most commonly used as column carrier gases. Purity is always critical to avoid damage to the column, and more stringent purity requirements may be imposed by the detector. Purity specifications of the instrument manufacturer should always be followed.

Helium and hydrogen requirements range from 99.999-99.9999% purity, depending on the detector. Even with the highest purity, oxygen traps, available from chromatography suppliers, are recommended; traps that change color when permeated with oxygen are ideal for alerting the analyst to potential problems.

Purchase of ultra high purity helium and hydrogen may not be necessary if specially designed purifiers are used. Purifiers are available that permit use of commercial grade gases (99.995%) at a much lower price, justifying the cost of the purifier. Different purifiers are needed for helium and hydrogen; they are not interchangeable. FDA has had successful experiences with:

> hydrogen purifiers: Model 560, AADCO Instruments, Inc., Clearwater, FL; Model 8372V, Consolidated Technologies, Inc., West Chester, PA
>
> helium purifiers: Product # HP, Valco Instrument Co., Houston, TX; Model 2-3800, Supelco, Bellefonte, PA

Nitrogen is used as a carrier gas only for packed columns (Section 502 B). Either nitrogen or argon/methane (95+5 or 90+10) is also required as a carrier and/or makeup gas for the electron capture detector (Section 503 B). Commercial grades of these gases are acceptable if oxygen and moisture traps are used between the gas tank and the chromatograph.

501 C: RESIDUE METHODOLOGY FOR GLC DETERMINATION

Applications of analytical methodology require consideration of many factors to assure compatibility of method steps. The following factors related to extraction and cleanup of food samples profoundly influence accuracy and reliability of GLC determinative steps.

Cleanup

Solvent extraction of pesticide residues also extracts food constituents ("co-extractives") from the sample. Cleanup steps are included in residue analytical methods to remove co-extractives that can interfere in the determinative step of the analysis or cause damage to the column and/or detector.

For many years, predominant use of the nonselective electron capture (EC) detector caused justifiable concern about potential detector response to nonpesticidal co-extractives. In addition, documented cases in which sample co-extractives damaged GLC columns and caused subsequent breakdown of injected residues supported the need for extensive cleanup prior to GLC determination [3].

More recently, several factors have reduced emphasis on cleanup. The more selective GLC detectors now in use have decreased the likelihood that sample or reagent artifacts might be mistaken for pesticide residues. In addition, use of capillary columns, which are more efficient than equivalent packed columns, result in increased peak height response for the same amount of analyte. The amount of extract injected can thus be reduced without changing the level of quantitation, and this in turn reduces the likelihood of damage to the GLC system. Inlet liners and adapters used with capillary columns (Section 502 C) also provide the column with some degree of protection from damage caused by co-extractives. Finally, there are many incentives to perform more analyses with the same or fewer resources and to minimize the volume of solvents that must be purchased and disposed of. These factors contribute to a trend toward performing only minimal cleanup of sample extracts during routine surveillance analyses, with the intention of cleanup with applicable step(s) if an extract is found to contain interfering materials.

Despite these compelling reasons to reduce cleanup, GLC systems that are not protected from co-extractives deteriorate faster than those into which only cleaned up extracts are injected. The column and/or detector may be damaged by injection of insufficiently cleaned up samples, especially when the method and the chromatograph are used repeatedly. Such detrimental effects can occur even when the chromatogram appears to be clean enough for residue identification and measurement. Experience with a variety of sample types should make the analyst aware of these occurrences.

Detector response to sample co-extractives (artifacts) is still possible even with element-selective detectors. Although a selective detector is less likely to respond to chemically unrelated artifacts than the nonselective EC detector, artifacts containing an element to which the detector responds can still interfere with residue analysis. This occurs most often with nitrogen-selective detectors because of the number of nitrogenous chemicals in foods, but it can occur with any detector. Likelihood of interferences and potential for mistaken identity increase with decreasing cleanup.

Insufficiently clean extracts may also affect quantitative accuracy when determining residues that are polar or otherwise subject to adsorption by active sites in a GLC column. Such chemicals usually exhibit poor chromatography when standard solutions are injected, because adsorption delays or inhibits the chemical during its passage through the column. Peak tailing and/or changes in retention times are caused by adsorption. The net effect is an apparently diminished detector

response, which is especially evident if peak height measurements are used rather than peak area.

In contrast, when an uncleaned extract containing the same analyte is injected into the GLC system, co-extractives compete for the column's active sites, and the analyte moves through the column in a tighter chromatographic band. Analyte concentration (per unit time) entering the detector thus increases, and detector response (peak height) is greater. Quantitation by the usual practice (*i.e.*, comparison of detector responses to residue and reference standard) results in calculation of an inaccurately high residue level, especially if peak heights are compared. Quantitative accuracy can be improved for such chemicals by employing more rigorous cleanup of the extract or by using a GLC column with fewer active sites.

An appropriate balance is needed between efficiency in processing samples and accuracy in determining residues. Every injected extract should be sufficiently clean that it (1) does not jeopardize the column beyond the point that it can be easily repaired; (2) does not introduce substances that will degrade co-injected or subsequently injected residues; (3) does not foul any part of the detector, including combustion tube, flame, radioactive source, *etc.*; (4) minimizes introduction of artifacts to which the detector will respond; and (5) does not cause a disproportionate response enhancement of the residue in the extract.

Reagent Blanks

The analyst must ascertain that no interference from reagents and/or glassware occurs during residue analysis. Scrupulous attention is required to eliminate all such contaminants, and routine analysis of reagent blanks should be specified in the laboratory quality assurance plan (Section 206).

Contaminants can be introduced from a variety of sources. Studies with the EC detector have identified interferences from impure solvents, adsorbents, sodium sulfate, glass wool, Celite, blender gaskets, laboratory air filters, and polyethylene containers. The more nonselective the detector, the more likely it is to respond to interferences introduced by reagents or the environment. A thorough examination of the reagent blank is also necessary for methods that use a relatively selective detector. One example demonstrated that chemicals extracted by petroleum ether from a polyethylene squeeze bottle caused response by both an EC and a halogen-selective detector [3]. Contaminants can even be pesticides themselves, present on glassware or microliter syringes used in prior analyses, or present in the laboratory environment because of pest control treatment.

When interferences are discovered and the source(s) identified, every effort must be made to reduce or eliminate the problem. Solvents can be purchased to meet requirements or may be redistilled. Solids frequently can be washed and/or heated prior to use. Section 204 provides purity tests and procedures for purifying certain commonly used reagents; other reagent purification procedures are included in pertinent method descriptions in Chapters 3 and 4. Sometimes the method cleanup step removes interferences added to the sample during previous steps, but whether this is accomplished must be determined by a complete investigation of the method reagent blank.

Equipment should be washed thoroughly and rinsed with solvent as soon as possible after use. Syringe plungers and needles should be wiped with lint-free wipers

dipped in an appropriate solvent (*e.g.*, acetone), and the barrel should be cleaned by drawing solvent through the needle and out the top by a vacuum applied to the top. Particular care should be taken to assure elimination of residues from glassware or syringes previously in contact with high concentrations of pesticides.

Choice of Solvent

The solvent in which the final extract is dissolved must be compatible with the detector(s) in the GLC determinative step(s). The most basic requirement is that the solvent not contain elements to which the detector responds. Specifically, no amount of chlorinated solvent, such as methylene chloride, can remain in extracts being examined by an EC or halogen-selective detector, and no trace of acetonitrile can be present in extracts examined with nitrogen-selective detectors.

Other effects besides element selectivity cause incompatibility between detectors and solvents. For example, acetonitrile has an unexplained adverse effect on response of the EC detector, and aromatic and halogenated solvents may increase detector response of the N/P detector and eventually render it useless.

Solvent volatility must also be considered when using a detector that requires a solvent venting time. For these detectors, the most volatile practical solvent in which residues are soluble should be chosen to minimize length of venting time and avoid potential loss of early eluting analytes.

Solvent volatility has another practical effect related to the ease with which the extract can be concentrated. Final volume of concentrated extract must be sufficiently small that the volume injected into the GLC system contains sufficient equivalent sample weight necessary to reach the targeted level of quantitation (Section 105). Sensitivity of a particular detector to residues of interest governs how much sample equivalent must be injected, and column type and arrangement limit the volume that can be injected. In cases where a very small final extract volume is needed, or where the concentration step begins with a very large solvent volume, practicality dictates the choice of a volatile solvent to minimize time needed for concentration.

501 D: INJECTION TECHNIQUES

The technique used to inject extracts and reference standards into the chromatograph is critical to system performance. Improper syringe handling can lead to myriad problems, including asymmetrical peak shapes and nonreproducible retention times or responses. Autoinjectors are increasingly used for residue determination, but manual injection is still practiced.

Manual Injection

If extracts and standards are injected manually, it is imperative that each analyst develop and follow good technique in syringe handling and sample introduction. This can be achieved through practice and care. Several methods presently in use for filling syringes and injecting include:

1) A volume of solvent greater than or equal to needle volume is drawn into the syringe, followed by a small amount of air. The extract (or reference standard solution) is then drawn completely into the syringe barrel, where

its volume can be measured by reading both ends of the liquid. Injection is then made. The initial solvent flushes the extract or standard into the chromatograph. This technique is referred to as the "solvent flush" or "sandwich" technique.

2) The syringe is filled by drawing extract (or standard solution) completely into the barrel (*i.e.*, none is left in the needle). Total volume is measured by reading both ends of the liquid. Injection is made, with the syringe removed quickly from the inlet. The syringe plunger is withdrawn until whatever volume of liquid remains is completely in the barrel of the syringe, where it is measured as before. The difference in liquid volume before and after injection is the amount actually injected. It is important when using this technique to remove the syringe from the heated injection port as quickly as possible after injection to avoid any evaporation of liquid remaining in the syringe.

3) The syringe is filled to the desired volume, the volume noted, and the injection made. The volume measured is considered to be the volume injected. This technique introduces error, because it ignores the volume in the needle and the volume that remains after injection. The effective error can be minimized by use of the same solvent for both sample extract and standard solution and by injection of the same volume of each.

Whichever injection technique is chosen, it must be performed reproducibly. Each analyst should choose the injection technique he/she finds most reproducible and use it routinely. Poor precision among chromatograms from repetitive injections may be caused by faulty syringes or poor analyst technique, as well as by inappropriate solvents or inadequate sample cleanup. Volume of liquid in the syringe should be measured by holding the syringe in the same manner each time while looking toward a light background. The same injection technique must be used for both the sample extract and the reference standard to which it will be compared.

Choice of injection technique is not solely based on personal preference; type of column being used (packed *vs* capillary) must also be considered. Any technique described above can be applied when using packed columns. However, too much solvent can overwhelm the small diameter capillary column, so injection volume must be limited. Several inlet systems and injection options are used with capillary columns to accommodate both column restrictions and volume requirements of residue determination (Section 502 C). Consistently good capillary column results have been achieved with manual injection and the solvent flush technique. The syringe needle should remain in the inlet 1 sec for each µL injected to allow the pressure surge from vaporization of solvents to dissipate.

The syringe manufacturer's recommendations for use and care of the syringe should be followed. Syringes must be kept free of traces of analyte. This should be checked occasionally by injecting a volume of pure solvent; if the syringe is clean, no peaks other than the solvent peak will appear.

Autoinjectors

The best injection performance is achieved using an autoinjector (also called autosampler). Various commercially available autoinjectors can be interfaced with

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GLC systems. For normal use of autoinjectors, extracts and standard solutions are placed in disposable glass vials with vapor-tight septum caps. The autoinjector wets the syringe completely and removes air bubbles by pumping extract (or standard solution) into the barrel. It then draws a precisely measured volume of solution into the barrel and injects it into the chromatograph. Between injections, the autoinjector flushes the needle with appropriate solvent to clean it. Beyond the improved reproducibility achieved with autoinjectors, their use permits unattended operation of the chromatograph and frees the chromatographer to perform other tasks.

501 E: REFERENCE STANDARDS

Section 205 provides information on pesticide standards. The importance of reliable standard solutions to accurate pesticide analyses cannot be overemphasized. Solvents used for GLC standard solutions are subject to the same requirements and limitations listed above for extracts.

The quality assurance plan for analyses involving GLC determination should include routine injection of a mixed standard solution. The mixture should include compounds normally used as markers for retention time and response and should also include compounds prone to adsorption or degradation. Vulnerable compounds serve as indicators of problems developing in the system; *e.g.*, the presence of p,p′-DDT in such a solution serves to alert the analyst when degradation to p,p′-TDE occurs. GLC systems used for determination of organophosphorus or other polar residues should be checked with a solution that includes, at a minimum, methamidophos, acephate, and monocrotophos. Response to acephate may disappear in systems that contain too much glass wool, and response to methamidophos may not be seen if it elutes with the solvent front or if column packing is of poor quality; both these situations can be avoided by monitoring the system with routine injection of an appropriate mixed standard. Frequency of injection of mixed standard, at least twice during an 8-hr period, should be specified in the quality assurance plan.

For best quantitative results, reference standards should be dissolved in the same solvent that is used for the final sample extract. In addition, reference standards should be injected within minutes of the sample containing the residue(s) to be quantitated, and responses to residue and standard should match within ±25% for accurate quantitation.

References

[1] *Standard Practice for Gas Chromatography Terms and Relationships*, ASTM E 355-77, reapproved 1983, ASTM, Philadelphia, PA

[2] Jennings, W. (1987) *Analytical Gas Chromatography*, Academic Press, Orlando, FL

[3] Burke, J.A., and Giuffrida, L.A. (1964) *J. Assoc. Off. Agric. Chem.* 47, 326-342

502: COLUMNS

502 A: INTRODUCTION

Separations among analytes in GLC are achieved within the column. Although choice of detector dictates which class of analytes can be determined, individual detection and measurement of multiple analytes would not be possible without the separations provided by the column.

Columns are available in several different physical configurations, each of which offers advantages and disadvantages to pesticide residue determination. The two basic types of GLC columns currently used in pesticide residue determination are (1) packed columns, in which liquid phase is immobilized as a film on particles of fine mesh solid support and packed into 2-4 mm id columns, and (2) open tubular capillary columns, in which liquid phase is immobilized as a film on the interior walls of a capillary tube. Capillary columns are further distinguished by internal diameter: wide bore (0.53 mm id), traditional (0.25-0.32 mm id), and narrow bore (≤0.25 mm id). Each type of column requires unique hardware and operating parameters.

In all GLC columns, identity of the liquid (stationary) phase is the primary factor dictating what separations are achievable. Carrier gas (mobile phase) is also integral to GLC operation and must be included in any discussion of columns. However, only inert gases are used as carrier gases, so few options exist. Operating parameters that affect column efficiency, including column temperature and carrier gas identity and flow rate, provide additional variables that can be adjusted to achieve separations required for the analysis.

Column Specifications

Descriptions of GLC columns and operating conditions must specify the following: type of column (packed or capillary); its length, in meters (or feet), and internal diameter (id), in mm; identity and amount of liquid phase; identity of solid support, including pretreatments and mesh size (packed columns only); operating temperature; and carrier gas identity and flow rate.

Liquid phases used in GLC are viscous materials able to be thinly dispersed on solid support or on an internal column wall. Many different liquid phases are available, but relatively few are in routine use for pesticide residue determination, because pesticide residues usually either chromatograph on one of these phases or are not amenable to GLC. The chemical structure of the most common phases consists of a polysiloxane backbone with various substituent groups; Figure 502-a illustrates several of these.

Liquid phase polarity, important to its separation capabilities, varies with polarity and concentration of substituent group(s) on the polysiloxane. Thus, in terms of polarity, methyl<5% phenyl<cyanopropylphenyl<50% phenyl<cyanopropyl. The 100% methyl-substituted phase, least polar of those in Figure 502-a, is best suited to separation of nonpolar analytes; it has been used for many years as a general purpose phase for a wide variety of pesticide residues. The phase with 50% cyanopropylphenyl-substitution is the most polar of those shown and is a better choice for more polar analytes.

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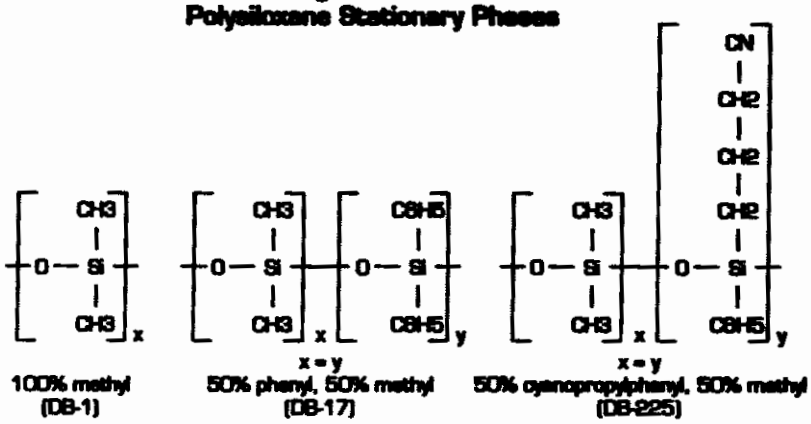
Figure 502-a
Polysiloxane Stationary Phases



Equivalent products suitable for different column configurations are commercially available for most common liquid phases; Table 502-a lists some of these products.

Although the table refers to liquid phases themselves, most pesticide residue laboratories no longer purchase liquid phases as materials for preparing columns in-house. Instead, laboratories that use packed columns usually purchase them prepacked or at least purchase packing material precoated with liquid phase. Residue laboratories always purchase commercially prepared capillary columns.

The liquid phase for a particular analysis is selected to take advantage of differences in chemical and physical properties of the analytes involved. No one liquid phase is universally applicable to the wide range of chemical and physical properties found in pesticide residues, so a variety of liquid phases of different polarities should be available in a residue laboratory.

For packed columns, the amount of liquid phase, often called "liquid load," is described as a percentage, *i.e.*, weight liquid phase × 100/(weight liquid phase + weight solid support). For open tubular columns, the amount of liquid phase is described as film thickness (µm) of the layer of liquid phase bonded to the internal wall of the column.

GLC columns are always heated to a temperature at which analytes remain in the vapor phase. Both isothermal and temperature-programmed operation are possible. Use of capillary columns with temperature programming is becoming increasingly common, but this operation will not be described further in this chapter because FDA has not yet validated its use on an interlaboratory basis. Maximum operating temperatures vary with specific stationary phases; information on each is provided by the manufacturer. Increasingly polar stationary phases (*e.g.*, cyanopropylphenyl) have significantly lower maximum operating temperatures than nonpolar phases (*e.g.*, 100% methyl). In use, maximum operating temperature is usually 20° C higher for temperature programming than for isothermal work.

Column Parameters

The following column characteristics or parameters are commonly used to describe chromatographic behavior or to measure column performance; terminology of these parameters is illustrated in Figure 502-b. Evaluation and comparison

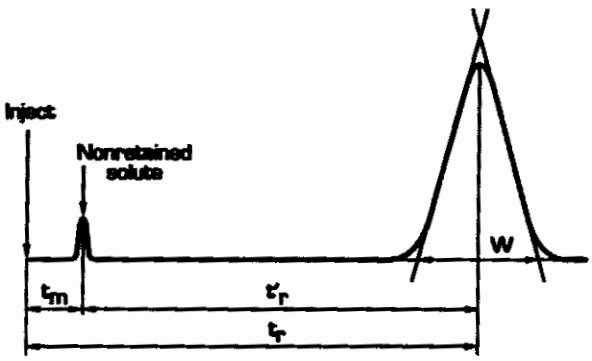
Table 502-a: Common GLC Liquid Phases Used in Pesticide Residue Determination

Equivalent Commercially Available Products[1]

Basic Structure, Substitutions	Capillary Open Tubular	Packed
Polysiloxane, 100% methyl	DB-1 (ht), HP-1, HP-101, 007-1 (MS), SP-2100 SPB-1, BP-1, CP-Sil 5CB, Ultra 1, RSL-150, RSL-160, Rtx-1, SP-2100, CB-1, OV-1, PE-1, SE-30, AT-1	OV-101, OV-1, SP-2100, DC 200, CP-Sil 5, SE-30
Polysiloxane, 50% phenyl, 50% methyl	DB-17 (ht), HP-17, PE-17, 007-17(MPS-50), AT-50, SP-2250, Rtx-50, RSL-300	OV-17, OV-11, SP-2250, OV-22, DC-710
Polysiloxane, 50% cyanopropyl-phenyl, 50% methyl	DB-225, HP-225, OV-225, SP-2330, CP-Sil 43CB, RSL-500, Rtx-225, BP-225, CB-225, PE-225, 007-225, AT-225	OV-225
Polysiloxane, 14% cyanopropyl-phenyl, 86% methyl	DB-1701, SPB-7, CP-Sil 19CB, Rtx-1701, BP-10, CB-1701, OV-1701, PE-1701, 007-1701	OV-1701
Polysiloxane, 5% phenyl, 95% methyl	DB-5 (ht), HP-5, Ultra-2, OV-5, SPB-5, Rtx-5, CP-Sil 8CB, RSL-2000, BP-5, CB-5, PE-5, SE-52, 007-2(MPS-5), SE-54	OV-3, OV-73, CP-Sil 8
Polysiloxane, 50% trifluoro-propyl, 50% methyl	DB-210, RSL-400, SP-2401	OV-210, SP-2401, OV-202, OV-215
Polyethylene glycol	DB-WAX, HP-20M, Carbowax, Supelcowax 10, CP-WAX 52CB, SUPEROX II, Stabilwax, BP-20, CB-WAX, PE-CW	Carbowax 20M, Supelcowax 10
Diethylene glycol succinate	No equivalent	DEGS (no longer produced)

[1] Commercial codes for each material are related to their manufacturer:
- 007: Quadrex, New Haven, CT
- AT, RSL, SUPEROX: Alltech Associates, Inc., Deerfield, IL
- BP: SGE, Inc., Austin, TX
- Carbowax: Union Carbide Corp.
- CB, CP-Sil, CP-WAX: Chrompak International BV, Middleburg, The Netherlands
- DC: Dow Corning Corp., Midland, MI
- DB: J&W Scientific, Folsom, CA
- DEGS: Analabs, Inc., New Haven, CT
- HP and Ultra: Hewlett-Packard, Co., Wilmington, DE
- OV: Ohio Valley Specialty Chemical Co., Marietta, OH
- PE: Perkin Elmer Corp., Norwalk, CT
- Rtx, Stabilwax: Restek Corp., Bellefonte, PA
- SE: General Electric
- SP, SPB, and Supelcowax: Supelco, Inc., Bellefonte, PA

Figure 502-b
GLC Column Parameters



Column parameters are calculated from measurements on a chromatogram produced by the column.

of columns can be based on such parameters. More detailed discussion of parameters and conditions affecting each are found in any basic chromatography text, such as those listed in Section 505, Bibliography.

Retention Time. The most basic measurement in chromatography is retention time, the time between sample introduction and elution of the analyte, measured at the peak maximum (t_r in Figure 502-b). Retention time is corrected for the time required for a nonretained solute to reach the detector (tm), often called dead time or holdup time. Corrected retention time (t'_r) is the difference between t_r and t_m. For practical convenience, the peak caused by the solvent is used as the nonretained solute in pesticide residue determinations.

Analyte retention time depends on the extent to which the analyte is retained by the particular stationary phase under a given set of conditions. Retention time is constant when column temperature and carrier gas flow are constant, so this characteristic is the GLC measurement that serves to identify the analyte; it can be measured electronically in seconds or manually in mm from the resulting chromatogram. Retention time measured from injection to peak maximum is often called "absolute retention time."

Absolute retention time is affected by many column conditions that can vary, including amount of liquid phase, temperature, carrier gas flow rate, column length, and system volume. Thus, absolute retention times are insufficiently reproducible to list in tables of data intended to assist an analyst in identifying analytes. Instead, "relative retention times" are calculated and listed, because they are far more reproducible from day to day and among different instruments or laboratories.

Relative retention time (rrt) of an analyte is the ratio of its corrected retention time (t'_r) to the corrected retention time of a "marker" (reference) compound. The pesticide chlorpyrifos, molecular formula $C_9H_{11}C_{13}NO_3PS$, is used in this manual as the marker compound for most systems, because it chromatographs well and contains all the heteroatoms to which selective GLC detectors respond; retention times relative to chlorpyrifos (rrt_c) for many pesticides and related compounds are listed in Appendix I, PESTDATA.

For the same (or equivalent) liquid phase, rrt of an analyte is independent of column type (packed *vs* capillary), liquid load, column length, or carrier gas flow rate change. The rrts for a particular liquid phase vary significantly only with column temperature; rrt_cs in Appendix I are valid only at the temperature specified for each column.

Capacity Factor. Capacity factor describes the retentive behavior of a sample component relative to the "retentive behavior" of a nonretained component. The

capacity factor of an analyte depends only on the time the analyte spends in the stationary phase, which is, chromatographically speaking, far more important than the time spent in the mobile phase. The capacity factor (k) of an analyte is calculated from analyte retention time as $k = (t_r - t_m)/t_m$.

(Capacity factor should not be confused with "sample capacity," which describes the maximum amount (*e.g.*, 50 ng) of an analyte that can be injected onto a chromatograph before column overload occurs. Column sample capacity depends on percent liquid load in packed columns and on column id and film thickness in capillary columns.)

Selectivity. Stationary phase selectivity is simply defined as the ability of a phase to differentiate between analytes in the same injection. The selectivity term is technically not interchangeable with polarity [1]. A polar column may exhibit very poor selectivity for a particular chemical species. In general, nonpolar stationary phases exhibit greatest selectivity for nonpolar analytes, and polar stationary phases exhibit greatest selectivity for polar analytes. Selectivity of a GLC system is defined by both the stationary phase and the analytes. In the literature, selectivity (α) is synonymous with separation factor, relative retention, and selectivity factor and is calculated as k_B/k_A, where k_B and k_A are capacity factors of two adjacent peaks. In this calculation, a is always ≥ 1.0, but a separation factor of 1.0 indicates that no separation is possible in that system [2].

Resolution. Resolution is the degree of separation between two chromatographic peaks and is related to time (capacity factor), selectivity, and efficiency. Considerable information about resolution and its related parameters is available in general textbooks on chromatography. For practical purposes, however, it is enough to know that optimizing selectivity by choice of stationary phase will optimize resolution. Despite the importance of column efficiency in analyzing complex samples, especially at low levels, increasing efficiency will not solve all separation problems and often will only increase analysis time. A different choice of stationary phase may solve a resolution problem more easily than a longer column will. Resolution is considered optimized when calculated k values range between 2-10.

Efficiency. In qualitative terms, column efficiency refers to the degree to which injected analyte is able to travel through the column in a narrow band. Visually, a more efficient column produces narrower, sharper peaks on the chromatogram. The more efficient the column, the better able it is to resolve analytes that elute close to one another. Greater efficiency results in greater signal-to-noise ratio and hence increases sensitivity. Efficiency is measured quantitatively by calculating theoretical plates according to the formula

$$N = 16\ (RT/w)^2$$

where N = total theoretical plates, RT = absolute retention time in mm, and w = width of peak base in mm, measured as the distance at the baseline between lines drawn tangent to the two sides of the peak. The analyte on which theoretical plates are calculated must be specified, because comparisons are only valid for analytes eluting at the same absolute retention time. Column efficiency can also be expressed as height equivalent of one theoretical plate (HETP), *i.e.*, column length (cm)/N; using this expression, smaller numbers represent more efficient columns. Calculation of theoretical plates/column length permits comparisons of different length columns.

Basic GLC texts, such as those listed in Section 505, provide additional explanations about theoretical plate measurements, qualitative effect of column efficiency on peak shape, and practical means of improving peak shape. Column efficiency is referred to in this chapter when discussing relative advantages of different types of columns.

502 B: PACKED COLUMNS

During most of the over 30 years of GLC use in pesticide residue determination, packed column GLC prevailed as the only practical option. During early development of open tubular capillary columns, when only traditional capillaries were available, packed columns offered distinct advantages in ease of use and capacity for injection of larger volumes of extract. Current availability of wide bore capillary columns has reversed the trend, however, and use of packed columns is diminishing.

Packed columns still offer advantages in ease of installation; no additional inlet adapters or other specialized hardware are needed to install packed columns into chromatographs designed for packed column operation. Packed columns can also still withstand repeated injections of extract better than capillary columns. However, recent improvements in inlet systems and operating parameters for wide bore columns have increased their capacity for injected extract. Combined with the innately greater efficiency and inertness of wide bore columns, these improvements are encouraging the shift from packed to wide bore columns for routine use.

Components of Packed Columns

Packed columns consist of packing material made by coating inert solid support with a thin film of stationary liquid phase, glass or metal tubing to contain the packing material, and silanized glass wool plugs used to hold the packing material in place within the tubing.

Solid Support. The solid support in packed GLC columns provides a large inert surface onto which the stationary liquid phase is deposited as a relatively uniform thin film. Solid support should provide as large a surface area as possible and should interact as little as possible with analytes. Desirable properties of solid supports are large surface area per unit volume, chemical inertness at high temperatures, mechanical strength, thermal stability, ability to be wetted uniformly by a stationary liquid phase, and ability to hold a liquid phase strongly.

The most frequently used solid supports for GLC column packings are derived from diatomaceous earth. The structure of the diatomaceous earth consists essentially of three-dimensional lattices containing silicon with active hydroxyl and oxide groups on the surface. Untreated diatomaceous earth has considerable surface activity that must be reduced before it becomes a suitable support material. Several techniques have been used to deactivate the surface activity of diatomaceous earth. Most frequently, the diatomaceous earth is acid-washed and then silanized with an agent such as dimethyldichlorosilane.

Different commercially available solid supports and even different lots of the same support may have different surface areas or variations in inertness toward particular analytes. Unpredictable behavior among solid supports provided the impetus

for most laboratories to purchase precoated packing. Variations in solid support activity are of greatest concern when determining pesticide residues that are difficult to chromatograph, because such analytes are easily adsorbed or degraded during chromatography. Adsorption or degradation of an analyte on a poor quality solid support can affect the relative retention time of the analyte and the size and shape of the resulting peak. The most inert solid support material available should always be used to prepare column packings.

Chromatographic solid supports are available in a variety of mesh sizes. A support material of 80/100 mesh contains particles that will pass through an 80-mesh screen but not through a 100-mesh screen. Experiments have shown that column efficiency improves as solid support mesh number increases (particle size decreases) [3]. However, to maintain the same gas flow through a column, carrier gas pressure must be increased as solid support particle size decreases. Mesh size of 100/120 was shown to produce optimum efficiency for a 6' column of 4 mm id. Columns 4-6' long and 2-4 mm id, filled with column packings prepared from 80/100 or 100/120 mesh solid supports, are routinely used for residue determination.

Liquid Phase Load. No matter what liquid stationary phase is used, liquid load influences column efficiency and capacity (amount of sample extract that can be injected onto the column). Packing materials with loads ranging from <1 to 5% are routinely used for pesticide residue determination.

Liquid phase load can be varied without changing relative retention times of compounds if the same column temperature is used. At the same column temperature and gas flow, a column with less liquid phase will allow compounds to elute more quickly than a higher load column. Carrier gas flow can be lowered when using columns with less liquid phase to permit compounds to elute at approximately the same time as from higher load columns operated at higher gas flows.

Laboratory observations indicate that compounds with a tendency to degrade on or be adsorbed by a column are more likely to do so when a lower liquid phase load is used, probably because the lower load is incapable of covering all solid support active sites. In these cases, analyte retention time and peak size will be affected, as described above. Residue analysts should be aware of the pitfalls of low load columns when dealing with compounds that are easily degraded or adsorbed.

Column Tubing. Almost all columns used in pesticide residue determinations are made from glass tubing. Although some gas chromatographs require metal columns, so many problems occur with metal that they should be avoided. In the past, new glass columns had to be cleaned and silanized in the laboratory to remove any residual caustic materials and to deactivate the column. Today, most glass columns are silanized by the manufacturer and are purchased ready to use. Inadequate deactivation of glass columns can cause peak tailing due to adsorption or degradation of the sample or standard on the active sites of the column itself.

Glass Wool. Glass wool for use in GLC columns must be silanized to prevent compound adsorption; presilanized glass wool is available commercially or silanization can be performed by the laboratory. A plug of silanized glass wool is always used at the outlet (detector) end of a packed column to hold the packing material in place. Glass wool can also be used in the inlet end of a packed column, but opinions vary about the advisability of this practice.

Used in the inlet end of a packed column, glass wool can cause adsorption or degradation of certain sensitive compounds. Problems with normally stable compounds can also occur when deposits of sample co-extractives collect on the glass wool.

In particular, when deposits of fatty extracts accumulate at the top of the column, analytes in subsequent injections can be partially trapped; errors in residue quantitation result. Elimination of glass wool at the inlet end of the column appears to minimize this problem by allowing injected co-extractives to spread over a portion of the column where subsequent analytes cannot be trapped so readily.

In other cases, glass wool in the inlet end of the column may prevent the rapid deterioration of columns caused by injecting co-extractives from fatty foods or other commodities that are difficult to clean up. Co-extractives trapped on the glass wool plug can be eliminated by replacing the plug, an easier, quicker, and less expensive process than replacing the packing material.

Choosing whether to use glass wool in the inlet end of the column appears to depend on several factors, including type of packing material used, commodity being analyzed, analytes of interest, type of detector, and method of analysis. Experience will dictate when the advantages of glass wool in the column inlet outweigh the disadvantages; a laboratory attempting to locate the source of problems in a GLC determination should definitely investigate the effects of glass wool in the column inlet.

Preparation of Packed Columns

Acceptable techniques for packing empty GLC columns are designed to fill the column with as much packing material as possible (*i.e.*, to pack the material as tightly as possible) while breaking the fewest particles. Column efficiency increases with the amount of properly coated support in the column, and adsorption and degradation problems are minimized when careful handling of the packing material creates the fewest broken (active) sites.

Poor packing technique causes visible differences in column performance (efficiency) and peak symmetry. Loosely packed columns or columns containing too little column packing are inefficient and a cause of inadequate separations. On the other hand, a column packed too tightly requires excessive carrier gas pressure, which can result in the column becoming plugged with broken particles.

To pack a glass column:

- Insert about 1-2" silanized glass wool into detector end of column, far enough from end to prevent packing material from extending into detector base where temperatures are usually much higher than column operating temperature.

- Use rubber tubing to connect detector end of column to vacuum source (aspirator or vacuum pump); attach funnel with short piece of rubber tubing to inlet end of column.

- Apply partial vacuum at detector end of column, and slowly add prepared packing material through funnel.

- Tap column gently while adding packing material, to settle it as tightly as possible; do not use a vibrator to help settle packing.
- Continue to tap gently along entire length of column while adding more packing, until column is full or within 1" of being filled at inlet end.

To reuse a glass column:

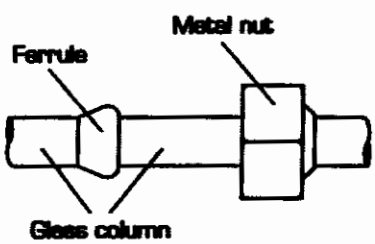
- Remove old packing.
- Rinse empty column successively with 5% potassium hydroxide/methanol and dilute hydrochloric acid.
- Rinse empty column thoroughly with successive portions water, alcohol, and ethyl acetate to eliminate accumulations of liquid phase and/or sample co-extractives from column walls.
- Dry empty column before repacking.

Installation of Packed Columns

In modern GLC equipment, glass columns, filled with packing material, are connected directly to the detector (metal) and injector (metal), an arrangement that eliminates dead space in the system. The availability of ferrules that are thermally stable at high temperatures makes these glass-to-metal connections possible and eliminates problems once associated with such connections. Ferrules with these capabilities include those made from Vespel, graphite, or Vespel/graphite mixture.

To install a glass column in the chromatograph:

Figure 502-c
Ferrules for Connecting Glass and Metal



[Reprinted with permission of Supelco, Inc., adapted from Supelex M-1 Packed Column Ferrules data sheet (1987).]

- Slide stainless steel or brass (usually 1/4" Swagelok) nuts onto detector and inlet ends of column followed by ferrule, as shown in Figure 502-c.
- Connect nuts on column to corresponding hardware on injector and detector.
- Tighten each nut finger tight.
- To seal ferrules, alternately tighten detector nut and injector nut using standard wrench, following instructions provided by ferrule manufacturer. Unevenly exerted pressure on either end of column may twist and break it.
- Turn on carrier gas (30-60 mL/min) and flush with gas for about 20 min.
- After all oxygen has been flushed from column by flow of carrier gas (and not before), turn on column oven to heat column.

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- Check connections for leaks after establishing carrier gas flow.

During installation, always hold the bottom of the column for support. Do not overtighten the nuts, which can force the column against the bottom of the injector/detector and break the column. Refer to the instruction manuals provided by the instrument manufacturer for more specific instructions on column installation.

Conditioning of Packed Columns

Column "bleed" is degradation of stationary liquid phase that causes a background signal as the detector responds to its presence. Column bleed occurs in all columns and is not in itself a symptom of damage. However, excessive or increasing bleed, seen as a rise in baseline, may be caused by damage to the column. Bleed increases when the column is operated at higher temperatures, and damage may be caused by operation at temperatures higher than allowed for a particular stationary phase.

To minimize column bleed, newly packed columns must be conditioned before they are connected to the detector; conditioning purges volatile components that could contaminate the detector and produce an unsteady baseline. Column conditioning involves heating the column above normal operating temperatures for an extended period prior to its use. The column must not be connected to the detector during conditioning. In most cases, a normal carrier gas flow is maintained during column conditioning. Excessively high conditioning temperatures will shorten column life.

Minimizing column bleed by conditioning is essential to good operation. If liquid phase is bleeding from the column, frequent detector cleaning will be necessary, sensitivity of the GLC system will change, quantitative results will probably be affected, baselines will drift, and good quality chromatograms will not be obtained.

"Stabilized" liquid phases are designed to be more thermally stable than their nonstabilized equivalents, because they bleed less at normal operating conditions. However, conditioning of stabilized packings is still required before use.

Conditioning procedures vary with the type of column packing and are provided by the manufacturer in the literature supplied with the packing.

Rejuvenation of Packed Columns

Column deterioration during use is most often caused by inadequate cleanup of samples injected onto the column (see Section 501 C). Extracts of materials containing large amounts of fats or oils (*e.g.*, dairy products, animal tissue, and fish oils) are difficult to clean up thoroughly. Injection of excessive amounts of oily extract can cause irreversible damage to a GLC column. Waxy or colored material co-extracted from nonfatty foods may also damage the GLC column, but this effect is not as readily apparent as that caused by oily co-extractives. Care should be taken to minimize the amount of any co-extractive material injected, including the use of additional or alternative cleanup techniques when original cleanup is inadequate.

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No matter how rigorous the sample cleanup, some accumulation of co-extractives on the column will occur. To prevent column deterioration, the column must be periodically cleaned. Packed columns are most often cleaned by removing up to several inches of packing at the inlet end of the column and replacing it with new (preferably conditioned) packing. To perform this operation:

- Turn off column oven heat and permit oven to cool.
- When oven is cool, turn off carrier gas and remove column.
- Remove contaminated packing with disposable pipet or other device, and swab inside of glass column with acetone using pipe cleaner or other appropriate device to remove fatty deposits or other matrix contamination that have adhered to interior column wall.
- Add fresh packing to column, in same way described above for packing new columns.

Criteria for Acceptable Packed Columns

Column performance must meet the following criteria for successful pesticide residue determination. Exact performance will vary somewhat as the column ages, but minimum criteria should be met through its lifetime; when the column no longer meets these standards, it should be replaced.

Some of these criteria relate to careful column preparation and conditioning and are important to check when the column is new. Others relate to the potential for gradual column deterioration and contamination during use. Some other part of the GLC system may be responsible for the system's failure to meet criteria, so all parts should be examined when the system is malfunctioning.

1) Chromatography of selected compounds should result in a single symmetrical peak with no breakdown. Endrin frequently chromatographs as two or three peaks when columns are not satisfactory, and methoxychlor breaks down to its olefin. DDT deteriorates to TDE or DDE or may be lost entirely on a contaminated column. None of these conditions should be tolerated.

2) Peak resolution of selected compounds should be complete. For example, dieldrin and endrin can be separated from one another on most columns that are performing well; a mixture of the two should be chromatographed routinely to monitor changes in resolution as the column ages.

3) Peak heights for several compounds should be reproducible when repetitive injections are made. Poor reproducibility (≥5%) can have several causes external to the column: improper injection technique, a faulty syringe, a faulty septum, or detector malfunction. Poor reproducibility can also indicate breakdown or adsorption of the compound on the column. Compounds used to test the column for general acceptability are those that may break down or be adsorbed by columns but can be successfully chromatographed, such as endrin. When a column is used to analyze for compounds that are hard to chromatograph, it should first be checked with a compound such as endrin.

Sometimes, injection of large concentrations of compounds that are difficult to chromatograph may improve their chromatography. Some pesticide chemicals may not chromatograph well until a column is more thoroughly conditioned by prolonged use. If a compound shows tailing, or little or no response, or if multiple peaks are obtained from injection of a single standard of known purity, adsorption, degradation, or some other column effect may be the cause. Chromatography of some compounds may not be satisfactory until the column has been used extensively.

4) Instrument response to varying amounts of a compound should be linear. A nonlinear response can have many causes, but breakdown or adsorption of the compound on the column may be indicated when the system is linear for some compounds but not for others. It is especially important to ascertain linearity for each compound of interest when the compounds are difficult to chromatograph.

5) A 4 mm id packed column should have about 500 theoretical plates/foot of column length, as measured on a peak produced by p,p′-DDT. (Retention time of the peak used affects theoretical plate calculation, so measurement of the p,p′-DDT peak at whatever time it elutes from an individual column is an admitted oversimplification, but is adequate for the purpose defined here.)

   Theoretical plate counts <500 do not necessarily render a column unacceptable, but performance of columns with <400 plates/foot should be closely observed. Routine measurement of theoretical plates will alert the analyst to unsatisfactory new columns or to deterioration of columns already in use and is recommended as a part of the routine check on instrument performance.

Recommended Operating Procedures for Packed Columns

Each GLC determinative step in Chapters 3 and 4 is described in terms of its specifications and operating conditions. Most of these describe wide bore capillary columns, now recommended for routine use in pesticide residue determination; only Sections 302 DG20-DG23 describe systems with packed columns, because the DEGS column of those modules has no wide bore equivalent. However, most GLC data (rrts and responses) included in Appendix I, PESTDATA, were developed with packed columns during the many years in which they were in use. Table 502-b provides operating conditions for packed columns useful in pesticide residue determination.

Column liquid phase and temperature are dictated by the analytes being sought in a particular method. Choice of carrier gas depends on the requirements of the detector; in some cases, argon/methane is used to accommodate the ^{63}Ni electron capture detector. Carrier gas flow rate is typically 30-60 mL/min. Injection volume is typically 3-8 µL.

Columns must be protected from damage that can occur when the stationary phase is exposed to oxygen at high temperature. Increased bleed of degradation products from oxidation will occur, and the phase can be damaged permanently. After any exposure to air, *e.g.*, during septum change, the column should be

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Table 502-b: Operating Conditions for Packed Columns[1]

	OV-101	OV-17	OV-225
Liquid load, %	5	3	3
Injector temperature, ° C	225	220	225
Target rrt_c[2] (marker compound)	3.1 ± 0.06 (p,p′-DDT) 2.56 ± 0.05 (ethion)	3.5 ± 0.07 (p,p′-DDT) 3.36 ± 0.07 (ethion)	3.6 ± 0.06 (p,p′-DDT) 3.9 ± 0.1 (ethion) 0.69 ± 0.02 (lindane)
Elution time, min[3]	4	4	5.5
Conditioning[4]	1° C/min to 250° 250° C, ≥16 hr	1° C/min to 250° 250° C, ≥72 hr	50° C; 2° C/min to 245° 245° C, ≥60 hr
Special requirements			Do not use with ElCD or N/P

[1] All columns are: 1.8 m × 2 or 4 mm id; liquid phase coated on 80/100 mesh Chromosorb W HP, or equivalent.
[2] Column temperature is 200° C, adjusted as needed to produce specified rrt_c for marker compound.
[3] Approximate elution time of chlorpyrifos with carrier gas (nitrogen, helium, or argon/methane, as required by detector) at about 60 mL/min.
[4] Conditioning performed with column disconnected from detector. Degas with 60 mL/min nitrogen for 1 hr; temperature program as specified; hold at specified temperature with nitrogen flowing for specified time period.

checked for leaks and then flushed with carrier gas for 15-20 min before restoring the column to operating temperature.

If it is necessary to change carrier gas tanks while the column remains at operating temperature, interruption of column carrier gas flow can be avoided by turning off secondary valve pressure, which is usually at 40-80 psi. While the gas flow continues bleeding into the column, the main tank valve can be turned off and the regulator moved to a new tank.

502 C: OPEN TUBULAR CAPILLARY COLUMNS

Capillary column GLC has existed almost as long as packed column GLC and is now preferred for determining pesticide residues in foods. Capillary columns provide greater inertness, chemical and thermal stability, column efficiency (and thus system sensitivity), resolution, operating temperature range, and column-to-column reproducibility than equivalent packed columns.

In addition to the nature of the liquid phase, many factors affect capillary column performance and applicability, including bore size, film thickness, operating temperature, column length, and carrier gas identity and flow rate. Most often, a change in one column parameter improves some features of column performance and diminishes others, so choice of column for a particular analysis is based on an assessment of the most important feature(s).

Capillary columns are available with internal diameters ranging from 0.050-0.53 mm. Efficiency increases as capillary column bore size decreases. Traditional (0.25-0.32 mm id) and narrow bore (<0.25 mm id) capillary columns are noted for extraordinary efficiency (≥5000 theoretical plates/m), which improves signal-to-noise ratio and thus sensitivity. High efficiency also provides the improved resolution necessary for analyses of complex samples. However, sample capacity decreases with decreasing bore size, and columns become less forgiving of improper handling. In addition, the low carrier gas flow rates used (≤0.9 mL/min for narrow bore and ≤3 mL/min for traditional) require specialized flow control hardware.

For certain determinations, advantages offered by narrow bore columns outweigh their disadvantages. Thin film, narrow bore capillary columns are ideal for specialized "ultra trace" determinations at levels of part per trillion and below, *e.g.*, for determination of dioxin residues. Once adjustments are made to accommodate requirements of narrow bore columns related to gas flow, sample capacity, and injection technique, they provide the ultimate efficiency, resolution, and sensitivity needed for these determinations.

The low gas flows required with narrow bore capillary columns also make them the best choice for use in certain instruments. For example, interfacing narrow bore columns directly to mass spectrometers has become an industry standard, because the low flow is compatible with the requirements imposed by vacuum conditions within the spectrometer (≤1 mL/min maximum flow). Use of narrow bore columns obviates the need to divert carrier gas before effluent reaches the spectrometer.

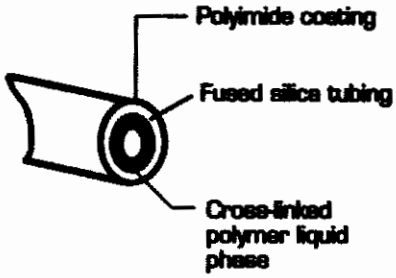
In contrast, either wide bore (0.53 mm id) or traditional capillary columns are preferred for routine pesticide residue determination, with wide bore the most popular. Although wide bore columns are less efficient than narrow bore, they offer greater sample capacity; depending on the film thickness, wide bore columns may have sample capacities comparable to packed columns. Carrier gas flow of ≤6 mL/min is recommended for optimum efficiency, but if this results in excessively long analysis time, the larger internal diameter of a wide bore column can accommodate 20-30 mL/min without generating excessive column head pressure. Wide bore columns can be operated at these higher gas flows ("packed column conditions") without the specialized pneumatics required for low flow rates. Performance of wide bore columns can be optimized by changes in carrier gas flow rates and other system parameters, such as injection technique.

Column Description

Early open tubular capillary columns were made from glass, with liquid (stationary) phase coating the interior wall. These columns were fragile and subject to significant liquid phase bleed. The columns assumed the shape of the "cage" on which they were mounted and thus required straightening before inserting the ends in inlets or detectors. A high degree of operator skill was necessary for their use.

The disadvantages of capillary columns were minimized or eliminated when several features were vastly improved. Columns are now made from fused silica, a synthetic quartz, coated on the outside with polyimide, which makes them rugged, flexible, and easy to handle. Stationary phases are now cross-linked polymers bonded to the interior column wall, effectively eliminating column bleed.

Figure 502-d
Capillary Column Cross-Section



Drawing (not to scale) of GLC capillary columns; id of fused silica tubing ranges from 0.05-0.53 mm.

Figure 502-d shows a cross-sectional view of a typical modern open tubular capillary column.

Tubing for capillary columns is produced by drawing fused silica through a furnace. The exterior of the drawn capillary tubing is then coated with a plastic polyimide coating and the interior cleaned and deactivated. Exact processes used by manufacturers are proprietary and beyond the scope of this chapter. The resultant tubing is very flexible, rugged, and reasonably inert and requires only minimal care in handling. It is easily cut and may be coiled around cages and flexed as necessary for instrument connections. When released, the tubing straightens, simplifying connections. Capillary columns are available in lengths from 10-60 m; 15 m or 30 m columns are usually used for determination of pesticide residues in foods.

Stationary phases are no longer simply coated on the interior walls. Individual stationary phase polymer "strands" are cross-linked, and the cross-linked stationary phase is covalently bonded to the deactivated interior wall of the tubing by proprietary processes. Columns prepared in this manner are more thermally stable than coated phases, so they can be operated at higher temperatures; they are also more efficient. Cross-linked phases exhibit minimal bleed and resist being stripped by solvent, to the degree that they can be rinsed with solvent to remove nonvolatile contaminants. The process of cross-linking also facilitates preparation of thicker films (*i.e.*, 1.0-8.0 µm) that are otherwise difficult to prepare. Chemically, the stationary phases are equivalent to those coated on solid support for packed column use, so relative retention times for analytes are essentially the same in equivalent packed and capillary columns, as long as column temperature is the same [4].

Capillary columns are available with films ranging from 0.10-5.0 µm thick. Columns with <0.32 mm id usually have film thickness of 0.10-1.0 µm, while those of ≥0.32 mm id have films 0.1-≥5.0 µm. Film thickness is proportional to sample (analyte) capacity, *i.e.*, thicker films accommodate more analyte without overload. Theoretically, a 0.53 mm id column has a sample capacity of 53, 130, 530, and 2600 ng for film thicknesses of 0.1, 0.2, 1.0, and 5.0 µm, respectively; sample capacity for a 0.25 mm id capillary column is about half as much for each film thickness [2].

Column efficiency, however, is inversely proportional to film thickness. Thick film columns are also more retentive than thin film columns, so retention times are longer and analyte peaks broader on the former. Thick film columns are also more susceptible to column bleed.

Because polar stationary phases (*e.g.*, cyanopropylphenyl) are difficult to coat onto column walls, they are usually only available in film thicknesses up to 1.0 µm. Polar stationary phases tend to bleed more than their nonpolar counterparts even under ideal conditions.

Film thickness for columns used in pesticide determination is normally 1.0 or 1.5 µm, which provides optimum balance between phase thermal stability, analyte

retention, and analyte column capacity. Relationships between film thickness and column efficiency, thermal stability, analyte retention, and capacity are discussed in detail in most modern GLC books (Section 505).

Each stationary phase has upper and lower temperature limits that define the operating range, but only the upper limit is of concern in pesticide determination. Operation at temperatures exceeding the upper limit accelerates phase degradation. Heating a column without carrier gas flow, or exposing it to any oxygen at temperatures ≥100° C, even for short periods, can damage phases rapidly and irreversibly.

Injection onto Capillary Columns

The small internal diameter of all capillary columns imposes specific requirements on how injection is performed; the narrower the diameter, the more rigid the requirements. (These injection options should not be confused with injection techniques discussed in Section 501 D. That section covers choices between manual and automatic injection and among various techniques for handling syringes. This section refers to ways to accommodate injection and vaporization of solvent into the restricted space available in capillary columns.)

Extensive research into means of introducing solutions onto capillary columns has produced four major injection techniques, called split, splitless, on-column, and direct. Each has advantages and disadvantages, and each has found uses in particular GLC applications. FDA studies, however, support recommendations that pesticide residue GLC determinations be performed with direct injection, using a retention gap, onto wide bore capillary columns [5]. This system eliminates or minimizes problems such as band broadening, peak splitting, and intolerance to variable injection volumes [6-9]. Direct injection involves introduction of the sample into a hot, vaporizing inlet with total transfer (no splitting) of injected materials onto the analytical column. GLC inlets designed for packed columns are easily converted to use with direct injection; kits for this purpose are commercially available. Injection volumes of 0.5-6.0 µL are used with direct injection.

Direct injection is not suitable for use with narrow bore columns or low gas flows, so references such as those in Section 505 should be studied for further information on the other injection techniques not covered here.

Capillary Column Systems

The practical necessities of residue determination require that a minimum weight of sample equivalent be examined by the determinative step. When capillary column GLC is used for determination, provision must be made to ensure that the volume of extract needed for injection of this weight does not overwhelm the capacity of the column. The following arrangements are required to accommodate physical limitations imposed by capillary columns. Because requirements become more stringent as internal diameter decreases, different recommendations may apply to wide bore and traditional capillary columns.

Retention Gaps (Guard Columns). Use of a "retention gap" [10] is recommended for capillary column GLC used in pesticide residue determination. A retention gap is a segment of deactivated fused silica tubing (without stationary phase) that is placed between the instrument inlet and the top of the capillary column; in effect,

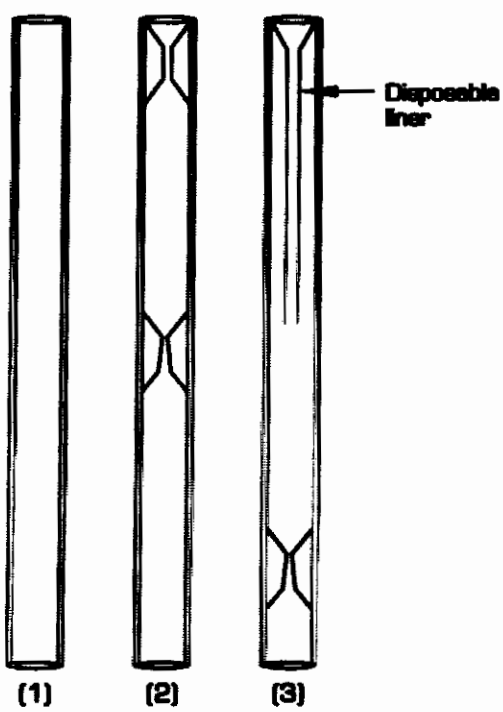
it serves as an extension of the column inlet. Tubing 0.53 mm id and 1-5 m long is commonly used; a length of 5 m is recommended for pesticide residue determination.

A retention gap serves at least two purposes: (1) It provides space for the injected solution to vaporize and expand, thus permitting injection of solvent volumes (>1 µL) that could not otherwise be injected into capillary tubing; and (2) it provides surface area for deposition of co-extractives, thereby protecting the analytical column from buildup of nonvolatiles that can cause loss of efficiency and analyte decomposition or adsorption; in this role, the retention gap is often called a "guard column." Properly installed, a retention gap will not noticeably reduce column efficiency.

Inlet Adapters. Direct injection of extracts and standard solutions onto capillary GLC columns requires a glass adapter to minimize analyte contact with hot metal surfaces. Adapter design has gradually evolved to meet the practical needs of trace level determinations.

Figure 502-e shows three styles of inlet adapters evaluated for use with direct injection. Adapter 1, the straight tube adapter, is simplest. A capillary column or retention gap is inserted into the bottom of this adapter with a stainless steel reducing union. This adapter, containing a small glass wool plug, was successfully used with various wide bore columns to determine pesticide residues in foods analyzed by the method described in Section 302 [11]. However, this style adapter is not recommended, because it allows exposure of analytes to the hot metal reducing union. In addition, injection of large volumes can result in flashback of solvent vapors and analytes into the instrument pneumatic systems.

Figure 502-e
Inlet Adapters for Capillary Columns



Inlet adapter used with capillary columns: (1) straight, (2) adapter with restrictors, and (3) adapter with disposable liner.

Adapters designed with tapered restrictors at the point where the column or retention gap connects are preferable to straight tube adapters; this design eliminates contact of analytes with the hot metal reducing union. Adapter 2 displays a commercially available direct flash injection liner [12] with a nontapered restrictor at the top and a tapered restrictor below for connection to a column. The top restrictor minimizes both flashback during injection and contact of analytes and solvent with the septum area of the inlet. This adapter was successfully used with extracts from Section 302 [13], and its performance was validated with an interlaboratory trial involving similar extracts cleaned up with Florisil [14]. The only drawback with this adapter is difficulty in cleaning.

A major improvement in inlet adapters is the addition of an easily replaced disposable liner. Originally, an adapter intended for use with on-column injection (not pictured in Figure 502-e) was inverted and modified to include a disposable Supelco PureCol™ inlet liner, a small amount of column packing, and a small glass wool plug for determination of organochlorine pesticides in fatty foods [15]. Subsequently, during application to analysis of nonfatty foods, the column packing material was found to be unnecessary. Successful application of this adapter-liner combination led to commercial production of Adapter 3, designed specifically for use with a replaceable liner, as shown.

Use of a liner protects the inlet adapter, because nonvolatile co-extractives deposit on the liner rather than the adapter. A contaminated liner is easily replaced without disturbing the connection between the adapter and the column; depending on instrument design, the liner is changed after removing the septum or after removing the adapter-liner combination from the GLC inlet.

Chromatographic efficiency using any of these adapters will deteriorate with repeated injections of food extracts. Efficiency can be restored by removing the adapter from the instrument, cleaning, and resilanizing. After resilanizing, the adapter (without a column attached) should be heated overnight to normal inlet temperature with 10 mL/min gas flow to remove excess silanizing reagent.

Septa. In GLC, injections are made by microliter syringes through septa made of materials that permit passage of a needle and then reseal after the needle is withdrawn. For troubleshooting purposes, chromatographers must be aware of the problems that can be caused by septa. Each septum has a limited useful life, after which it leaks and must be replaced. Leaking septa cause inaccuracies in quantitation, problems with chromatography, and exposure of the system to air. Materials from which septa are made can contribute to system bleed and/or can become brittle with use. Shards from damaged septa can also pass into wide bore columns and block gas flow.

Connections. Any adapter installed in the GLC inlet is sealed by means of a nut and high temperature ferrule. Ferrules are available in various sizes, shapes, and materials; GLC instrument manufacturers specify requirements for ferrules to be used in each instrument. Typically, ferrules of 100% graphite are used, though ferrules consisting of graphite and Vespel are also common and sometimes required (*e.g.*, graphite/Vespel is used in GC-MS because graphite ferrules out-gas).

Analytical columns are connected to retention gaps with "low dead volume" or "zero dead volume" butt connectors. Various styles are available from chromatographic supply companies, including ferrule, adhesive, and "press-in" types. The simplest and least expensive are the press-in types, in which each tube is pushed into opposite ends of a flared connector to form a seal. Press-in connectors are suitable for most applications and are ideal for connecting 0.53 mm retention gaps to smaller diameter analytical columns. The "universal" style of press-in connectors, *i.e.*, those that connect tubing of any sizes, have been found to work best.

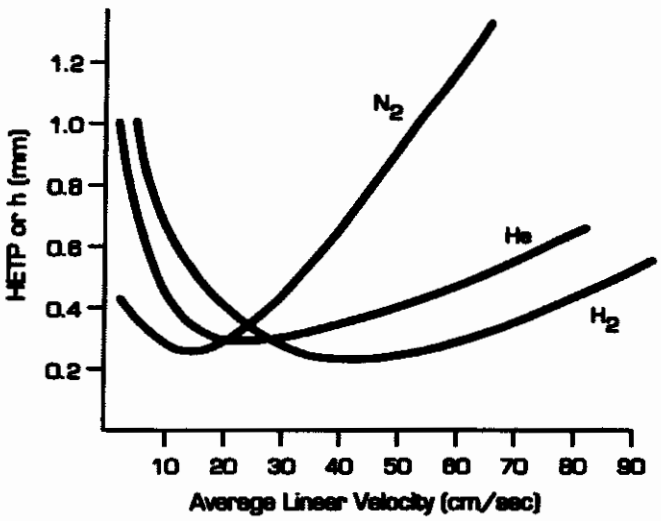
The two most critical connections in capillary GLC are those that connect the analytical column to the inlet and to the detector. Connections not only must be leak-free, but positioning is critical to optimum performance. Most manufacturers of gas chromatographs provide detailed instructions for positioning the column outlet at a specific location in detectors. These instructions must be followed exactly. If the manufacturer's instructions for proper positioning of capillary

column ends are not available, optimum positioning must be determined experimentally.

Inlet column positioning is also critical but is simplified with inlet adapters or liners that have tapered glass restrictors for column seating. The restriction is positioned at the proper location in the inlet, and the column is inserted firmly to form a seal between the polyimide coating and tapered fitting. Adapters or liners with no tapered glass fitting require careful, precise measurements for proper column positioning. Manufacturer's instructions for positioning tubing in inlet adapters or liners must be followed exactly.

Carrier Gases. Hydrogen and helium are the carrier gases of choice with capillary columns, because their flow rates can be increased with less loss of efficiency than is seen with nitrogen. The van Deemter curves for nitrogen, helium, and hydrogen (Figure 502-f) display the effect on column efficiency (HETP) of increasing average linear velocity (cm/sec, calculated as column length in cm/retention time in sec, of an unretained peak) in a typical capillary column.

Figure 502-f
Van Deemter Curves



Effect of carrier gas flow rate on column efficiency for several gases, measured using 30 m x 0.25 mm id column, 0.25 mm film thickness.

Minimum HETP (*i.e.*, maximum efficiency) for nitrogen carrier gas occurs at very low linear velocity (flow rate) and over a narrow range. Any increase of flow causes a substantial decrease in column efficiency. Chromatography at a flow rate required for usable column efficiency results in unacceptably long analysis time.

Compared to nitrogen, van Deemter curves for helium and hydrogen show greatest efficiency at higher flow rates. Use of these gases at their optimum flow reduces analyte elution time. The much shallower curves for these gases also demonstrate that increasing the flow above optimum to further reduce analysis time results in acceptable losses of efficiency. For these reasons, helium and hydrogen are commonly used as carrier gases for most capillary GLC applications.

Two different modes of operation, differentiated by carrier gas flow rate, are possible with wide bore capillary columns. Maximum column efficiency is achieved in "capillary column mode," *i.e.*, with carrier gas flows ≤6 mL/min. However, at these low flow rates, chromatographic time is considerably longer than the time to which pesticide analysts are accustomed with packed columns.

Operation of wide bore columns in "packed column mode," *i.e.*, 10-25 mL/min carrier gas flow, at 200° C (isothermal) combines the advantages of packed columns' faster elution with open tubular columns' greater deactivation (fewer active sites). In this mode, column efficiency equals or exceeds that of equivalent packed columns. At the same time, polar pesticides that require polar liquid phases (*e.g.*, DEGS) for packed column chromatography can be successfully chromatographed on capillary columns, even with nonpolar liquid phases [13, 14]. Because analyte relative retention times vary only with stationary phase and column temperature, extensive data (Appendix I, PESTDATA) compiled over the years for packed columns may be used for tentative identification of residues found with capillary columns operated at the same temperature [4]. Directions for operation of a wide bore capillary column in packed column mode, validated by interlaboratory study [5], are presented below.

Carrier gas should be of the highest possible purity, because use of highest purity gas will extend capillary column life. Moisture and oxygen traps should be used for all gases.

Makeup Gases. Because modern GLC detectors are designed for optimum performance at gas flows greater than those preferred for capillary columns, some systems require additional "makeup gas" to be added before effluent enters the detector. In addition to providing proper flow rate for optimum detector performance, makeup gas efficiently sweeps analytes from the end of the column into the detector. A gas different from the carrier gas may be used if the detector requires a specific moderating gas, such as argon/methane or nitrogen for an electron capture detector.

Makeup gas used for detector moderation should be of the purity level recommended by the manufacturer. As with carrier gases, moisture and oxygen traps should be used for all gases.

Installation and Conditioning of Capillary Columns

Regardless of the connection being made, proper cutting of the column and retention gap is critical. Square, clean cuts minimize flow disturbances and allow tubing of the same or different diameters to connect smoothly. Cleaving tools for cutting polyimide-coated fused silica capillary tubing are available from most chromatography supply companies. After scribing the polyimide coating with a cleaving tool, the tubing is cut by applying gentle pressure to bend the column opposite where it was scribed. If properly scribed, the column will break cleanly at that point. New cuts should be examined with a 10-20X magnifier to ensure that the cut is square and clean with no ragged edges or chips in the polyimide coating. Tubing should be recut if necessary to achieve a proper finish. All cuts should be made after installing any ferrules (especially graphite) onto the tubing to eliminate the possibility of small shaved ferrule particles being deposited in the end of the tubing.

Two techniques can be used to facilitate marking critical measurements for proper column positioning with either detectors or inlets. Water-soluble typewriter correction fluid can be used to mark columns at the desired length; the fluid does not react with the polyimide coating and is easily removed. Alternatively, a small slice of septum pushed onto the column can act as a marker and simultaneously hold the nut and ferrule in place for easier maneuvering in the oven.

After the inlet, column, retention gap, and detector have been connected, carrier gas flow should be established and all connections thoroughly checked for leaks. Every connection should be treated as the source of a potential leak, so connections should be minimized. Manufacturer's instructions must always be followed carefully to obtain leak-free connections. Soap solutions must never be used to detect leaks, because they can be aspirated into the system; the resulting contamination with nonvolatile materials can be removed only by rinsing the contaminated area. Electronic leak detectors are preferred. All leaks should be eliminated prior to heating the column.

Capillary columns with cross-linked, bonded stationary phases do not require the extensive conditioning of packed columns, because the stationary phases are more stable than those in packed columns and less susceptible to bleed. Usually, purging the column thoroughly with carrier gas, then heating it to 20-30° C above the maximum operating temperature for 1 hr is sufficient to condition the column. Conditioning is performed with carrier gas flowing; the detector may be connected during conditioning of capillary columns. It is critical that the upper temperature limit for the column not be exceeded.

Rejuvenation of Capillary Columns

With time and use, nonvolatile residues accumulate in all capillary columns, regardless of the use of retention gaps or other protective measures. Efforts to improve deteriorated chromatography should always begin with removal of portions of a contaminated retention gap or replacement of the retention gap. If the analytical column is also contaminated and replacement of the retention gap is insufficient to improve chromatography, a portion of the inlet end of the analytical column can be removed by cutting the column as previously described. If a capillary column ≥5 m is used, removal of a relatively short segment does not significantly affect its overall length or behavior, even if segments are removed repeatedly.

When removal of a contaminated segment of column is insufficient to restore appropriate chromatography, the column can be rinsed with solvents to help remove accumulated residues; rinsing may be performed with the retention gap attached. Column rinsing is possible because of the stability of the cross-linked, bonded phase.

Kits for rinsing columns are commercially available. Most kits consist of a vial that serves as a solvent reservoir; the vial has fittings for insertion of the column and for connection to a gas supply that pressurizes the solvent. The detector end of the column is inserted into the vial containing the rinse solvent, and gas pressure forces solvent backward through the column. The column should be rinsed with a sequence of solvents in order of decreasing polarity, starting with water and ending with hexane. Each solvent should be miscible with the preceding one. After the column is rinsed and excess liquid purged with gas flow, the column is re-installed and purged with carrier gas at ambient temperature for 30-60 min. The column should be conditioned by heating to 20° C above operating temperature for at least 15 min, but the upper temperature limit for the column must not be exceeded.

Recommended Operating Procedure for Wide Bore Columns (Isothermal)

The following procedure describes the setup and operation of a wide bore capillary column in packed column mode. Both direct injection adapters (below) were successfully validated [5, 14], and either may be used. Option 1 is recommended, because the disposable liner can be easily replaced once it has become contaminated with injected co-extractives.

Apparatus and Reagents.

column, fused silica capillary column, bonded with one of the substituted polysiloxane phases (Table 502-a), 30 m long × 0.53 mm id, 1.0 or 1.5 µm film thickness

retention gap, deactivated fused silica tubing, 5 m long × 0.53 mm id

capillary column connectors, low dead volume or zero dead volume, suitable for connecting analytical column to retention gap

direct injection adapter. Use new adapter as is without silanizing. Resilanize used and cleaned adapters with 10% dimethyldichlorosilane/toluene; after resilanization, heat to 240° C overnight with gas flow before use. Optional adapters are:

1) Silanized direct injection adapter (Figure 502-e, Adapter 3), 1/4" od, 4 mm id (Cat. No. 210-1071, J&W Scientific, Folsom, CA). This adapter is specially made to FDA specifications and is available with the special order part number. Direct injection adapter is 220 mm total length. Inlet end, 125-130 mm long measured from top of restrictor, has notches. Column oven end descends 75 mm below flared end of tapered restrictor. Column oven end may be shortened if desired; if cut, leave at least 20 mm tubing to attach adapter/column reducing union and lightly fire polish cut end. With Option 1 only: column inlet liner, PureCol™ for 4 mm id columns (Cat. No. 2-0540M, Supelco, Inc., Bellefonte, PA, or equivalent)

2) Double restrictor adapter, 1/4" od (Figure 502-e, Adapter 2). Adapter has two restrictors. Top restrictor allows passage of syringe needle into chamber formed by two restrictors. Lower restrictor is tapered for connection of column end into adapter. Silanize adapter prior to use if not silanized by manufacturer or if adapter has been cleaned. These adapters may be purchased cut to specified length or as longer version to be cut by user.

pesticide grade silanized glass wool; see Section 204 for silanization in laboratory; must be free of nitrogen, chlorine, phosphorus, or sulfur contaminants

capillary installation kit, required if chromatograph was designed for packed column. Kit should include necessary fittings to attach retention gap to inlet adapter and, if necessary, to detector, with provision for makeup gas at detector connection.

Gow-Mac Leak Detector, available from chromatography suppliers

Magnifier, 20X

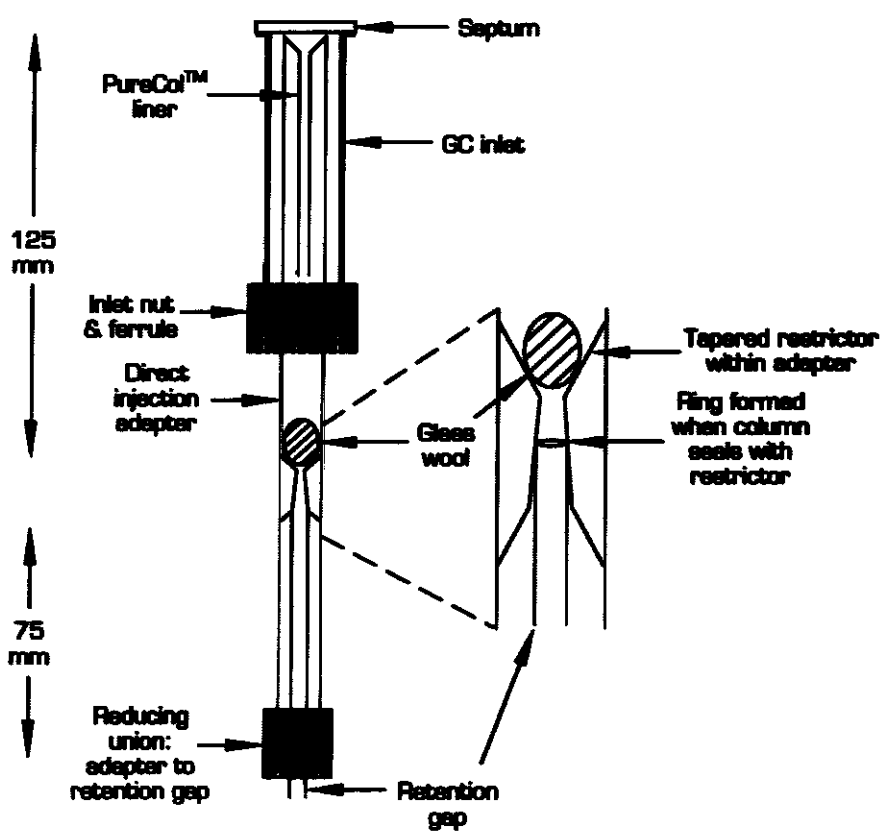
typewriter correction fluid, water based (if needed to mark correct positions on capillary tubing)

capillary column cleaving tool

Instrument Setup. Connect apparatus according to following directions; review instrument manufacturer's instructions and adjust directions as necessary to accommodate specific equipment. Figure 502-g shows the Option 1 inlet system arrangement:

- Place ferrules and nuts on end(s) of retention gap and column.
- Cut ends of column and retention gap with capillary cleaving tool. It is imperative that ends be cut *after* placement of ferrules to eliminate possibility of ferrule fragments becoming settled in tubing. Examine new cuts with 20X magnifier to assure that ends are square and smooth. Recut as necessary to obtain smooth, square ends.

Figure 502-g
Capillary Column Inlet System



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- Clean ends of column and retention gap with wiper wetted with methanol.
- Attach retention gap to column, using appropriate capillary column connector.
- Secure retention gap to column cage.
- If using injection adapter (1), place small plug of glass wool in inlet end and push down as far as restrictor, then place disposable PureCol™ liner in inlet end of adapter. If using injection adapter (2), use as is without additional equipment.
- Gently insert adapter into instrument inlet or fitting until it touches top of injector. Move it back about 1-2 mm and tighten nut. Exercise care not to fracture end of adapter or liner when tightening nuts and ferrules during installation; overtightening can cause leaks and deform expensive hardware. If considerable effort is necessary to tighten fitting, it may already be deformed and should be replaced. (Follow manufacturer's instructions on how to tighten ferrules, check for leaks with leak detector, then tighten in small increments, *e.g.*, 1/4 turns, using correct size wrench to obtain leak-free connection.)
- Attach reducing union (for connecting adapter to retention gap) to column oven end of adapter.
- Insert end of retention gap through reducing union and into adapter. Push retention gap firmly into flared portion of restrictor until seal is formed between polyimide coating of retention gap and adapter restrictor wall. Formation of seal is evidenced by visible "ring" at contact point between tubing and restrictor wall (Figure 502-g, enlarged area). Tighten column nut on reducing union.
- If necessary, install makeup gas fitting to detector inlet using appropriate length and diameter of silanized narrow bore (1 mm id) glass tubing. Install column into detector as directed by detector manufacturer's instructions (if available) for positioning column. See section on connections, above, for additional cautions about effects of column positioning.
- Use helium carrier gas to get best column efficiency and compatibility with various detectors. For 30 m × 0.53 mm id columns, set initial flow to 20 mL/min.
- Use makeup gas as needed to accommodate optimum detector operation. Nitrogen, helium, argon/methane, or other gases may be used, as required for proper detector operation. Adjust flow of makeup gas so that total flow of carrier and makeup gases equals optimum gas flow recommended by detector manufacturer. Makeup gas flows of 5-40 mL/min are typical.

System Startup and Inspection.

- After installation and establishment of initial carrier and makeup gas flows, check all connections and fittings for leaks with electronic leak detector. Do *not* heat system until it is leak-free and has been thoroughly purged with carrier gas for 15-20 min to avoid damage from oxygen.

- Heat column to 230° C for 1 hr or until detector baseline has stabilized. Reduce temperature to 200° C and recheck both carrier and makeup gas flow rates. Adjust carrier gas flow and column temperature as necessary to meet retention time and rrt_c requirements for specific system being used. Re-adjust makeup flow as necessary to maintain optimum detector flow.

- Evaluate system for linearity, repeatability, and tolerance for varying injection volumes. System should be linear over at least one full scale deflection on integrator/recorder. For accurate quantitation, responses to repetitive injections of standard reference material should have percent relative standard deviation ≤5%. Limitations on injection volumes must be determined for each system. Experience has shown that injection volumes of 1.0-6.0 µL are normally tolerated without adverse affects on analyte response.

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503: DETECTORS

503 A: INTRODUCTION

GLC detectors are devices that indicate the presence of eluted components in the carrier gas emerging from the column. Depending on the way in which they measure the quantity of the analytes, detectors are classified as differential concentration, differential mass, or integral [1]. The electronic equipment associated with the detector amplifies the signal and causes the response to be recorded.

Definitions of Detector Characteristics

Characteristics of detector operation that are critical to qualitative and quantitative determination of residues include sensitivity, selectivity, and linearity. Certain terminology is common to the discussions of these characteristics in different detectors.

Sensitivity. Detector sensitivity refers to the relationship between amount of analyte injected and response of the detector. Detector response is the change in measured detector signal that results from a change in amount of analyte present within the detector volume; measured detector signal includes the amplification provided by associated electronics. Sensitivity is often described by referring to the smallest amount of a specific analyte that causes a measurable detector signal.

FDA methodology has traditionally specified detector sensitivity in terms of ng of a specified compound that causes 50% full scale deflection (FSD) on a recording or data system. That convention is continued in this chapter and in the determinative step descriptions in Chapter 3 methods.

Selectivity. Detectors must be selective to be suitable for use in determining any trace residue, including pesticides. Selectivity refers to the detector's preferential response to one or more elements ("heteroatoms") or functional groups that might be present in analytes of interest. Response of the detector to these moieties must far exceed its response to carbon, hydrogen, and oxygen if the resulting chromatogram is to distinguish between residues and food co-extractives present in the same extract.

Nonselective detectors, such as flame ionization (FID) and thermal conductivity (TC), respond to solutes in proportion to the mass of each that elutes from the column. Such detectors are impractical for most residue determinations.

Among detectors that are suitable for residue determination, selectivity to the moiety of interest varies considerably. Probably no detector is completely "specific" to one heteroatom or functional group; instead, degrees of selectivity can be described in terms of the relative response of the detector to the same weight of different compounds or moieties.

In practical terms, the greater the detector selectivity, the less sample cleanup is needed (within the boundaries discussed in Section 501 C) and the greater the inherent degree of confirmation that is provided by the determination. Conversely, the less selective the detector to the type of analyte being detected, the greater the precaution needed in preparing samples, avoiding reagent contamination, and confirming residue identity.

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Some relatively selective detectors are subject to interferences from co-extractives that contain the heteroatom to which they respond, in particular those detectors that respond to the presence of nitrogen or sulfur. Sulfur-selective detectors are subject to considerable interference from organic disulfides present in foods such as onions, rutabagas, and brassicas. Use of cleanup steps to remove or react the interfering co-extractives may also cause the analyte(s) to be lost. Nitrogen-containing compounds are extractable from all foods; for this reason, no detector that selectively responds to elemental nitrogen can ever be totally successful in analysis of foods for trace-level contaminants. Several HPLC determinative steps have been developed for particular nitrogen-containing functional groups (Sections 401 and 403), and these have been more successful because interference from other forms of nitrogen is avoided.

Linearity. Use of a detector within its linear range is a prerequisite for the simplified way in which residues are routinely quantitated in pesticide residue determinations (Section 504). Terms associated with detector linearity are: dynamic (response) range, over which a change in the amount of chemical present within the detector volume produces a measurable change in detector response, and linear (response) range, the portion of the dynamic range over which a change in the amount of a chemical present within the detector volume produces a *proportional* change in detector response. A detector's linear range is the range of analyte weight over which the sensitivity of the detector is constant to ±5.0%, as determined from a linearity plot of response/weight *vs* log weight [2].

FDA laboratories evaluate the dynamic range of a detector and then operate in a segment of that range that exhibits appropriate linearity. For added assurance that quantitation is accurate, sufficient extract and reference standard solutions are injected to cause detector responses to residue and standard to agree within 25%.

503 B: ELECTRON CAPTURE DETECTOR

The electron capture (EC) detector has been used for many years to analyze foods for organohalogen pesticide residues. The earliest EC detector in common use had a ^{3}H (tritium) radioactive source; this was later replaced by detectors using a ^{63}Ni source. The continued popularity of the EC detector results from its high sensitivity to halogen and certain other moieties, as well as its ruggedness and low maintenance needs. Its sensitivity makes it applicable to determination of residues at the ppb and even ppt level, its wide dynamic response range facilitates its use with automatic data systems, and its high operating temperature (≤400° C) minimizes detector contamination by sample co-extractives and column bleed.

These advantages are offset by the EC detector's low selectivity compared to other detectors used in residue determination. When using the EC detector, appropriate methodology precautions are necessary to prevent introduction of interferences from food samples, reagents, or the environment (Section 501 C).

EC detectors used in FDA laboratories have ^{63}Ni sources and constant current, variable frequency design. Several manufacturers produce and market such detectors, all of which operate on the principles described below but vary in source activity, cell volume, and geometry. Most EC detectors currently in FDA laboratories are from Hewlett-Packard (HP), Wilmington, DE; Tremetrics, Inc. (formerly Tracor, Inc.), Austin, TX; or Varian Associates, Sunnyvale, CA. Discussions of detector characteristics in this section refer to detectors from these manufacturers.

Principles

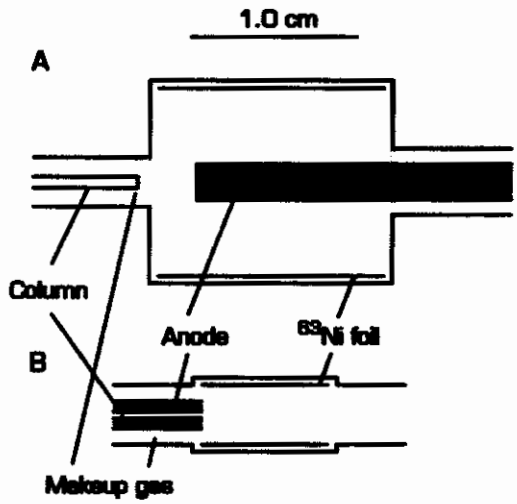
High energy beta particles, emitted by the ^{63}Ni source, collide with carrier gas molecules to produce low energy electrons. These electrons are continually collected at the cell anode by applying voltage pulses to the cell electrodes. Cell current thus produced is measured and the pulse interval (frequency) adjusted to maintain constant cell current. A standing pulse frequency describes the equilibrium condition that exists when only carrier gas is passing through the cell.

When molecules of an electrophilic substance enter the detector, electrons are "captured" to a degree dependent on the amount and electron affinity of the substance. As the electron supply is thus decreased, pulse frequency increases to generate the exact number of electrons necessary to maintain the established constant current. Change in frequency required to maintain constant cell current is converted to voltage, and this signal is sent to the recording device as the detector's response to the analyte.

Design

Two basic differences exist in EC cell design, one a pin-cup with ^{63}Ni plated on the cell wall and an anode suspended in the center of the cell cavity from the top, and the other with ^{63}Ni plated onto a cylinder aligned parallel to the column and cell flow. The electrode leads enter the cell cavity at right angles to the source and gas flow. Figure 503-a, diagrams A and B, display these respective designs.

Figure 503-a
Two EC Detector Designs



A, pin-cup EC, ^{63}Ni plated on cell wall; B, ^{63}Ni plated onto cylinder.

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Apparatus and Reagents

Section 501 B provides general information on apparatus and reagents required for GLC. Further materials or specifications for this detector are described below.

Radioactive Source: Special Requirements. The presence of radioactive material in EC detectors brings them under the authority of the Nuclear Regulatory Commission (NRC). The following special procedures must be followed by a laboratory with an EC detector:

1) Labeling. According to NRC regulations, each chromatograph containing a ^{63}Ni detector must have a label signifying the isotope, activity, and date at which the activity was determined and the words "Caution: Radioactive Material." The NRC will accept the manufacturer's label on the detector if it contains the necessary information. If this information is not present, stick-on labels must be applied to the gas chromatograph. Appropriate labels have been provided to FDA laboratories by FDA's

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Winchester Engineering and Analytical Center (WEAC), Winchester, MA. In addition, the Department of Health and Human Services (DHHS) requires that a sign with the following notice be posted on each gas chromatograph: "This equipment contains a radioactive source registered with the RSO (*i.e.*, the WEAC Radiation Safety Officer) as required by license from the NRC. Notify the RSO before removing the source from this location or upon any change in custodial responsibility" [3].

2) Venting. In addition to the labeling requirement, DHHS also requires that chromatographs with ^{63}Ni or other radioactive sources be vented through plastic tubing into a chemical hood or room exhaust [3].

3) Wipe tests. Any laboratory with a ^{63}Ni EC detector is required, as part of the licensing procedure, to perform a wipe test of all accessible exterior parts of the detector each January and July. WEAC supplies FDA laboratories with cotton-tipped swabs for performing the wipe tests. Each detector is wiped with an alcohol-moistened swab using moderate pressure, with particular emphasis on potential leak areas such as the outlet terminus and joints. Each swab is returned to WEAC in a mailing tube so that radiation removed from the detector exterior can be measured. A Certificate of Inspection for each detector is provided by WEAC and returned with new swabs.

4) Cleaning. NRC licenses in effect in FDA laboratories permit use of EC detectors containing ^{63}Ni but do not allow their dismantling and cleaning. All FDA ^{63}Ni EC detectors are shipped to the WEAC facility for cleaning [4].

Laboratories outside FDA must either make arrangements with a properly licensed laboratory for detector cleaning services or obtain the appropriate NRC license. Laboratories that have an existing license for use of ^{63}Ni EC detectors may be able to obtain from NRC an amendment that permits cleaning. Proof that the laboratory is capable of handling such materials safely is required before NRC will grant such an amendment.

Carrier and Makeup Gas. EC detector manufacturers recommend the use of argon/methane (95+5 or 90+10) for greatest detector linear range; however, nitrogen is used satisfactorily in some FDA laboratories. An external switch on the chromatograph permits selection of pulse width and cell current to accommodate whichever gas is predominant upon reaching the detector. Only reliable, high purity grade gas should be used, with oxygen and moisture traps on all gases going to the detector.

Often, the carrier gas and flow rate chosen for optimum column efficiency do not result in the best detector operation. Most EC detectors are configured to allow makeup gas to be added to the flow from the column so that detector operation is enhanced. In the most common example, argon/methane is essential to operation and is added to helium column carrier gas to produce optimum EC detector response and stability.

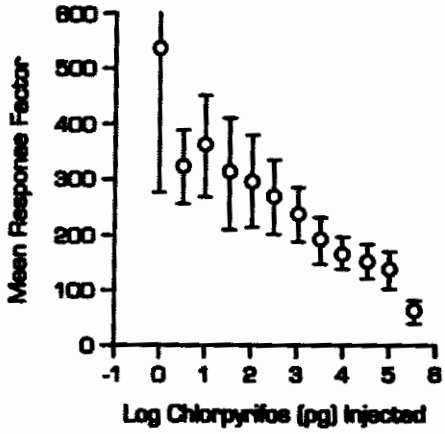
Detector Characteristics

The following basic characteristics of EC detectors must be understood for proper application to pesticide residue determination:

Sensitivity. Magnitude of response of a ^{63}Ni EC detector to a particular compound is dependent on the electron-capturing ability of the compound. Under conditions described below, the minimum detectable amount of an electron-capturing compound to which the detector will respond is typically in the 1-10 pg range.

Reproducibility of response at this low level is not as good as that for larger amounts of the same compound. A series of injections of 1-300,000 pg chlorpyrifos was made monthly over a 6-mon period [5]; variation in response factor over that time is shown in Figure 503-b. Standard deviations of response factors for each weight indicate that response stability was considerably better at levels ≥3 pg than at the 1 pg level, although response to 1 pg was reproducible over the course of any one day. Although this experiment was performed with an HP 5713A instrument, minimum response is expected to be equivalent with other ^{63}Ni constant current detectors.

Figure 503-b
Reproducibility



Mean and standard deviation of different weights of chlorpyrifos injected monthly (6 mon) into GLC with ^{63}Ni EC detector.

Selectivity. EC detectors respond to molecules containing an electrophoric group, *i.e.*, a highly polar moiety that provides an electron-deficient center in the molecule; examples include halogen, sulfur, phosphorus, and nitro- and α-dicarbonyl groups [2]. Because the response is not to a single element nor is it proportional to the amount of an element in a molecule, statements on detector selectivity can refer only to ranges or to relative responses to particular analytes.

Relative to its response to hydrocarbons, an EC detector may display 100-1000 fold greater response to mono- and disubstituted halogens and up to 10^6 times greater to polysubstituted halogens [6]. However, other molecules that contain only carbon, hydrogen, and oxygen may also be electrophoric, and EC detector response is far less selective to halogen relative to these molecules; examples include quinones, cyclooctatetracene, 3,17-diketosteroids, o-phthalates, and conjugated diketones [2].

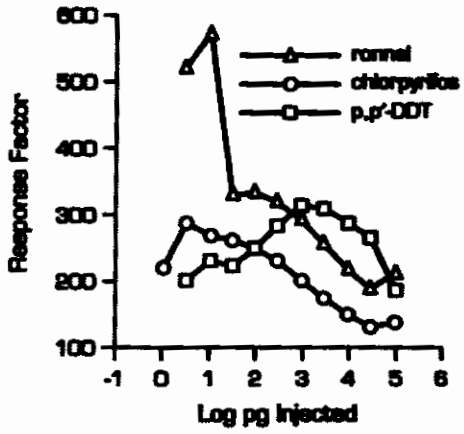
The relative lack of selectivity of the EC detector provides a bonus of applicability to a variety of analytes; *e.g.*, if an extract contains residues of pesticides containing halogen and also other residues containing sulfur, use of EC-GLC permits simultaneous determination of each. Lack of selectivity is more often a detriment to residue analysis, however; in practice, the EC detector's value is dependent on how free of interfering co-extractives the final extract is. Food co-extractives or environmental contaminants with electrophoric characteristics compromise the determination by causing responses that interfere with residues or that are mistakenly interpreted as residues.

Many examples of the interfering substances have been documented during long use of EC detectors. In addition to examples noted in Section 501 C, artifacts from plastics, rubber products, hand lotions, and cleaning solutions have been seen.

Certain fruits and vegetables also contain nonhalogenated substances that cause EC response, and these are not always removed by Florisil column cleanup (Section 303 C1, C2). Some of the sample types known to contain artifacts that cause an EC response include cabbage, radishes, and lettuce (6% Florisil eluate), carrots (15% Florisil eluate), and onions (both 6 and 15% eluates). Recommendations for avoiding interferences are included in the recommended operating procedures for EC, below.

Linearity. The mode of operation of the ^{63}Ni constant current detector produces a dynamic response range of greater than five orders of magnitude. Experiments in which an automatic data system was interfaced with the detector [5] showed that detector response to increasing quantities of injected material was still increasing when the data system became saturated. In order to plot the dynamic response over such a large range, the response (units in which response is measured/pg injected) is plotted *vs* log pg injected. A linear dynamic response range would produce a straight line parallel to the x-axis in such a plot. Plots of typical dynamic response ranges of the HP ^{63}Ni detector to three pesticides are shown in Figure 503-c.

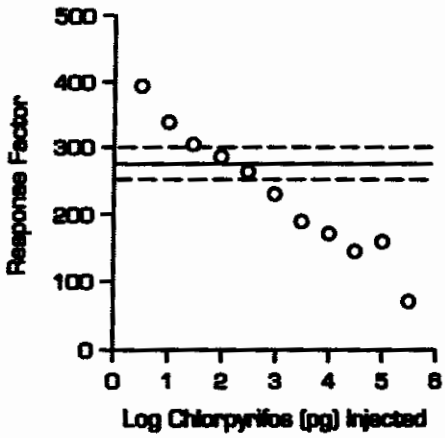
Figure 503-c
Dynamic Response Ranges



Response range of ^{63}Ni EC detector to three halogenated pesticides.

Instead of straight horizontal lines, the plots indicate a variation in response factor with amount of pesticide injected. These plots show that the detector is not linear over its entire dynamic response range. Within smaller segments of this range, however, the detector displays acceptable linear response.

Figure 503-d
Linear Response Range



Response range of ^{63}Ni EC detector is linear (±10%) to amounts of chlorpyrifos injected over a limited portion of the dynamic response range.

The range over which response is considered linear is dependent on the definition of linearity chosen. For example, in Figure 503-d the linear range is constant within ±10%; detector response is linear from approximately 30-500 pg chlorpyrifos. With this same definition, detector response can also be considered linear over other segments of the dynamic range. A change in definition (*i.e.*, different % variation permitted) would change the ranges for which detector response is considered linear.

The general rule that detector response to residue and reference standard should match within 25% is especially important when using the EC detector.

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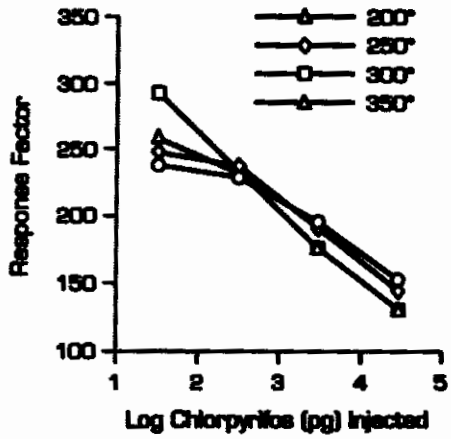
Other Influences on Detector Performance

The following parameters were studied for HP ^{63}Ni constant current detectors [5] and, in a more limited study, for a Tracor ^{63}Ni constant current detector [7].

Detector Temperature. Experiments with the HP ^{63}Ni constant current detector [5] documented its dynamic response range for seven pesticides at four detector temperatures; Figure 503-e displays results for chlorpyrifos. Detector temperature caused only slight changes in response to any particular amount of pesticide and caused no consistent change over the whole dynamic range. Thus, there is no reason to choose detector temperature on the basis of enhanced response.

Figure 503-e
Effect of Detector Temperature

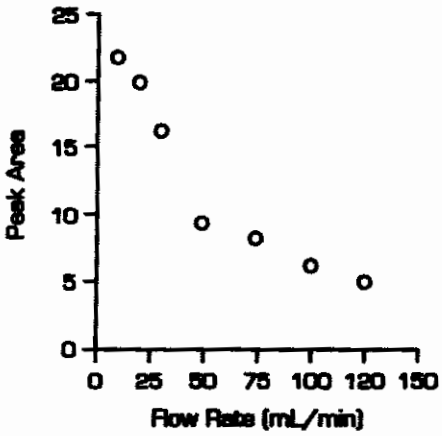


Dynamic response range of ^{63}Ni EC detector at four different temperatures.

Manufacturers provide recommendations for operating temperatures of detectors. Varian recommends operating the detector at 30° above column temperature, HP recommends 250-300° C, and Tracor recommends operation at 350° C. Detector contamination by materials eluting from the GLC column can be minimized with use of higher detector temperatures, but the ^{63}Ni radioactive foil must not be operated at >400° C.

Flow Rates. Column flow rate is usually chosen to optimize column efficiency and permit reasonable analysis time. The effect of flow rate on detector operation must also be considered, however, because response of the concentration-sensitive EC detector decreases with increased flow rate.

Figure 503-f
Effect of Carrier Gas Flow Rate



Response range of ^{63}Ni EC detector to 100 pg chlorpyrifos at different carrier gas flow rates.

Plots in Figure 503-f illustrate the effect of flow rate on response for the HP detector; a similar plot was obtained with the Tracor detector. In each case, the optimum flow rate range is that which provides for response stability, *i.e.*, flow at which a small change in rate does not cause a large change in response.

HP and Varian recommend a minimum flow rate of 30 mL/min through their detectors, and Tremetrics recommends 60-100 mL/min [6, 8, 9]. Each recommends use of makeup gas to bring the total flow through the detector to 60-100 mL/min. Makeup gas is usually needed for capillary columns (Section 502 C) and may be either the same as the carrier gas or different. For example, when hydrogen

or helium carrier gas is used to obtain maximum resolution and efficiency on the column, makeup gas of argon/methane or nitrogen must be used for proper detector operation.

Recommended Operating Procedures

The following steps should be taken for a new detector and after each time a clean detector is installed. (See above for special requirements for radioactive sources.)

- ^{63}Ni constant current detectors are usually delivered already installed in the chromatograph. To re-install after cleaning, follow manufacturer's directions for setting up the instrument and conditioning the column [6, 8, 9]. Ensure that all heaters, temperature sensors, and electrical connectors are properly positioned. Never connect a column to a cool detector.

- Heat detector until it reaches the maximum operating temperature recommended by the manufacturer, then attach column and equilibrate overnight at operating temperature and flow rate.

- Determine the instrument attenuation required to cause 40-80% FSD in response to 1.5 ng chlorpyrifos.

- Determine detector dynamic response range to chlorpyrifos and other standards of interest by plotting response factor (response/unit weight) *vs* weight injected on a semilogarithmic scale. Do not operate instrument outside the dynamic response range.

Earlier Tracor models allow for determining the pulse frequency profile and saturation current. To operate these models, refer to the instructions in the manufacturer's operation manual. Newer models have these parameters preset, so adjustments are not necessary or possible.

To minimize interferences that can occur during determination with EC detectors, follow these rules:

- Exercise extreme caution to avoid introduction of contaminants from reagents, apparatus, and environmental sources; routine inclusion of reagent blanks in laboratory quality assurance procedures will monitor success of these precautions.

- Employ suitable cleanup procedures for extracts that will be examined by EC detectors. Elution through Florisil is usually required before EC determination, though even this is not a guarantee that artifacts from foods will not cause response.

- Always confirm the identity of residues that have been tentatively identified by EC GLC; confirmation may include GLC with element-selective detectors or other techniques (Section 103).

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503 C: FLAME PHOTOMETRIC DETECTOR

The flame photometric detector (FPD) is the detector of choice for determination of organophosphorus residues and the only practical detector for organosulfur compounds. In its phosphorus-selective mode (FPD-P), the detector is one of the most element-selective GLC detectors available, although large amounts of sulfur will cause a response. The sulfur-selective mode (FPD-S) offers less selectivity (phosphorus can interfere) and is linear only on a semilogarithmic scale, but it provides useful confirmation for sulfur compounds tentatively identified by EC determination. Neither nitrogen nor chlorine cause any practical interference in either mode. In addition, the ratio of FPD-P and FPD-S responses can be used to calculate an analyte's P:S ratio for confirmatory purposes.

Methods designed for use with FPD determination sometimes include only minimal cleanup. However, column contamination can be caused by repeated injections of extracts from such methods, and the cautions outlined in Section 501 C must be observed.

The majority of FPD detectors in use in FDA laboratories were produced by three manufacturers: HP, Tremetrics (formerly Tracor), and Varian Associates. Discussion of detector characteristics in this section is limited to these models.

Principles

GLC column effluent is burned in a flame fed by a mixture of hydrogen and air. Characteristic optical emissions are produced when compounds containing phosphorus or sulfur are decomposed in the flame, and these emissions are viewed by a conventional photomultiplier tube through a narrow bandpass (interference) filter of appropriate wavelength. Choice of filter determines whether emissions produced by phosphorus or sulfur reach the photomultiplier tube. A filter with maximum transmittance at 526 nm, corresponding to the emission wavelength of HPO, permits detection of phosphorus compounds, while one with maximum transmittance at 394 nm, the emission wavelength of S_2, detects sulfur compounds. A single optical filter and photomultiplier tube may be used, or two filters and photomultiplier tubes can be assembled to permit response to both phosphorus and sulfur simultaneously.

Design

Figures 503-g and 503-h are diagrams of single and dual flame FPDs, respectively. In each, column effluent enters the detector from the bottom, where it is mixed with hydrogen gas. Air is added before the effluent and hydrogen emerge from the jet or at the same time they emerge. The emission from the resulting flame is measured by a photomultiplier tube after passing through the proper filter.

Figure 503-g
Single-Flame FPD

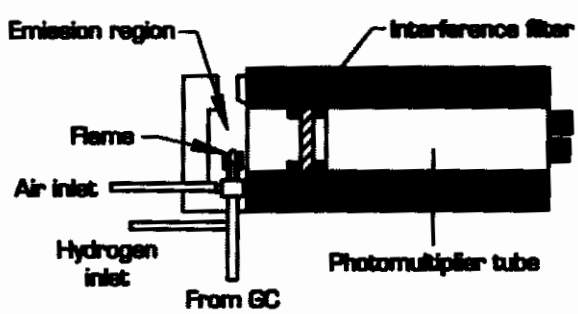

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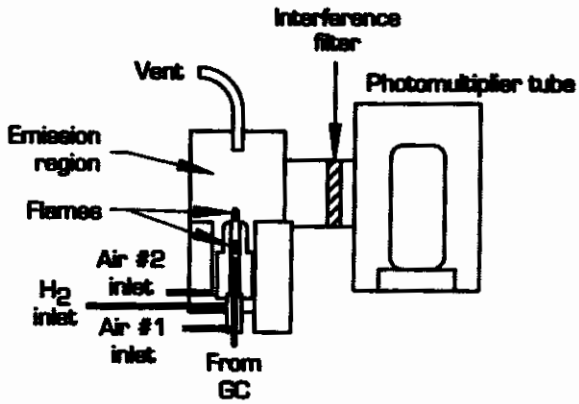
Figure 503-h
Dual-Flame FPD



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The Varian design differs from the others in that two air-hydrogen jets are used, "to separate the region of sample decomposition from the region of light emission" [10]. Sample decomposition occurs in the presence of air and hydrogen in the first combustion. Above that area, additional air is added to the combusted mixture; this supports a second flame, whose optical emission is filtered and measured as detector response.

Apparatus and Reagents

Section 501 B provides general information on apparatus and reagents required for GLC. Nitrogen or helium, of at least 99.998% purity, are usually used as carrier gases for columns connected to FPDs. Hydrogen and air are used as reactant gases in the detector flame. Manufacturers' recommendations for hydrogen purity vary from 99.995-99.999%. Air purity should be zero grade (maximum total hydrocarbon <2 ppm) or CGA Grade E [10-12].

Detector Characteristics

Sensitivity. The minimum amount of phosphorus detectable by the FPD-P is about 0.01 ng; for the FPD-S, about 0.04 ng sulfur. Detector sensitivity is greatly dependent on the condition of the photomultiplier tubes. Response varies among tubes, and the use of a variable voltage output with the power supply (or a variable voltage power supply) makes precise attainment of specific sensitivities easier to accomplish. Light leaking into a photomultiplier tube will increase the noise level and decrease the detector's effective sensitivity by making it less able to detect small amounts of analyte.

Response of the FPD-S (394 nm filter) to sulfur is proportional to the square of the concentration of sulfur. When the 526 nm filter is used (FPD-P mode), response to sulfur is also proportional to the square of the concentration. This relationship affects both selectivity ratios and linearity of the detector in both the P and S modes.

Selectivity. The response of the FPD-P detector is about 10^5 times greater to phosphorus than to carbon. The selectivity of phosphorus to sulfur in the FPD-P mode varies with the amount of sulfur present because of the square root relationship of response to sulfur concentration. For the Varian detector, P:S selectivity varies from $>10^4$ for very low concentrations of sulfur to about 50 for very high concentrations. Preliminary experience with the Varian detector indicates that it has a greater P:S selectivity than the other models. It is assumed that this increased selectivity is due to the stacked flame arrangement of this detector.

Selectivity of the FPD-S also varies with concentration of sulfur because of the square root relationship. The sulfur-to-carbon ratio varies from $>10^6$ at high sulfur concentration to 10^4 at low concentration. The S:P selectivity varies from $>10^4$ at high sulfur concentrations to 10 at low concentrations [10].

Selectivity of the FPD-S varies with the ability of the individual filter to screen out wavelengths associated with phosphorus. Experimentally, FPD-S detector response to a mixture of pesticides was compared using several different 394 nm filters. When conditions for each system were set to cause equal response to propargite (sulfur only), all filters permitted FPD-S response to methamidophos, chlorpyrifos, acephate, and omethoate (both phosphorus and sulfur), and most permitted response to monocrotophos (phosphorus only). Only one filter of those tested did not permit FPD-S response to monocrotophos; *i.e.*, the particular filter was far more selective to sulfur than the others. Further examination of the spectrum of light passed by the different 394 nm filters showed a distinct difference in the amount of absorbance at 526 nm; as expected, the filters that permitted FPD-S detection of monocrotophos passed much more 526 nm light than did the filter that did not detect it [13].

Linearity. Response of the FPD-P (526 nm filter) to phosphorus is linear over about four orders of magnitude.

Because of the square root relationship, response of the FPD-S to sulfur can be plotted as a straight line only if semilog paper is used. Most newer instruments provide a switch that automatically converts the detector output signal to its square root for an apparently linear response. However, quantitation using this converted signal is accurate only if the signal is carefully "zeroed," and the detector response is less sensitive at this setting. Many laboratories choose to measure the unconverted signal and plot response *vs* weight injected on semilog paper. Quantitation using the FPD-S is always less reliable than with other detectors.

Other Influences on Detector Performance

Detector Temperature. Each of the three manufacturers recommends a minimum detector operating temperature of 120° C. Recommended maxima range from 250° C (Tremetrics) to 350° C (Varian), with HP intermediate at 300° C.

Physical deterioration of parts of the detector can occur or be accelerated at higher temperatures. Both O rings and the casing for the photomultiplier tube have been seen to deteriorate at high temperatures. During routine operation, O rings should be changed periodically (about every 6-12 mon); all O rings in a particular detector should be changed at the same time. All manufacturers warn against continued operation at maximum temperature. Normal detector operating temperature should be about 20° C above that of the column, usually ≤250° C.

Gas Flow Rate. Optimum gas flow rate varies among detectors, and directions provided by the manufacturer of the specific detector should be followed. Additional experimentation may be required to optimize flow rates for any particular detector.

Detector Voltage. Satisfactory operation of the FPD requires use of a highly stabilized voltage power supply. Depending on the manufacturer, voltage may range from 350-850 V and may be obtained from either a variable or set voltage

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supply source. Detectors are usually shipped with the recommended voltage preset at the factory. Manufacturers' operations manuals give specific instructions for varying voltage when this is an option.

Sensitivity of the detector can be increased by increasing the voltage, but an upper limit is imposed by the simultaneously increasing noise. Optimum voltage can be determined by comparing detector response to an amount of compound as the voltage is varied.

Adequate Chromatography. Nonlinear response of FPD-P to oxygen analogs of organophosphorus pesticides (P=O compounds) is often noted. Because the detector (526 nm filter) is linear over a wide range for P=S compounds (*i.e.*, most parent organophosphorus pesticides), the difficulty is assumed to be caused by degradation of P=O compounds. Once attributed to a defect in detector design, this problem is now considered to be caused by poor chromatography of these polar compounds, and thus a column problem. Use of wide bore capillary columns (Section 502 C) minimizes the effect.

Recommended Operating Procedures

FPD-P. The following steps should be taken for detector operation:

- Install detector if necessary, according to instructions provided in manufacturer's manual [10-12]. FPD usually comes installed in chromatograph.
- Set detector temperature as recommended by manufacturer, at least 20° C above column temperature.
- Establish flow rate of column carrier gas as suitable for proper column operation (Section 502). Set flows of hydrogen and air as recommended by detector manufacturer or as determined from experimentation to provide optimum operation. Connect column to detector.
- If voltage is set by user, follow manufacturer's directions.
- Ignite flame after all instrument temperatures are equilibrated and with carrier gas flowing into detector.
- Turn on air.
- Depress ignitor and hold.
- Slowly turn on hydrogen.
- Release ignitor after hydrogen has been turned completely on. Slight increase in signal should occur when flame is ignited. Alternatively, check for lighted flame by holding mirror or other shiny object at exhaust end of detector. Presence of condensed moisture indicates that flame is present.
- If flame does not light, turn off hydrogen and repeat previous steps. Increasing air flow and/or decreasing carrier flow may help in igniting flame.

- Turn on auxiliary gas if needed.
- Determine detector response by injecting 1.5 ng chlorpyrifos. Adjust electrometer sensitivity so that 1.5 ng gives about 50% FSD (40-80% is satisfactory). Adjust voltage to change response, if variable power supply is available. At given voltage, changes in flow rates may improve sensitivity and chromatography.

FPD-S.

- Follow procedures above for FPD-P, but use 394 nm filter.
- If more than one 394 nm filter is available, test to determine which is most selective to sulfur over phosphorus by injecting mixture of methamidophos, chlorpyrifos, acephate, omethoate (each containing S and P), and monocrotophos (P only). A filter that does not permit response to monocrotophos, or that permits least response to it, is the best choice for sulfur selectivity.
- For greatest sensitivity, do not use electrometer square root function; instead, plot response *vs* amount injected on semilog paper and quantitate from that calibration. FPD-S is sufficiently insensitive that it should be set up to provide the greatest sensitivity possible while still maintaining reasonable baseline noise; this will vary from instrument to instrument.

Troubleshooting

Consult the manufacturer's operation and service manual for recommendations specific to detector model being used. Note the following additional suggestions:

Symptom	Possible Solution
Noisy and/or wandering baseline	Install flow controllers to prevent gas flow fluctuation; normal baseline is very straight with <1% noise in P mode and <2% in S mode.
	Check by shining flashlight on detector. Recorder will show response if leak exists. Replace O rings. If this does not work, seal light leaks with black tape or other material. Photomultiplier tube should never be exposed to light when connected to power supply or it will burn out.
	Clean detector.
Low sensitivity	Check for photomultiplier tube light leaks as above.
Peak broadening or tailing, poor response reproducibility	Improve chromatography by changing to capillary column or other column suited to chemistry of analyte.
	Rejuvenate capillary column.

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503 D: ELECTROLYTIC CONDUCTIVITY DETECTOR

The electrolytic conductivity detector (ElCD) is capable of operating in modes selective to halogen, nitrogen, or sulfur. ElCDs can also be configured for selective detection of nitrosamines or esters or for the nonspecific detection of carbon-containing compounds.

For pesticide residue determination, the ElCD is most often used in the halogen mode (ElCD-X), where it exhibits much greater selectivity to halogen than does the EC detector and yet responds to <1 ng of most organohalogen pesticide residues in foods. Although ElCD in the nitrogen mode (ElCD-N) has been shown suitably sensitive for use in residue determination, it is used routinely for that purpose in only a few laboratories, because adequate operation is more difficult to establish and maintain. In addition, problems associated with interferences from nitrogen-containing commodity co-extractives (Section 503 A) apply to ElCD-N.

Only ElCDs in the halogen and nitrogen modes are discussed in detail in this section.

Presently, two manufacturers market ElCDs, Tremetrics and OI Corp., College Station, TX. The Tremetrics "Hall 1000" and "Hall 2000" replaced the original "Hall 700A," which was marketed by Tracor Inc. (now Tremetrics); the latter model is no longer commercially available but continues to be used in many residue laboratories. OI markets the "4420" and a newer "5200." FDA experience is limited to the Hall 700A, Hall 1000, and OI 4420, so only these models will be discussed in this chapter.

Principles

GLC column effluent is pyrolyzed in a nickel reaction tube at >800° C in the presence of hydrogen reactant gas. Heat causes most of the compounds in the reaction tube to be pyrolyzed to their elemental form, but the presence of reactant gas results in other chemical reactions. Products formed during reaction of the analytes are either removed by appropriate scrubbers prior to entering a conductivity cell or are swept into the conductivity cell *via* carrier gas where they are dissolved in a circulating conductivity solvent (electrolyte).

In the conductivity cell, electrolyte conductivity is constantly monitored for changes caused by dissolution of the reaction products. Change in conductivity is converted to a voltage signal that produces an electrical peak at the detector output. ElCD hardware is configured into various operating modes by appropriate selection of reactant gas, electrolyte, ion exchange resin, and chemical scrubbers used to remove interferences. Detector sensitivity is affected by reaction conditions as well as by electrolyte flow rate and reactant gas flow rate.

Design

Figure 503-i displays a block diagram of a GLC system with ElCD; terminology is generalized to display the basic system arrangement that applies to all models of ElCD; some design differences exist between models. Figure 503-j displays a simplified diagram of the ElCD reactor and conductivity cell.

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Figure 503-i
Block Diagram of the EICD

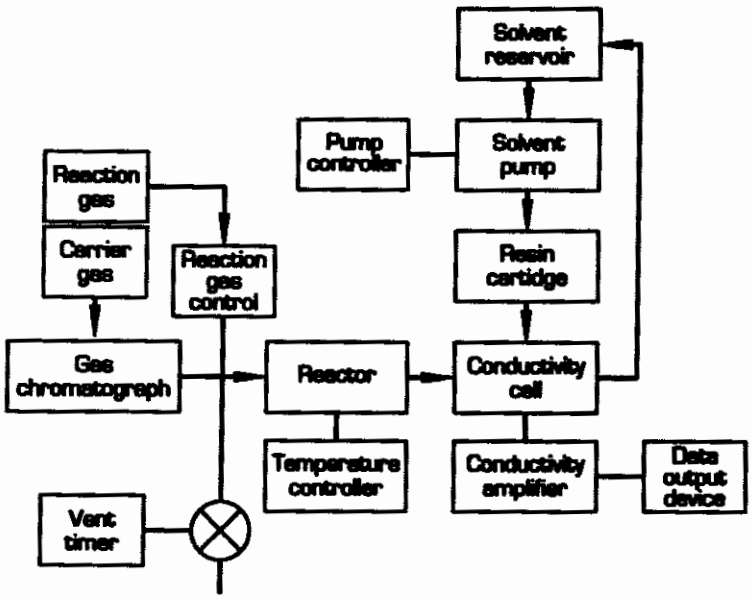

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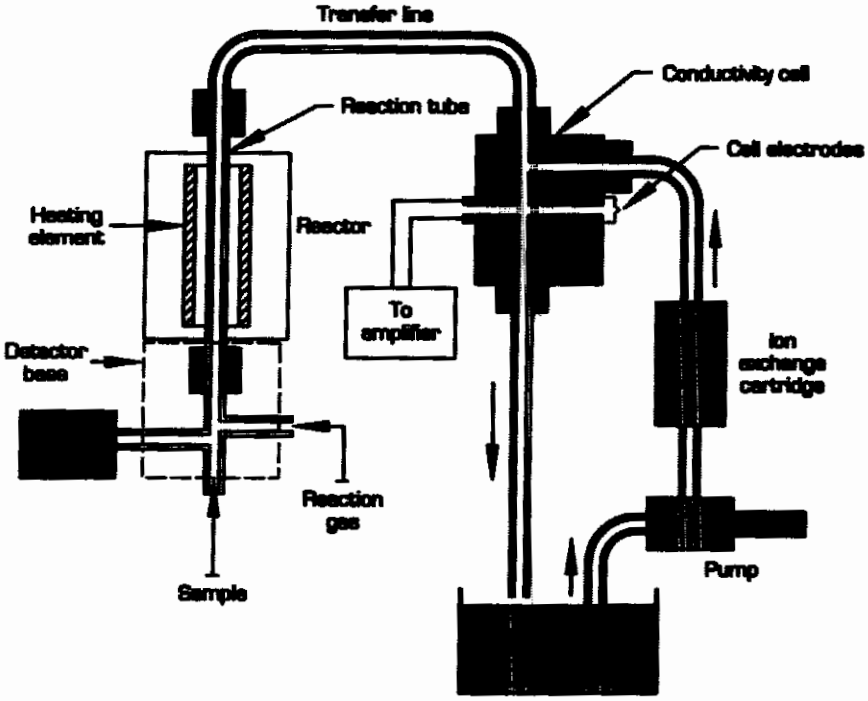
Figure 503-j
EICD Reactor and Conductivity Cell



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Each model ElCD includes four major units:

1) Reactor unit, including reactant gas inlet, nickel reaction tube, and solvent vent to prevent injection solvent from entering the reaction tube.

2) Electrolyte unit, including solvent reservoir, ion exchange tube, and solvent pump. The OI 4420 uses resin in two stages, a "roughing" resin within the electrolyte reservoir and a "finishing" resin through which the electrolyte passes before entering the conductivity cell.

3) Conductivity cell.

4) Signal processing unit, which converts conductivity cell signal for display and provides power for other detector components.

The Hall 700A differs from other ElCDs in that it has a reference cell measuring the conductivity of the electrolyte without any dissolved reaction products; electrolyte conductivity is subtracted from that of the analytical cell, so that background signal is removed from the final measurement. The Hall 1000 and OI 4420 measure absolute conductivity with no subtraction of signal related to electrolyte conductivity.

ELCD-X

Principles

ElCD response in the halogen mode results from formation of HF, HCl, HBr, or HI by catalytic reduction of analytes containing fluorine, chlorine, bromine, or iodine, respectively. The heated nickel reaction tube provides all the ingredients for reaction: a chamber for mixing analyte and hydrogen gas, heat, and the nickel surface for catalysis.

Carrier gas transports the acid formed in the reaction tube into a conductivity cell. The acid dissolves in deionized n-propanol electrolyte, increasing electrolyte conductivity and producing a measurable response (peak) at the detector output.

To prevent neutralization of the acid formed in the reaction tube, pH of the n-propanol electrolyte must also be slightly acidic. Electrolyte acidity is maintained by circulation through ion exchange resin.

Apparatus and Reagents

Section 501 B provides general information on apparatus and reagents required for GLC. Consult appropriate instrument manuals for purchasing information and proper procedure for replacing the following reaction tubes, resins, scrubbers, and electrolyte:

additional nickel reaction tubes. Tubes are purchased from the instrument manufacturer or from other suppliers of nickel tubing. Use of tubing not designed for analytical purposes will probably require additional cleaning and/or polishing to be suitable [14]; laboratory prepared tubing has not been successfully used in the OI 4420 models.

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gases, helium, ultra high purity, 99.999%, used as column carrier gas; hydrogen, ultra high purity, 99.999%, used as reactant gas

gas filters, capable of removing oxygen and water from carrier gas

n-propanol, distilled from all-glass apparatus, for electrolyte

resin materials, to replace resin tube when selectivity deteriorates. Some extra resin material should be provided with the detector and more can be ordered from the manufacturer, or a system that replaces the whole unit can be purchased to avoid the inconvenience of replacing the resin [15-17]. Consult appropriate detector manual for instructions on replacing resin. (Resin and electrolyte are sold as a unit by Tremetrics, Inc., because the resin bed is contained within the conductivity reservoir.)

Resins are subject to deterioration and are dated; they may be unsuitable for use after storage, even if refrigerated as directed.

Detector Characteristics

Sensitivity. An FDA interlaboratory trial involving eight Hall 700A ElCDs in the halogen mode, each operated at the same basic parameters, showed that each detector was different in terms of the minimum amount of halogenated material to which it would respond [18]. In this study, the most responsive detector was 10-25 times more sensitive than the least responsive detector to the same amount of the same compound. However, each properly functioning detector was capable of detecting 0.05 ppm lindane in the presence of sample extract. The more sensitive detectors could readily measure 0.01 ppm lindane. No comparable study has been performed with the other model ElCDs.

The following parameters affect ElCD sensitivity:

1) Reactant gas purity. Impurities in the reactant gas can undergo chemical reaction in the reaction tube, and resulting products may be soluble in the electrolyte. If this occurs, conductivity may be raised sufficiently to obscure measurement of small amounts of analyte (*i.e.*, the signal-to-noise ratio will be reduced). Adherence to manufacturers' purity recommendations is critical. Gases meeting manufacturers' specifications occasionally contain traces of hydrochloric acid that can destroy the detectors; use of appropriate gas filters is required even on high purity gases.

2) Reactant gas flow rate. ElCD-X response was shown to increase with increasing reactant gas flow rate, up to about 60 mL/min, in a study of the Hall 700A ElCD [19]. Above that flow rate, response remained essentially constant up to 100 mL/min. Manufacturers suggest reactant gas flow rate of 50-75 mL/min for the Hall 700A [15], 25 mL/min for the Hall 1000 [16], and 100 ± 10 mL/min for the OI 4420 [17]. FDA laboratories routinely use 60-80 mL/min for the Hall 700A and the Hall 1000 and 100 mL/min for the OI 4420.

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3) Reaction tube condition. The nickel reaction tube catalyzes the reaction and must be free of contamination. After a period of use, nickel tubes become contaminated because of fouling by bad gases, sample reaction products, septum or column bleed, or other causes; chromatograms at this point characteristically show a slow return to baseline after venting, peak tailing, and loss of response. Once contaminated to this degree, the tube must be replaced, because no successful reconditioning process has been developed.

4) Reaction temperature. In a study using the Hall 700A in the halogen mode, reaction temperature was not found to affect detector sensitivity significantly. No significant differences in detector response occurred when chlorinated compounds were injected at temperature control potentiometer settings of 850, 730, and 630° C [19]. However, a minimum potentiometer setting of 900° C is recommended for ElCD-X, because it is suspected that potentiometer setting is not an accurate reflection of the actual temperature of the reaction tube and because it is reasonable to assume that more efficient reduction of halogen occurs at higher temperatures. Reaction tubes whose operation is compromised by other problems (*e.g.*, contamination from samples, column bleed, or poor quality nickel tube) may show fluctuations in sensitivity with changes in temperature.

   Reaction furnaces ("reactors") of OI 4420 detectors have been subject to repeated burnout, requiring replacement. The manufacturer offers a smaller, cartridge-style heating element as a reactor replacement in an upgrade to the detector; this model is also expected to have a limited lifetime but will be easier and less expensive to replace than the original reactors.

5) Electrolyte flow rate. Electrolyte flow rate significantly affects detector sensitivity by affecting the length of time the dissolved reaction products spend in the conductivity cell. As the electrolyte flow decreases, response increases. Below a certain flow rate, however, baseline noise increases and further decrease in flow rate results in no additional improvement in the signal-to-noise ratio.

   Respective manufacturers recommend 0.5 mL/min electrolyte flow rate for the Hall 700A, 0.6 mL/min for the Hall 1000, and 0.02-0.05 mL/min for the OI 4420. FDA laboratories usually use 0.35 ± 10% mL/min flow for the Hall 700A and the Hall 1000 and 0.035-0.050 mL/min for the OI 4420.

Selectivity. The ElCD is made selective to halogens by using hydrogen reactant gas and n-propanol electrolyte. HX, formed by pyrolysis of halogenated compounds in the presence of hydrogen, is readily soluble in n-propanol. Other compounds formed in the reactor, such as H_2S and NH_3, do not usually cause detector response because they are not ionized in n-propanol and therefore cannot change solvent conductivity. No scrubber is needed to remove interfering reaction products from the gas flow in the halogen mode. Large quantities of nitrogen and possibly carbon dioxide may cause a response, however.

(An optional oxidative mode operation for halogen selectivity, using oxygen as reactant gas, is far less selective, produces greater noise, and requires use of scrubbers capable of removing SO_2/SO_3 from the reaction products. This operation has

never been implemented for residue determination because it is far less preferable than the reductive mode.)

Because detector selectivity to halogen is dependent on reaction conditions and on solubility of reaction products in the electrolyte, all the following factors affect selectivity:

1) Gas purity. Impurities in column carrier gas and/or hydrogen reactant gas can introduce other chemical species that may interfere with halogen detection; only ultrapure gases are acceptable. Gases must be free of any level of halogenated compounds.

2) Reaction tube condition. The nickel reaction tubes that contain and catalyze the reaction are known to vary from one another in their ability to convert halogen to HX. A tube that initially produces an acceptable response may deteriorate over a period of use because of contamination. As previously discussed, the tube must be replaced when this occurs.

3) Reaction temperature. Reaction temperature may affect selectivity by influencing the degree to which reduction of halogen to HX occurs. Reaction temperature setting of 900° C is recommended to achieve efficient reduction.

4) Ion exchange resin. The ion exchange resin affects selectivity by controlling pH of the electrolyte and by continuously removing ionized reaction products from the electrolyte. The resin used for the halogen mode maintains the n-propanol electrolyte at a slightly acidic pH. Presence of the ionized HX then produces a measurable change in solvent conductivity. When the resin fails to control pH of the electrolyte appropriately, certain species other than HX are also able to ionize, and detector selectivity deteriorates. Failure to maintain slightly acidic electrolyte results in negative or "V"-shaped peaks. Replacement of the resin with fresh material re-establishes the necessary selectivity.

For unknown reasons, addition of n-propanol to the reservoir of the OI 4420, to replace evaporated solvent, can cause severe damage to the resin. When this occurs, resin and electrolyte must be replaced [20].

Linearity. Linear dynamic range of the Hall 700A ElCD-X varies with the compound and with the individual detector [19]. Moreover, the typical degree of linearity and length of linear range are not sufficiently reliable to eliminate the need for matching peak heights of residue and standard when quantitating. Each system should be tested to measure its linear range. For accurate quantitation of residues, peak sizes must be within 25% of one another.

Other Influences on Detector Performance

Solvent Venting. ElCD reactor units include a vent line positioned just before the heated reaction tube. The relatively large volume of injection solvent, eluting through the column prior to the analytes, is diverted through this port to prevent its entry into the reaction tube. Venting prevents combustion of hydrocarbon solvent in the reaction tube and thus protects the tube from carbon deposition that decreases catalytic performance, nickel tube lifetime, and detector response.

Contamination of the reaction tube and transfer line is most severe when acetone is used and then not completely vented.

Efficiency of venting is affected by several factors, including reactant gas flow rate, combustion tube diameter, and the percentage of flow that is vented. Both increased flow of reactant gas and decreased combustion tube id improve vent efficiency by increasing back pressure.

The percentage of total effluent that is vented is critical. While efficient venting of most solvent is necessary, not all solvent can be vented, because lack of positive pressure permits electrolyte to flow from the reservoir and enter the nickel reaction tube, contaminating it. Vent rate is preset by the manufacturer, but the OI 4420 includes a vent system with a pressure-regulated "T" that permits the user to adjust the vent rate by turning a threaded rod. Manufacturer directions specify adjusting the rate so that about 50-60 mL/min total gas flow exits the vent; frequent monitoring of the flow is necessary, because the vent split ratio fluctuates.

Many of the recurring problems with the OI 4420 detector were traced to the vent system. It is now recommended that the original vent flow valve be replaced with a constant flow port, which is capable of maintaining a constant vent flow while the vent is open; the detector upgrade offered by the manufacturer includes this replacement. Even this change, however, does not vent most of the solvent, and its combustion in the reaction tube may cause subsequent problems [20].

Position of Capillary Column. Results observed during evaluation of a capillary column with OI 4420 ElCD-X indicate that the most critical element for successful operation is proper positioning of the column outlet in the reactor [21, 22]. When the capillary column is installed as directed in the detector manual (*i.e.*, column outlet placed about 0.5" into the nickel reaction tube) a noisy baseline with frequent "spiking" is observed.

These studies demonstrate that positioning the column outlet between the solvent vent and the reactant gas inlet produces optimal results. In this position, the column is outside the nickel tube and away from the extremely high temperatures of the reactor. This positioning also ensures efficient venting, because the reactant gas takes the path of least resistance and flushes the injection solvent through the vent rather than through the small id transfer line to the combustion tube; the possibility of tube contamination is thus reduced. A steady baseline is maintained when the column is installed between the vent and the reactant gas inlet. This same positioning is also optimal when wide bore capillary columns are used. A redesigned base for mounting the detector on the chromatograph may minimize the importance of user attention to positioning the column. Operation of ElCDs with packed columns is not as sensitive to column position.

Transfer Line Cleanliness. More often than not, broad, tailing peaks are caused by a dirty transfer line between the reactor and the conductivity cell. Contaminants (*i.e.*, unreacted hydrocarbons) can deposit in the transfer line and produce active (adsorptive) sites. When hydrochloric or other gaseous acids pass through the transfer line, they may be adsorbed; this phenomenon causes tailing or, in severe cases, total loss of detector response. Transfer lines can be rinsed with the injection solvent being used [23]; the ease with which this can be performed varies with the detector model.

Recommended Operating Procedures

Except for positioning the column in the reactor (above), follow directions from the appropriate detector manual to set up and operate the GLC system with ElCD. The following additional recommendations are based on experiences in FDA laboratories:

- Reaction tube temperature: Set potentiometer that controls reaction tube temperature to 900° C to ensure that temperature is high enough to completely reduce analytes. To protect reaction tube from possible deactivation by column bleed, do not allow reactor temperature to drop below that of column.

- Reactant gas flow: Maintain hydrogen flow of about 60-100 mL/min through reaction tube to ensure complete reduction of sample. Measure flow rate using bubble meter and stopwatch, either at point where gases enter conductivity cell (with column carrier gas turned off) or at return line to solvent reservoir (with both column carrier gas and solvent pump turned off). Column temperature should be reduced to room temperature if carrier gas is off for any length of time.

- Solvent flow rate: For optimum performance, pump n-propanol electrolyte through conductivity cell at 0.35 ± 10% mL/min for Hall 700A and Hall 1000 and 0.035-0.050 mL/min for OI 4420 ElCD-Xs. Measure flow rate by placing line that usually carries solvent to reservoir into a graduated cylinder and measuring accumulation over known time.

System Suitability Test

Monitor detector selectivity by regularly injecting aliquot of mixed standard solution containing the following: 100 ng diisobutyl phthalate, 100 ng ethion, 1 ng chlorpyrifos, 100 ng methyl palmitate, 100 ng caffeine, and 2 µg octadecane. Properly operating detector will respond only to chlorpyrifos and possibly to caffeine. If response to caffeine is seen, calculate selectivity ratio as:

$$\frac{\text{detector response to chlorpyrifos} \times 100}{\text{detector response to caffeine}}$$

If selectivity ratio for chlorpyrifos:caffeine is <500:1 or if any response to other compounds is seen, improve selectivity by following the suggestions for troubleshooting, below.

Routinely monitor detector response to halogen by injecting solutions containing at least lindane, chlorpyrifos, and p,p′-DDT. If response decreases, follow directions in troubleshooting section to determine cause. While monitoring halogen response, also note peak shapes on chromatograms. Deteriorating shape (*i.e.*, increased tailing) of all peaks may be caused by various factors covered in troubleshooting section. Breakdown of p,p′-DDT (evidenced by smaller peak plus appearance of another peak at retention time of p,p′-TDE) has been found to be caused by prior injection of extracts prepared by the method of Section 302. This condition disappears over time if no further extracts from that method are injected.

Troubleshooting

Each detector manual [15-17] contains a section on troubleshooting that should be consulted when problems occur. Another reference [24] also contains excellent information on operation of the ElCD and potential problem areas. Beyond the advice offered in those references, these additional suggestions for optimum detector performance are offered:

Symptom	Possible Solution
Selectivity of chlorpyrifos: caffeine is <500:1	Replace resin in cartridge and n-propanol electrolyte.
	Change nickel reaction tube. Several different tubes may have to be tried, because each converts halogen to HX to different extent.
	Elevate reaction tube temperature to determine whether sample is being completely reacted.
	Replace column if liquid phase contains halogen or nitrogen.
Loss of detector sensitivity	Verify purity of gases; use only ultra high purity gases.
	Remove transfer line and rinse with injection solvent or replace transfer line.
	Replace column if liquid phase contains halogen or nitrogen.
Breakdown of p,p′-DDT to p,p′-TDE	Remove first 1-2" of packing from GLC column and replace with fresh, conditioned packing, *i.e.*, clean front end of column (Section 502).
Broad, tailing peaks	Replace or clean transfer line.
	Clean front end of column.
	Replace nickel reaction tube.
	Replace column if liquid phase contains halogen or nitrogen.
Slow return to baseline after venting	Replace nickel reaction tube.
Breakdown of analytes (but normal return to baseline after venting, normal peak shape)	Clean front end of column.

ElCD-N

Principles

ElCD response in the nitrogen mode results when organic nitrogen is pyrolyzed to ammonia with hydrogen reactant gas at high temperature. Acidic or sulfur gases, resulting from pyrolytic reduction of halogen and sulfur compounds in the reactor, are selectively removed by quartz threads coated with potassium hydroxide. Carrier gas transports the ammonia into the conductivity cell, where it dissolves in an aqueous electrolyte to form ammonium hydroxide:

$$NH_3 + H_2O \longrightarrow NH_4^+ + OH^-$$

As a weak base, ammonium hydroxide readily ionizes in the aqueous electrolyte and becomes a conducting species. The change in electrolyte conductivity caused by dissolution/ionization of ammonia produces a measurable response (peak) at the detector output.

Apparatus and Reagents

Section 501 B provides general information on apparatus and reagents required for GLC. The section above on ElCD-X provides additional information about replacement nickel reaction tubes, gas purity, and gas filters. Consult appropriate instrument manuals for purchasing information and proper procedure for replacing the following reaction tubes, resins, scrubbers, and electrolyte:

stainless steel, nickel, or copper gas lines from gas cylinders to the instrument

water, HPLC grade, prepared from water purification equipment, or equivalent commercial product; 18 megaohm resistance required

fresh electrolyte, prepared from reagent grade t-butanol and HPLC grade water

additional scrubbers for use in nitrogen mode. Nitrogen scrubber generally lasts 3-6 mon. However, under certain circumstances scrubber may last <1 mon.

resin, may be ordered from detector manufacturer, as described above for ElCD-X. Nitrogen mode operation is particularly sensitive to failures caused by resin deterioration, so use of fresh resin is critical.

Detector Characteristics

Sensitivity. The ElCD-N detector is capable of producing as much as 50% FSD response to 1 ng carbaryl or 0.5-1 ng chlorpyrifos. As with the ElCD-X operation, sensitivity of the ElCD-N depends on reaction conditions, reactant gas flow rate, and electrolyte flow rate. In addition, the condition of the chemical scrubber used in the nitrogen mode will affect detector sensitivity.

The control module (signal processing unit) of the OI 4420 offers several sensitivity settings, labelled according to the different modes of operation. Despite the

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label, the setting for the halogen mode should be used during nitrogen mode operation for greatest sensitivity.

Selectivity. Selectivity for N:P is much better than that of the N/P detector and is the chief virtue of the ElCD-N; selectivity of the OI 4420 in the nitrogen mode was found to be 2400:1 for chlorpyrifos (molecular formula $C_9H_{11}Cl_3NO_3PS$):bromophos ($C_8H_8BrCl_2O_3PS$) [25]. Selectivity for N:X is also very high as long as the scrubber is efficiently removing HX from the reaction products prior to dissolution in electrolyte; N:X selectivity is higher in this mode than is X:N selectivity in the halogen mode. Selectivity was found to be 4800:1 for chlorpyrifos: aldrin ($C_{12}H_8Cl_6$) [25]. As with the ElCD-X operation, selectivity of the ElCD-N is affected by reaction conditions and by parameters that affect ionization of the reaction products in the electrolyte.

Any parameter that affects the conversion of organic nitrogen to NH_3 influences selectivity. The most important of these parameters are reactant gas purity, condition of the reaction tube, and reaction temperature. In addition, the chemical scrubber (*i.e.*, quartz threads coated with potassium hydroxide), placed between the reactor and conductivity cell, prevents interferences from reaching the electrolyte, dissolving in it, and causing detector response.

Conditions that affect ionization of the reaction products, including nature of the ion exchange resin and electrolyte type and pH, also influence detector selectivity. Electrolyte pH must be slightly basic to prevent neutralization of the basic ammonium hydroxide; appropriate pH is maintained by passing the electrolyte through an ion exchange resin before it enters the conductivity cell. Aqueous electrolyte is used in the nitrogen mode because water is one of the few neutral solvents capable of ionizing a weak electrolyte like ammonia. Water purity is critical to detector selectivity; the high purity water specified above in Apparatus and Reagents is necessary.

Incorporation of carbon dioxide in the electrolyte affects its pH and thus detector selectivity. Carbon dioxide can enter the electrolyte from improper venting or from permeation through tubing during transfer to the conductivity cell. Early versions of the OI 4420 included a permeation chamber filled with ammonia in water, through which the Teflon tubing transferring the electrolyte passed. This arrangement was used only for nitrogen mode operation, with the intent of permitting ammonia to permeate the electrolyte and keep it sufficiently basic, but it was not satisfactory and the permeation chamber is no longer included with the system. The Hall 2000 uses a stainless steel transfer line to minimize permeation of gases into the electrolyte. Purging the electrolyte with hydrogen or helium for 1 hr, after t-butanol and water are mixed, may also be used to dispel carbon dioxide and air and improve detector performance [26].

Linearity. The linearity of ElCD-N detector response to any particular chemical is approximately three orders of magnitude, within the range of 10 pg-100 ng. Response to each chemical, depending on its chromatography and percentage nitrogen, has a lower threshold of linearity; below that level, response can be measured but is not linear. Response to an amount beyond the upper limit of linearity often appears as a double peak [20].

Other Influences on Detector Performance

Factors that influence operation of the ElCD-X detector, *i.e.*, venting efficiency and transfer line cleanliness, are also important parameters in ElCD-N operation,

although use of a scrubber in the nitrogen mode minimizes transfer line contamination. As with the ElCD-X, the optimal position for a capillary column in the OI 4420 detector was found to be between the vent line and the reactant gas inlet [25]. In addition, the detector operations described below are critical to acceptable detector operation.

Composition of Electrolyte. An important parameter for acceptable performance is electrolyte composition [25]. During early studies of the OI 4420, 50% n-propanol/water was recommended as a replacement for 0.1% hexanol/deionized water, originally recommended by the manufacturer. In the meantime, however, 10% t-butanol/water has become the recommended electrolyte for OI 4420 nitrogen mode operation; 50% n-propanol/water is recommended for Hall 700A and Hall 1000 detectors. As discussed above, water purity is critical in the nitrogen mode.

Condition of Scrubber. The scrubber can become exhausted and must be replaced when the detector begins responding to halogenated compounds. Solvents containing halogen or sulfur should not be used in the nitrogen mode because they will rapidly deplete the scrubber.

Recommended Operating Procedures

Follow the explicit directions from the appropriate detector manual to set up and operate the GLC system with ElCD, and incorporate special directions discussed above, including use of high purity (18 megaohm resistance) water and hydrogen purging of the mixed electrolyte prior to use, whenever electrolyte is changed.

System Suitability Test

Currently, there is no standardized system suitability check performed by FDA laboratories for ElCD-N detectors. Suggested system suitability tests may be found in detector operation manuals. It is recommended that a solution containing at least one compound containing nitrogen as the only heteroatom, one halogenated compound, and one hydrocarbon be injected into the system; a properly functioning system should show no response to the halogenated compound or to the hydrocarbon and should have no inverted (below baseline) peaks.

Troubleshooting

Detector operations manuals and Reference 24 each contain sections on troubleshooting. In addition, the following suggestions for optimum detector performance are based on the FDA evaluation of wide bore column and OI 4420 ElCD-N for determination of nitrogen-containing pesticide residues in food:

Symptom	Possible Solution
Peak tailing	Replace scrubber.
	Replace nickel reaction tube.
	Replace older OI 4420 detector base.
Poor linearity	Replace scrubber.

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	Replace electrolyte.
	Replace older OI 4420 detector base.
Excessive noise	Replace electrolyte.
	Replace gas line filters.
	Replace gas.
	Check for temperature fluctuations and correct as necessary.
	Replace nickel reaction tube.
	Check for and correct gas flow instabilities.
	Remove bubbles in OI cell by turning pump switch off for 1-2 sec, then turning on, or increase pump speed to maximum for 1-2 min.

General Precautions for ElCDs

The following precautions should be followed to ensure optimum performance of the ElCD in both halogen and nitrogen modes:

- Avoid column liquid phases that contain halogen or nitrogen, because the phase may bleed and de-activate the reaction tube and/or raise the conductivity of the electrolyte.
- Avoid injecting standards or sample extracts in solvents containing halogen or nitrogen. Even though the solvent is vented, traces may remain and affect detector operation. This effect becomes critical in cases where detector selectivity is already poor.
- Maintain constant carrier gas and reactant gas flow at all times. Reducing gas flows overnight to conserve gas may result in diminished responses when detector conditions are re-established the next day.
- When carrier gas flow must be interrupted, *e.g.*, to change columns or septa, cool reactor furnace first. Exposure of nickel reaction tube to oxygen at high temperature invariably damages performance and usually requires subsequent replacement of tube. Before reheating furnace, thoroughly purge system with carrier gas; 15 min is sufficient when capillary columns are used.
- Do not allow solvent return line to dip below surface of solvent in reservoir. Violating this rule will lead to backup of solvent into reaction tube anytime gas flow is inadvertently stopped.
- Vent injection long enough to ensure removal of solvents or volatile sample co-extractives that can interfere with determination. Adequate venting also protects reaction tube and conductivity cell. Vent time of 0.5-0.75 min is adequate for wide bore column and Hall 700A detector; 0.75->1.3 min is required for OI 4420.

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503 E: NITROGEN/PHOSPHORUS DETECTOR

The nitrogen/phosphorus (N/P) detector is selective to residues containing nitrogen and/or phosphorus atoms. Modern N/P detectors evolved from Kolb and Bischoff's 1974 design [27], itself an evolution of the potassium chloride thermionic detector (KCITD); the KCITD, introduced in the mid-1960s, was the first selective detector for phosphorus residues [28]. Most N/P detectors are more responsive to phosphorus than to nitrogen, but this section emphasizes use as a nitrogen-selective detector, because the FPD-P (Section 503 C) is preferred for phosphorus residues.

Although N/P detectors are selective and sensitive, problems associated with their reliability and performance have deterred their routine application for pesticide residue determination in FDA laboratories. In addition, the N/P's ability to distinguish residues from sample matrix unequivocally is hindered by the presence of nitrogen in many commodity co-extractives, a dilemma common to all nitrogen detectors. Despite these shortcomings, an N/P detector, optimized for nitrogen selectivity, can play a valuable role in examining extracts for residues; many pesticides contain no other heteroatom than nitrogen. Response of the N/P detector also provides complementary evidence about element(s) present in a residue, information often needed for confirmation of identity (Section 103, Table 103-a).

Several different manufacturers produce N/P detectors. Among these are: Chrompack, Inc., Raritan, NJ; DETector Engineering & Technology, Inc., Walnut Creek, CA; Hewlett-Packard Company, Wilmington, DE; Perkin Elmer Corporation, Instrument Division, Norwalk, CT; Shimadzu Scientific Instruments, Inc., Columbia, MD; Tremetrics, Inc., Austin, TX; and Varian Instrument Division, Walnut Creek, CA.

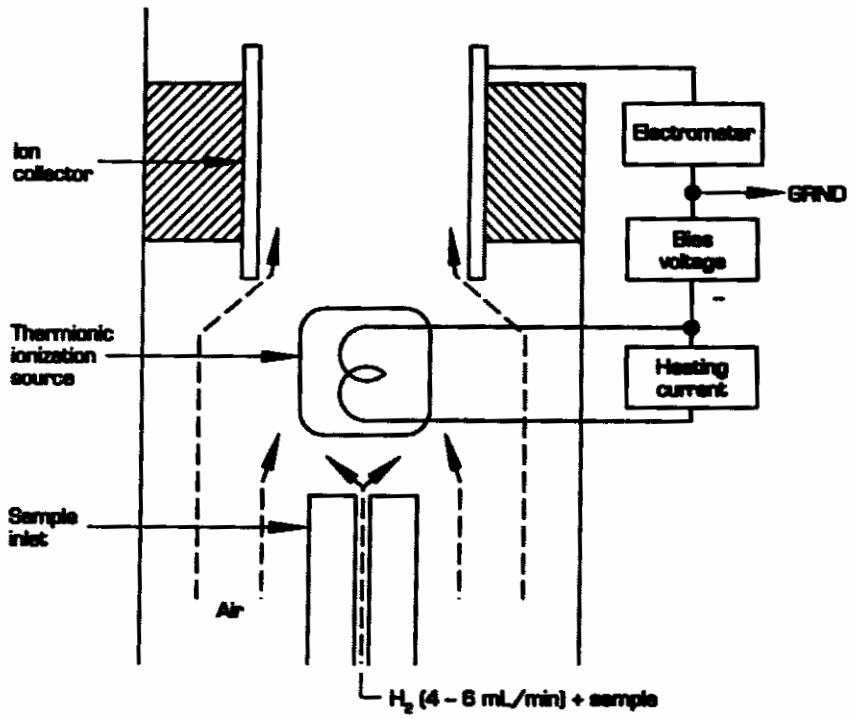
Principles

GLC column effluent impinges onto the surface of an electrically heated and polarized alkali source in the presence of an air/hydrogen plasma; ionization occurs and the flow of ions between plasma and an ion collector is amplified and recorded. Detector response to analytes results from the increased ionization that occurs when compounds containing nitrogen or phosphorus elute from the column. At gas flow rates used for N/P operation, the degree of ionization of compounds containing nitrogen or phosphorus is >10,000 times greater than for hydrocarbons. Mechanisms that explain the enhanced response to nitrogen and phosphorus are not yet fully understood and are beyond the scope of this manual; both gas phase ionization and surface ionization processes have been proposed [29].

Design

An N/P detector is similar to an FID to which an electrically heated source of alkali has been added between the jet and the ion collector; Figure 503-k provides a schematic diagram of typical components. Commercially available N/P designs vary considerably, with different collector electrodes, collector polarity, and optimum potential between jet and collector; Figure 503-l displays several of these variations. The most important component, the alkali source, is usually manufactured by impregnating a glass or ceramic matrix with an alkali metal salt. Variations among alkali source designs represent attempts to optimize selectivity to

Figure 503-k
N/P Detector Components



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nitrogen (selectivity to phosphorus over hydrocarbons is adequate for most designs), detector operating stability, and source ruggedness for extended operating life. Some but not all detector models permit adjustment of the alkali source height above the jet for optimization of sensitivity and selectivity.

All N/P detectors provide electronic heating of the alkali source to 600-800° C. The plasma in the region of the salt is sustained by flows of hydrogen and air. The alkali source exhibits longer operating life and more stable and reproducible response under these conditions than in the presence of a flame.

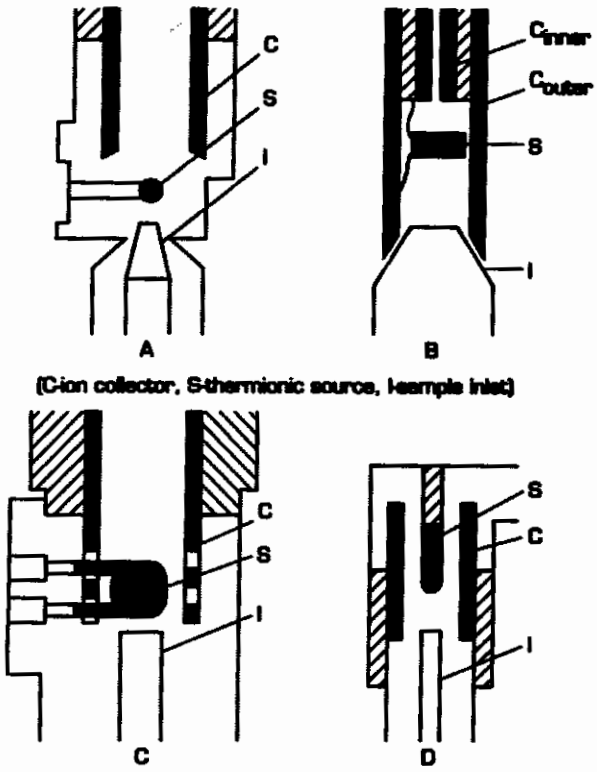
Apparatus and Reagents

Section 501 B provides general information on apparatus and reagents required for GLC.

Detector Characteristics

Sensitivity. N/P detectors are capable of producing detectable peaks in response to as little as 5-10 pg nitrogen-containing compounds or to 1-5 pg phosphorus-containing compounds [30]. FDA experience indicates that the pesticide for which the greatest N/P response occurs is diazinon, which contains two nitrogen atoms and one phosphorus atom; 25 pg diazinon should cause a response of approxi-

Figure 503-I
N/P Detector Configurations



(C-ion collector, S-thermionic source, I-sample inlet)

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mately 5% FSD; tebuthiuron, with four nitrogen atoms (no phosphorus), requires about 50 pg for 5% FSD response when chromatographed on a wide bore capillary column at standardized sensitivity (Appendix I, PESTDATA).

N/P sensitivity is most influenced by hydrogen flow and the magnitude of heating current supplied to the alkali source. Alkali source position may affect sensitivity, but not all designs permit adjustment of source height. Response diminishes over the lifetime of the alkali source. Variations in response are also seen among individual alkali sources.

Detector response to nitrogen is most affected by hydrogen flow rate, with response increasing as hydrogen flow decreases; optimum flow for the particular detector must be determined experimentally. Typical hydrogen flow rate for optimum nitrogen sensitivity is 1-5 mL/min. Response also increases with increasing alkali source current, but little improvement is realized, because detector background can also increase. Lifetime of the alkali source may also be shortened by operation at higher current.

Response to nitrogen compounds is not strictly proportional to the amount of elemental nitrogen in the molecule; variations based on molecular structure occur. Although the reactions that occur within the detector plasma are effective in decomposing analytes into common species, those compounds that easily decompose to the cyano radical usually cause higher response than do amides or nitro compounds [29].

Selectivity. Selectivity of the N/P detector is about 10^3–10^5 for N:C response, 10^4–5×10^5 for P:C response, and 0.1–0.5 for N:P response [29]. Factors that affect detector sensitivity do not always affect selectivity similarly. While both sensitivity and selectivity to nitrogen improve with decreasing hydrogen flow, only sensitivity (but not selectivity) improves with source heating current, because background noise and response to other elements increase simultaneously.

Linearity. Manufacturers of N/P claim linearity of response over four or five orders of magnitude. No FDA studies have been done on modern N/P detectors to measure detector linearity relative to amount of pesticides. Laboratories using N/P detectors must evaluate the linear range, work within that range, and match

peak sizes of residue and standard within 25% for accurate quantitative determination.

Other Influences on Detector Performance

Detector Temperature. Detector output is very sensitive to temperature changes within the active zone where ionization occurs; for stability of operation, conditions that permit variations in temperature should be avoided. Temperature of the alkali source is controlled by the electrical current at which it is operated but is also affected by hydrogen flow and, to a lesser extent, the rate of air and column carrier gas flowing past the source. The detector walls are heated separately. Stability is improved when the N/P detector itself is operated at a high temperature, because this minimizes the temperature gradient between the alkali source and the surrounding wall; reducing the gradient minimizes change in source temperature that occurs when high concentrations of analytes pass through the detector [29].

Age of Alkali Source. Each alkali source has a finite lifetime; eventually each must be replaced. Both sensitivity and selectivity decrease as the source ages, so regular calibration of detector performance is required. Source activity can be conserved by reducing hydrogen flow when the detector is not in use; however, manufacturer's instructions regarding source current and gas flow must be followed carefully to avoid destruction of the source. Operation of the detector at the lowest source current compatible with desired sensitivity is also recommended, as is maintenance of the detector at 100-150° C when not in use to prevent water condensation. Because degradation occurs more rapidly with higher source heating current, increasing the electrometer sensitivity to maintain constant detector sensitivity is preferable to increasing source heating current [29].

Replacement of the alkali source and re-establishment of optimum operating conditions can be troublesome and time-consuming with some detector designs. Design quality is at least partly judged by the stability of the source itself and even more so by the ease with which the source can be replaced and stable operation re-established.

Gas Flow Stability. Stable flow of hydrogen and air is critical for constant and linear response. High precision gas flow valves, standard equipment on some chromatographs, may be required for acceptable operation.

Position of Column Outlet. For maximum sensitivity and optimal peak shape, the GLC column should be positioned about 1-3 mm from the tip of the detector jet. The column should not protrude into the flame, because the polyimide coating on capillary columns will decompose and the resulting nitrogen products cause high background signal and noise. If the column outlet is too far below the tip, peaks may tail and/or be reduced in size because of the dead volume between the column and the alkali source [30].

Solvents and Reagents. Use of certain materials can have a detrimental effect on efficient operation of N/P detectors and should be avoided. For example, injection of extracts containing even trace amounts of acetonitrile can cause large detector response and preclude examination of the early eluting portion of the chromatogram; such extracts must be evaporated or azeotroped to remove all acetonitrile before injection. In addition, halogenated solvents may destroy the alkali source

and thus should not be injected. (Some N/P detectors are designed to permit use of halogenated solvents, but this must be ascertained prior to injection.)

Packed column stationary phases containing cyano groups (*e.g.*, OV-225) are unacceptable for use with N/P detectors. Equivalent bonded phase capillary columns have little bleed and may be acceptable, however. Other materials to avoid include those known to cause problems in many GLC systems, *e.g.*, septa not designated for high temperature use, impurities in gases, and leak-detecting solutions.

Certain common materials can appear as contaminants in determinations using N/P detectors. Nicotine is usually detected when cigarette smoking occurs in the vicinity; if phosphate detergents are used to wash glassware, or if the GLC column or glass wool is treated with phosphoric acid, trace amounts remain and are detectable during determination.

Recommended Operating Procedures

The following directions, adapted from the instrument manual for one N/P detector [31], have not yet been tested within FDA but are proposed as a way of optimizing detector operation:

- Follow manufacturer's directions for installation and operation. Pay particular attention to recommendations related to the alkali source, including situations that should be avoided to prevent its destruction. Use of wide bore capillary column with retention gap (Section 502 C) is recommended; makeup gas should not be necessary if column carrier flow rate of 10-20 mL/min is used.

- Follow manufacturer's directions to establish detector operation selective to nitrogen. Adjust detector parameters and instrument attenuation so that 1.0 ng chlorpyrifos causes 50% FSD.

- Prepare test solution containing 2.0 ng/µL azobenzene (containing 310 pg N), 2.0 ng/µL parathion-methyl (110 pg N and 230 pg P), 4.0 ng/µL malathion (380 pg P), and 4 µg/µL n-heptadecane (3.4 µg C) in isooctane.

- Inject 1 µL test solution, and adjust detector attenuation and range to keep peaks on scale. Examine relative responses of detector to four components; negative deflection of pen is normal in area of solvent peak.

- Experiment with effect of hydrogen flow on detector selectivity to nitrogen by re-injecting test solution after changing hydrogen flow rate in increments of 0.5 mL/min.

- Based on experimental results, use hydrogen flow rate that produces greatest ratio of response for parathion-methyl:malathion, as long as azobenzene peak is ≥4 times heptadecane peak at that flow; malathion peak can be expected to always be larger than parathion-methyl peak.

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504: QUANTITATION

504 A: INTRODUCTION

Accurate quantitation of pesticide residues identified by GLC is always of critical importance. Whether the analysis has been performed for purposes of monitoring or for enforcement of regulations, the consequences always have potential long term impact. All analyses that claim to produce quantitative results must be calculated in a consistent, reliable manner.

Accurate quantitation depends on use of accurate reference standards, use of a GLC system whose response is linearly proportional to the weight of chemical being detected (or for whose nonlinear response adjustment can be made), and use of proper technique for measuring detector responses. Given these conditions, quantitation is based on a simple proportion equation, *i.e.*:

$$\frac{\text{quantity of analyte}}{\text{detector response to analyte}} = \frac{\text{quantity of standard}}{\text{detector response to standard}}$$

Quantity of analyte, the unknown value, is readily calculated from the known quantity of standard and the measured detector responses.

This section assumes that the first two conditions for accurate quantitation, *i.e.*, accurate reference standards and a linear GLC system, are met. Only techniques for measuring detector response are discussed here.

Measurement of detector response for use in the above formula has traditionally involved manual measurement of the peak that represents detector response on a chromatogram drawn by a strip chart recorder. Section 504 B provides directions for the most practical ways of manually measuring peaks.

Modern automated data handling systems electronically integrate the detector output signal and produce a numerical representation of peak size. Step-by-step directions for such systems are not included in this manual, however, because each is unique; analysts using electronic integration must follow the directions provided by the manufacturer. Section 504 C provides general guidance to the appropriate application of electronic integration and advice about avoiding pitfalls that can occur.

Whether the detector response (peak) is measured manually or electronically, proper positioning of the baseline below the peak is critical. Accuracy of the measurement depends in part on how well the detector's response to the residue can be distinguished from its response to sample co-extractives and co-eluting residues. Typically, a residue peak in a sample chromatogram may occur on a sloping baseline, on top of another peak, or incompletely separated from another peak; in contrast, the reference standard solution usually causes a single symmetrical peak. Quantitative accuracy is sacrificed if the residue peak's baseline is not properly delineated. To measure peaks manually, the analyst must literally draw the baseline on the chromatogram before measuring; to use automated measurement, the analyst must configure the system to include only that part of the signal that can reasonably be assumed to represent the residue.

Appropriate setting of the baseline is integral to the directions below for measuring the peaks. In some cases, choice of appropriate baseline for particular residues will be shown by example.

504 B: MANUAL QUANTITATION

Many methods of quantitating gas chromatographic peaks have been presented in the literature, but through the years most laboratories that perform manual measurements of peaks have relied on the two simplest approaches: measurement of peak height and measurement of area of the triangle that best fits the peak ("triangulation"). Peak area is the more accurate representation of detector response, but peak height is a justifiable approximation of area when peak shape makes height proportional to area. Advances in column techniques (Section 502) have resulted in improved peak symmetry and resolution, thus encouraging use of peak height for quantitation.

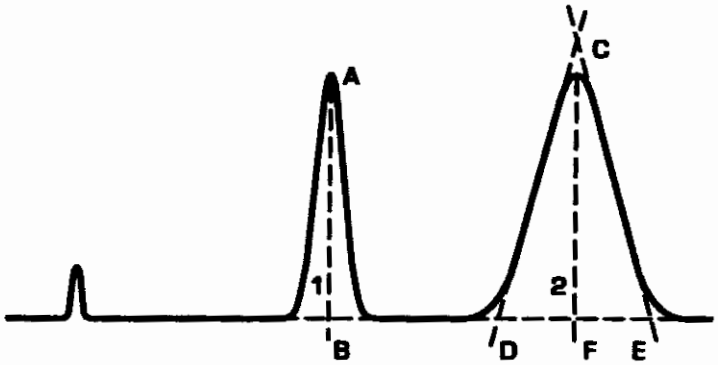
Other techniques for manual measurement of peaks have been described in various chromatographic texts; these include calculation of the product of peak height and width at half height, product of retention time and peak height, weight of peaks cut from chromatogram, peak area measured by a planimeter, and peak area measured by a mechanical integrator attached to the recorder. Comparison of results among some of these techniques indicated their validity [1], but none are described in this section because they are time-consuming and more difficult and offer no significant advantage over those presented here.

Measurement of Peak Height

Peak height measurements are recommended for early eluting peaks, peaks of width <10 mm, and very small peaks. If analyte and reference standard peaks are narrow and approximately the same size, comparison of peak heights is less subject to measurement error than is triangulation. Peak height measurements are very sensitive to changes in operating conditions, so operating parameters must be closely controlled for accurate quantitation.

To measure peak height, construct a baseline beneath the peak and measure the length of the perpendicular from peak apex to midpoint of the constructed baseline. In Figure 504-a, this is represented by line AB on Peak 1.

Figure 504-a
Manual Peak Measurement



Measurement by: (1) peak height and (2) triangulation.

Measurement of Area by Triangulation

Measurement by triangulation involves drawing a triangle that approximates a peak's dimensions and calculating the area of the triangle. This method requires

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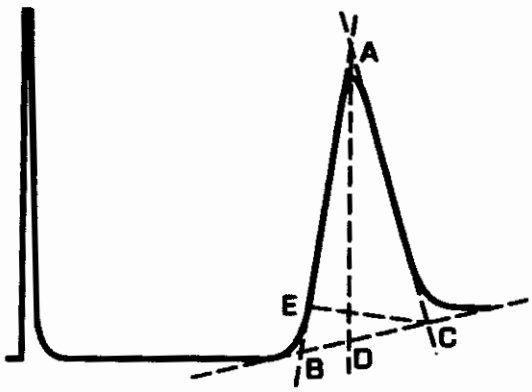
extreme care in construction of the triangle and in measuring its dimensions. Special treatment is required for peaks on sloping baselines and for skewed (asymmetrical) peaks. The technique is subject to error when the peak is narrow but is preferred over measurement of peak height when the peak is >10 mm wide at the base.

To construct the triangle, draw a baseline below the peak and draw inflection tangents to the peak, as shown in Figure 504-a, Peak 2. Drop a perpendicular bisect from the constructed apex to the baseline. Measure triangle height (length of bisect from baseline to constructed apex, CF in the figure) and the base (length of baseline between its intersection with the tangents, DE in the figure). Calculate area as 1/2 (base × height), *i.e.*, 1/2 (DE × CF).

Figure 504-b
Triangulation of Peak on Sloping Baseline



When the chromatogram baseline slopes under a peak, the line dropped from the intersection of tangents does not serve as an accurate measure of height because it is not perpendicular to the baseline; *e.g.*, Figure 504-b, line AD in triangle ABC. To measure the area of such a triangle, draw a perpendicular to one of the tangents (CE in Figure 504-b). Then use its length as the triangle height and the length of the tangent (AB) as the base. Calculate area from these values using the standard formula, *i.e.*, 1/2 (AB × CE).

Skewed peaks present another challenge to the validity of area measurement by triangulation. As a peak becomes more skewed, less and less of its area is included within the triangle drawn to approximate it. Skewed peaks may be tailing or fronting, depending on what physical phenomena caused the poor chromatography. The preferred solution to quantitation of skewed peaks is to improve chromatography sufficiently to cause peaks to be symmetrical. Use of a more polar column, changing column or inlet temperature, or optimizing the injection system may effect the improvement.

If manual quantitation must be performed on a skewed peak, measurement of the area using the formula for calculating area of a trapezoid is preferred [2]. In this system, peak widths at 15 and 85% of height are measured and used in the formula: area = 1/2 × height × (width at 15% + width at 85%). Calculations performed in this way have been shown to accurately represent peak area even for increasingly skewed peaks [3].

504 C: ELECTRONIC INTEGRATION

Electronic integration devices provide laboratories with powerful tools to accomplish their work more efficiently. Over the years, technology has progressively improved from simple desktop integrators to software programs operated by computers at all capability levels. The more powerful "automated data handling systems" can automate the entire determinative step, including monitoring of instrument temperatures and flow rates, operation of autoinjectors, acquisition of

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retention times and detector responses, and interpretation of those values for residue identification and quantitation. Unattended operation of instruments is common when automated systems are available; some systems are capable of simultaneous management of multiple instruments. Automated data handling is often incorporated into computerized laboratory information management systems capable of producing both the final laboratory report and the documentation necessary for quality assurance requirements (Section 206).

Discussion of entire systems is beyond the scope of this section. The focus is instead on measurement of peaks by electronic integration of the signal produced by a detector, *i.e.*, summation of the change in electronic signal per unit time. Beyond that generalized description of the integration process, each system operates under a unique "integration algorithm" that specifies how it will choose what part of the signal to integrate. The accuracy with which the system can measure detector response to a particular analyte depends on the algorithm itself, on the configuration options available to the user, and on the user's conscientiousness in choosing appropriate options. If an electronic integrator is properly configured, its measurement of peaks is the fastest, most accurate, and most reproducible available.

Major pitfalls exist, however, in the uncritical acceptance of results generated by electronic integration. Proper configuration of the algorithm, to the extent permitted by the system, is critical. After chromatograms have been run and results presented, review by a competent analyst is essential, because no integration algorithm can ever handle perfectly all the variations that occur in the chromatographic environment. The analyst must understand the concepts incorporated into the algorithm, be able to interpret the visual display of the chromatogram provided by the system, and evaluate whether integration was appropriately performed.

Data systems that perform electronic integration vary in the amount of "memory" available for storing data. Although simple integrators have only enough memory to process one chromatogram at a time, computer-based systems can usually store data associated with many chromatograms. In the latter case, when review of a chromatogram suggests that the original integration was performed improperly, the system can be reconfigured and a new calculation made from the stored data. If the system lacks the memory required to permit recalculation, the sample must be rechromatographed with the integrator reconfigured. Alternatively, the peak(s) can be measured manually from a printed chromatogram.

Optimum quantitation accuracy with any electronic integrator is dependent on the operator's making complete use of options available within the integrator. The following approach is recommended:

- Configure integrator for the GLC system. At the minimum, configure the integrator for the particular GLC system in use, rather than operating with default settings. Develop an integrator configuration for each GLC system routinely used. Store integrator settings as a "program," if the system permits, or keep a written record if necessary.

- Optimize peak and baseline recognition by considering the typical chromatograms the GLC system can be expected to produce. Chromatographic features and the conditions that determine them include: baseline noise, varying with type of detector; expected peak widths, dependent on column

and conditions; and inclusion of a solvent peak, dependent on whether or not solvent is vented. Most electronic integrators can be configured to specify the following options:

1) Size (in whatever value the integrator generates) below which a response is not recognized as a peak; sometimes called "area reject."

2) Range of peak base widths within which detector response is recognized as a peak.

3) Increase in baseline slope above which detector response is recognized as a peak; referred to as "threshold."

4) Appearance of multiple inflection points before the apex, used to identify the existence of two or more peaks when no "valley" exists between them. Some systems can classify such "shoulders" as front or rear.

5) Slope of peak above which response is recognized as the solvent peak; can be specified because solvent peak rises faster than most other peaks; value depends on detector, sensitivity, and column efficiency. May also permit recognition of peaks that appear on the tailing edge of the solvent.

- Use integrator features that demonstrate its operation. Most electronic integrators offer the option of displaying, on the chromatogram, an indication of exactly where the measurement started and ended. Some integrators can also be configured to show where the baseline was drawn. The analyst should take advantage of these features by choosing the option to print such indicators and should then use them in subsequent comparison of integrator measurements to the chromatogram.

- Configure integrator to accommodate particular chromatograms. An integrator configured by a pre-established program for a particular GLC system may not measure peaks accurately if the chromatogram includes responses to co-extractives or an unexpectedly complicated pattern of residues. Choose other options for configuration if experience with the commodity, method, or likely residues suggests in advance what type of chromatogram can be expected. For example, if the chromatogram is likely to contain isolated, symmetrical peaks on a flat, quiet baseline, configure the integrator to match peak width selection to measured width of the peak at half height, and set the threshold a few units below the highest value still capable of detecting the peak. In contrast, if the chromatogram is likely to contain peaks clustered together or with a noisy or sloping baseline, configure the integrator to accommodate those conditions. Table 504-a lists the effects produced when the two most important integrator settings, peak width and threshold, are varied.

- Review integrator measurements and reconfigure for accuracy. The analyst is ultimately responsible for accurate quantitation, so review and evaluation of chromatograms and integrator reports are essential. If examination reveals that the integrator inappropriately included or excluded portion(s) of the chromatogram, the following integrator options should be changed and the peak recalculated:

- Reposition baseline to appropriate base of the peak(s).
- Measure peak(s) appearing on top of much larger peak from baseline constructed to represent remainder of larger peak; sometimes called "tangent skim."
- Identify point at which to split incompletely resolved peaks, *i.e.*, where to end integration of one response and start integration of the next; sometimes called "split peak."
- Delete one or more peaks from integration; this does not remove peak from chromatogram.
- Integrate area within chromatogram as single number; useful when multicomponent residues, such as toxaphene, are being measured.

Table 504-a: Effects of Changing Electronic Integrator Settings

Setting		Result
Peak Width	Threshold	
High	High	Only major peaks detected; random noise eliminated
High	Low	Trace-level peaks detected; noise also recognized as peaks
Low	High	Peaks on sloping baselines detected; noise not detected
Low	Low	Narrow and broad peaks both detected (low peak width permits recognition of narrow peaks, while low threshold permits detection of broader peaks)

504 D: SPECIAL CONSIDERATIONS FOR COMPLEX CHROMATOGRAMS

Chromatograms that display residues of multicomponent chemicals or mixtures of two or more residues challenge the chemist to perform accurate measurement of peak size. Quantitative accuracy is further challenged when the residue has undergone degradation and the pattern of peaks does not match that of the most appropriate reference standard. The following procedures for quantitation of certain difficult residues have been developed during years of practical experience.

BHC (also known as HCH, hexachlorocyclohexane)

Technical grade BHC is a mixture of six chemically distinct isomers and one or more heptachlorocyclohexanes and octachlorocyclohexanes [4]; as a practical matter, the isomers α, β, γ, and δ are the only ones ever reported by FDA. The γ isomer is also known as lindane and is marketed as a separate pesticide. Currently, U.S. tolerances for BHC have been revoked, but residues are still found in imported commodities; U.S. tolerances for lindane remain on several commodities.

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Residues of BHC can be expected to vary in relative amounts of the individual isomers for several reasons: (a) Separate use of both BHC and lindane is possible, (b) commercial formulations vary in the percentage of individual BHC isomers present, and (c) isomers undergo different rates of metabolism or environmental degradation; *e.g.*, the elimination rate of isomers fed to rats was 3 weeks for the α, γ, and δ isomers and 14 weeks for the β isomer [5]. This difference in animal metabolism rates explains the typical finding of β isomer as the predominant BHC residue in dairy products.

Detector response to the same amount of different isomers may also vary. When BHC isomers were chromatographed individually on a wide bore methyl silicone column, relative response of an electroconductivity (halogen mode) detector (ElCD-X) to each isomer ranged from 0.58-1.00, while ^{63}Ni electron capture (EC) detector responses at the same conditions varied from 0.43-1.30 (Table 504-b). Both detectors responded less to ß-BHC than to the other three isomers [6].

Hexachlorobenzene, an industrial chemical and impurity associated with the pesticide quintozene, elutes near the BHC residues on all commonly used GLC systems. Although hexachlorobenzene has only occasionally been found in the same sample as BHC, it is important to ascertain that it is not present before BHC residues are quantitated. Several packed columns were once cited as capable of separating hexachlorobenzene and the four important BHC residues from one another [7, 8]. Among the GLC systems described in Section 302, the best choice for separating these residues is DG18 (50% cyanopropylphenyl, 50% methyl siloxane column at 200° C, electron capture detector). The column of DG18 is not compatible with ElCD-X, so DG22 (DEGS column at 180° C, ElCD-X) is recommended for confirmation of BHC residues as long as β- and δ-BHC, which do not separate, are not both present.

To quantitate BHC most accurately:

- Choose GLC system that separates residues in the sample from one another; if possible, use a halogen-selective detector, such as ElCD-X.
- Quantitate each isomer separately against a standard of the respective pure isomer.

Table 504-b: Response of Two Detectors to Four BHC Isomers

BHC Isomer	Ng Required for 1/2 FSD		Response Relative to Lindane	
	^{63}Ni EC	ElCD-X	^{63}Ni EC	ElCD-X
Alpha	0.24	0.41	1.30	0.71
Beta	0.72	0.50	0.43	0.58
Gamma (lindane)	0.31	0.29	1.00	1.00
Delta	0.33	0.49	0.94	0.59

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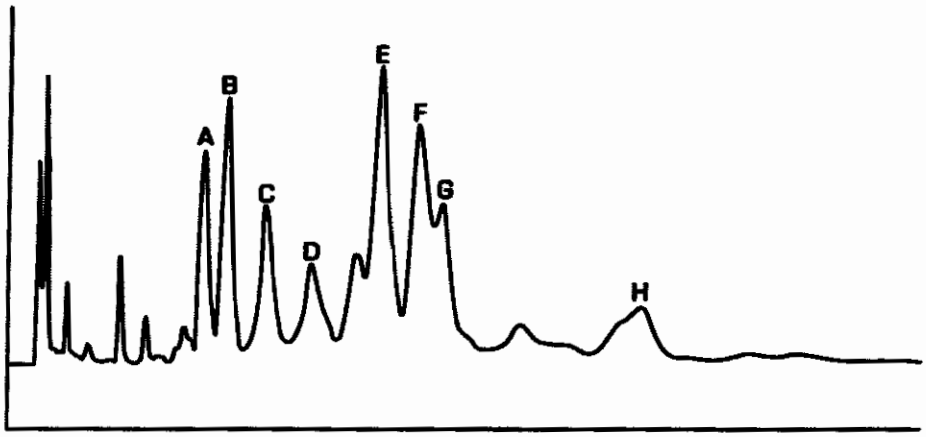
Chlordane

Chlordane is a technical mixture of at least 11 major components and 30 or more minor ones; Figure 504-c is a chromatogram of technical chlordane. Structures of the many chlordane constituents have been elucidated using GLC-mass spectrometry and nuclear magnetic resonance analytical techniques [9, 10]. The two major components of technical chlordane are trans- and cis-chlordane (Figure 504-c peaks E and F, respectively); the exact percentage of each in the technical material is not completely defined and is inconsistent from batch to batch.

At one time, heptachlor, a component of technical chlordane, was also marketed as a separate pesticide. When residues of heptachlor and its metabolite heptachlor epoxide were found in the same commodity as chlordane, the source of the former was in question. Currently, neither chlordane nor heptachlor is registered for use on foods in the United States, and tolerances for both have been revoked. Most residues that are now found occur in fish as a result of lingering environmental contamination.

The GLC pattern of a chlordane residue may differ considerably from that of the technical standard. Depending on the sample substrate and its history, residues of chlordane can consist of almost any combination of constituents from the technical chlordane, plant and/or animal metabolites, and products of degradation caused by exposure to environmental factors such as water and sunlight. Only limited information is available on which GLC residue patterns are likely to occur in which commodities (*e.g.*, References 11 and 12), and even this information may not be applicable to a situation where the route of exposure is unusual. For example, fish exposed to a recent spill of technical chlordane will contain a residue drastically different from a fish whose chlordane residue was accumulated through normal food chain processes.

Figure 504-c
Technical Chlordane



Chromatogram of 1.8 ng technical chlordane, chromatographed on system 302 DG 1. Labeled peaks are thought to represent, respectively: A, monochlorinated adduct of pentachlorocyclopentadiene with cyclopentadiene; B, co-elution of heptachlor and alpha chlordane; C, co-elution of beta chlordane and gamma chlordane; D, a chlordane analog; E, trans-chlordane; F, cis-chlordane; G, trans-nonachlor; H, co-elution of cis-nonachlor and "Compound K," a chlordane isomer.

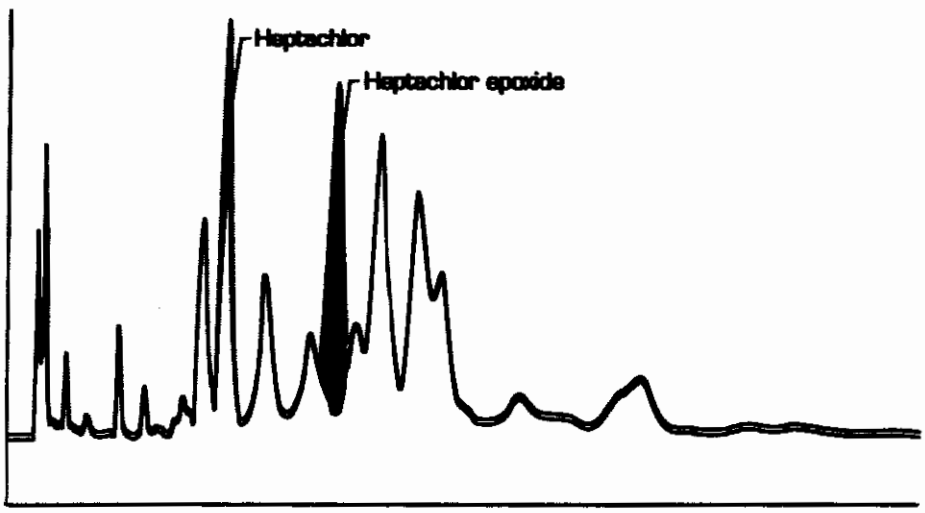
Because of this inability to predict a chlordane GLC residue pattern, no single method can be described for quantitating chlordane residues. The analyst must judge whether or not the residue's GLC pattern is sufficiently similar to that of a technical chlordane reference standard to use the latter as a reference standard for quantitation, then:

- When the chlordane residue does not resemble technical chlordane, but instead consists primarily of individual, identifiable peaks, quantitate each peak separately against the appropriate reference standard. Reference standards are available for at least 11 chlordane constituents, metabolites, or degradation products that may occur in the residue.

- When the GLC pattern of the residue resembles that of technical chlordane, quantitate chlordane residues by comparing the total area of the chlordane residue from peaks A through G (Figure 504-c) to the same part of the standard chromatogram. To define appropriate measurable area of chromatograms, adjust amount of extract injected so that the major residue peaks are about 50% full scale deflection (FSD), then inject an amount of reference standard that causes response within ±25% of that; peaks E and F in the two chromatograms should be about the same size. Construct the baseline beneath the standard from the beginning of peak A to the end of peak G. Use the distance from the trough between peaks E and F to the baseline in the chromatogram of the standard to construct the baseline in the chromatogram of the sample.

Peak H may be obscured in a sample by the presence of other pesticides. If H is not obscured, include it in the measurement for both standard and sample. If the heptachlor epoxide peak is relatively small, include it as part of the total chlordane area for calculation of the residue. If heptachlor and/or heptachlor epoxide are much out of proportion, as in Figure 504-d, calculate these separately and subtract

Figure 504-d
Chlordane, Heptachlor, Heptachlor Epoxide



Chromatogram of 1.8 ng technical chlordane, 0.1 ng heptachlor, and 0.3 ng heptachlor epoxide, superimposed on chromatogram of technical chlordane only; system 302 DG1.

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their areas from total area to give a corrected chlordane area. (Note that octachlor epoxide, a metabolite of chlordane, can easily be mistaken for heptachlor epoxide on a nonpolar GLC column.)

(When measurement of total peak area by integration was compared to addition of peak heights for quantitation of chlordane residue in several samples, the results of the two techniques were reasonably close; results justify the use of the "peak height addition" technique for calculating total chlordane when no means of measuring total area is available. To quantitate by peak height addition, measure heights of peaks A, B, C, D, E, F, and G, in mm, from peak maximum of each to the baseline constructed under the total chlordane area, then add heights. The technique has inherent difficulties because not all the peaks are symmetrical and not all are present in the same ratio in the standard and in the sample.)

PCBs

Polychlorinated biphenyls (PCBs) were manufactured for many years in the United States by the Monsanto Co. and marketed under the trade name Aroclor. Each Aroclor product was a mixture of chlorobiphenyl congeners into which 1-10 chlorine atoms were substituted; 209 different congeners were possible. Common commercial products included Aroclor 1221, 1242, 1248, 1254, 1260, and 1262, with the last two digits in the name indicating the average percent chlorination in the particular mixture; Aroclor 1016, purportedly a purified version of Aroclor 1242, was also marketed. Aroclors are no longer used or marketed in the United States, but their residues remain in the environment, in foods like fish and shellfish, in animals, and in human tissue.

GLC chromatograms of PCB residues contain many peaks, and patterns vary considerably, because residues can result from any combination of Aroclor mixtures. Variations in residue patterns are also caused by degradation from weathering or metabolism. Different congeners vary in the degree to which they are excreted by or retained within an animal and by the degree to which they volatilize. This multiplicity of potential PCB residue patterns makes the task of identifying and quantitating residues extremely challenging. The presence in the extract of residues from chlorinated hydrocarbon pesticides further complicates the determination. Residues of p,p'-DDE are most likely to interfere in determination of PCBs, because both residues are often present in the same commodity and because their structural and behavioral similarities make them difficult to separate with normal analytical methodology.

Quantitation of PCB residues is best achieved by following these steps:

- Isolate PCB residues from sample co-extractives and from other residues to the degree possible before GLC determination. Certain cleanup step options in Chapter 3 methods are designed to separate PCBs from pesticide residues of similar structure; these options should be used to analyze any commodity in which PCB residues are likely to occur, especially fish and shellfish.

- Select the reference standard that most closely resembles the residue pattern. A single Aroclor or, more often, a mixture of Aroclors that produce the most similar pattern is used for quantitation. Judgment must be made about what proportion of different Aroclors should be combined to produce the appropriate reference standard.

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- Use a GLC system that separates peaks efficiently. Packed or capillary columns may be used; wide bore capillary columns provide the best compromise of speed and efficiency.

- Choose from the following quantitation options the one that best suits the residue pattern. Both have been successfully collaborated in interlaboratory tests [13, 14]; choice depends on the degree to which the residue and reference standard match:

  1) When PCB residue pattern closely resembles that of a single Aroclor or mixture of Aroclors, quantitate by comparing total area or height of residue peaks to total area or height of peaks from appropriate reference standard(s). Measure total area or height response from common baseline under all peaks. Use only those peaks in the residue that can be attributed to chlorobiphenyls. These peaks must also be present in chromatogram of reference standards.

  2) When PCB residue pattern is significantly different from that of any Aroclor or mixture of Aroclors, quantitate by comparing area of each peak in residue to peak at same retention time in a specially calibrated lot of Aroclor reference standard (Table 504-c). To each peak thus measured, apply weight factor associated with that peak in particular reference standard, as listed in Table 504-c. Sum individual peak values to obtain total ppm PCB. This option can also be used when residue and reference standard chromatographic patterns match. The special Aroclor reference standards were calibrated using the separations effected by packed column chromatography, but the weight factors are also valid with chromatography on the equivalent wide bore capillary column operated in the packed column mode (Section 502 C).

Other quantitation techniques are sometimes used. One system makes use of capillary column chromatography, capable of separating most PCB congeners from one another, and a precalibrated reference standard mixture for which identity and weight percent of each congener have been established [15]. This "individual congener" capillary column method is significantly more time-consuming than measurement of individual peak areas or heights obtained by packed column GLC, and results from the two approaches are not significantly different when total PCBs are calculated [16, 17]. Several European countries use variations of the individual congener method by measuring, in sample and standard, only selected peaks [18]; in these countries, legal limits on PCB residues are defined in terms of results from the established quantitation method.

Accurate quantitation of both p,p'-DDE and PCBs in the same sample is possible only when chromatographed on a narrow bore capillary column. Quantitation of only the PCB residue, when p,p'-DDE is present, can be accomplished by first eliminating p,p'-DDE with derivatization and column chromatography [19].

Figure 504-e, a chromatogram of PCB residues isolated from chinook salmon, demonstrates the challenge of PCB determinations. Quantitation was performed by comparison to a mixture of Aroclors 1254 and 1260.

Table 504-c: Weight Percent Factors for Individual Gas Chromatographic Peaks in Aroclor Reference Standards

	Aroclor				
R_{DDE} (100x)[1]	1016 (77-029)[2]	1242 (71-696)[2]	1248 (71-697)[2]	1254 (71-698)[2]	1260 (71-699)[2]
11	0.2				
16	3.8	3.4	0.3		
21	8.1	10.3	1.1		
24	1.2	1.1	0.2		
28	16.8	15.8	6.0		
32	7.6	7.3	2.6		
37	18.5	17.0	8.7		
40	14.6	13.0	7.4		
47	11.6	9.9	15.7	7.1	
54	7.7	7.1	9.3	2.7	
58	6.4	4.4	8.3	1.2	
70	3.4	8.7	18.2	14.7	2.4
78		1.9	6.4		
84			4.6	18.6	3.6
98			3.4	8.3	2.8
104			3.3	14.1	
112			1.0		
117					4.4
125			2.3	15.6	11.0
146			1.2	9.0	13.3
160					5.5
174				7.4	10.0
203				1.3	10.9
232-244					11.2
280					12.5
332					4.2
360-372					5.4
448					0.8
528					2.0

[1] Peaks are identified by their retention times relative to p,p'-DDE=100 at conditions described in Section 302 DG1.

[2] Food and Drug Administration Lot Nos. (Weight factors are valid only for these FDA Lot Nos.) Aroclor reference standards are available from Food and Drug Administration, Division of Pesticides and Industrial Chemicals, HFS-337, 200 C Street SW, Washington, DC 20204. Aroclor 1016 (77-029) was referred to as KB-06-256 in *J. Assoc. Off. Anal. Chem.* (1978) 61, 272-281.

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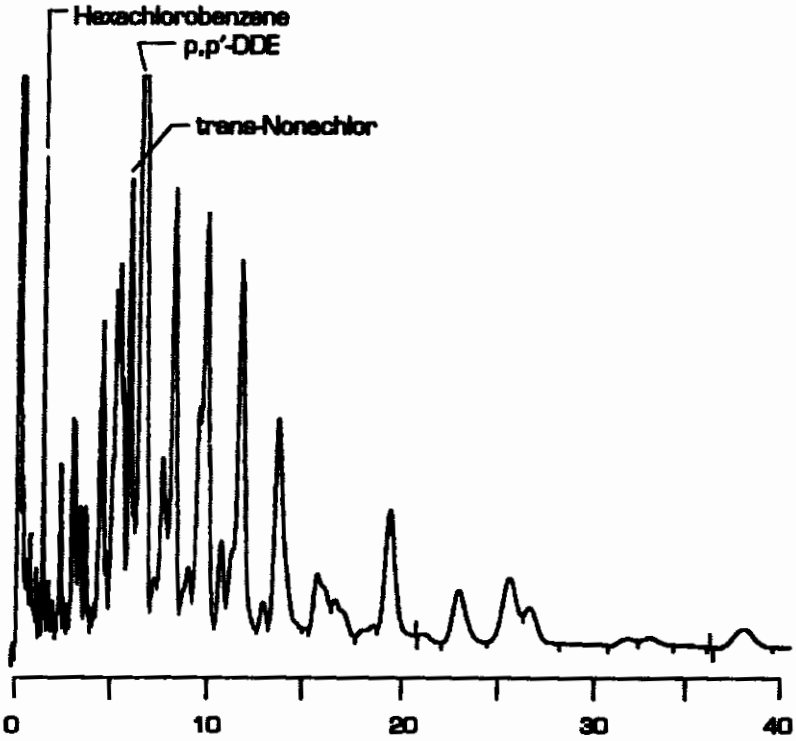
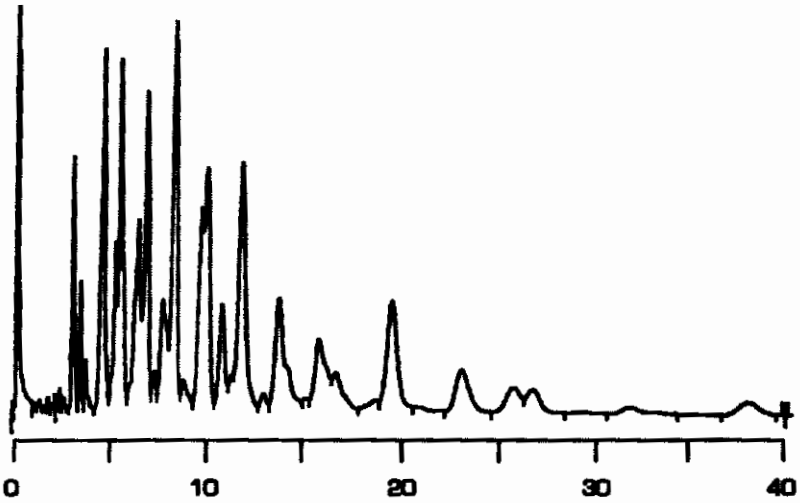
Figure 504-e
PCBs in Chinook Salmon



25 mg chinook salmon, extracted and cleaned up by method 304 E1+C3, chromatographed on system 302 DG1, with detector sensitivity greater than normal to permit measurement of low levels. Injection represents petroleum ether eluate from Florisil, which separates PCB residues from most but not all pesticides. Total PCB is 0.087 ppm, calculated using total area measurement, 0.090 using factors of Table 504-c; comparison is to mixed Aroclor standard below. Pesticides were identified but not quantitated.



1.23 ng Aroclor 1254 and 0.745 ng Aroclor 1260, chromatographed on same system as above.

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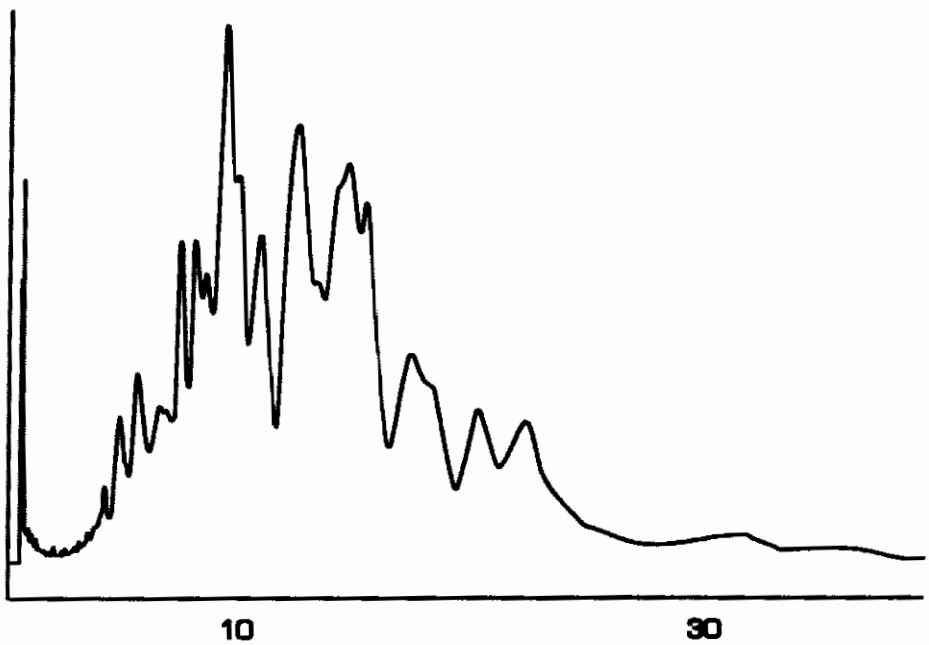
Toxaphene

Toxaphene is a complex mixture that results from the chlorination of camphene. The GLC chromatographic pattern for toxaphene does not display any individual peaks to simplify quantitation, but instead appears as a series of incompletely resolved peaks (Figure 504-f). Presence of other residues in the same extract as toxaphene requires estimation of baseline placement for quantitation. Reasonable accuracy is possible, but no truly quantitative technique has been developed.

To quantitate residues of toxaphene:

- Adjust amount of sample injected so that major residue peaks are 10-30% FSD.
- Inject amount of reference standard that causes response within ±25% of that of residue.
- Construct baseline for standard toxaphene between its extremities.
- Construct baseline under residue peaks, using distances of peak troughs to baseline on standard chromatogram as guide.
- Measure areas above baseline in sample and standard chromatograms for calculating level of residue. Relative heights and widths of matching peaks in the residue and reference standard will probably differ.

Figure 504-f
Toxaphene



Chromatogram of 11.4 ng toxaphene, chromatographed on system 302 DG1.

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