


Organic Trace Analysis by Liquid Chromatography



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To Jessie and Jimmie

Preface

During the past decade, modern high-performance liquid chromatography (LC) has expanded greatly, especially in the area of formulations analysis; this includes quality control of pharmaceutical preparations, cosmetics, food colors, pesticide formulations, and other sample materials where the compounds of interest are present in relatively clean matrices at concentrations of about 1% or greater. The application of LC to trace analysis has been slower to develop because of the difficulty in isolating and sufficiently purifying the analyte prior analysis. Of the many LC books available up to now, there is none completely devoted to organic trace analysis. It is hoped that this volume will go a long way toward filling the gap.

The introductory chapter provides an overview of the special requirements of LC for trace analysis and compares the approach to gas chromatography and formulations analysis, noting the essential differences. The chapters concerning LC equipment discuss the best conditions and types of instrumentation suitable for trace analysis. The detector chapter is particularly detailed, since the choice of the most appropriate detection system, as well as optimum operating conditions are important to the trace analytical chemist—especially at present when the selection of useful detectors is limited.

The chapter on chromatography theory is brief and includes mainly descriptive accounts of the principles of the various forms of chromatography, including recent developments (mainly through the work of Scott and Kucera) in the understanding of adsorption, reversed-phase, and ion-pair chromatography. Rigorous theoretical treatment of chromatography is not included in light of the abundance of texts already available on this subject. Chemical derivatization is an important tool for the trace analytical chemist, since it enables him to improve detection limits by forming a suitable derivative. The chapter concerning derivatization outlines many reactions applicable to trace analysis of a large number of substances in a variety of sample materials.

Sample preparation, including extraction and cleanup, is treated in some

depth. This is a very important area for trace analytical methodologies employing LC. The chapter includes many diverse approaches to sample extraction and extract purification and should provide the analyst with some guidelines for solving his analytical problems.

The final chapter illustrates typical approaches that have been used for trace analysis and shows how the various parts of a complete LC method are integrated to create a successful determination, including initial sample preparation, chromatographic separation, and detection. The chapter is divided into three major areas of organic trace analysis: clinical, environmental, and food analyses; it is intended to serve as a guide to the reader and is not a comprehensive compilation of the literature.

The book should be of value to all analysts involved in the determination of trace organics in many different substrates. It will be of particular interest as a teaching aid for those entering the field of trace analysis with the intention of employing LC.

I would like to express my sincere thanks to the Bureau of Chemical Safety, Food Directorate, Health Protection Branch, Department of National Health and Welfare, Canada for their support in preparation of this book.

James F. Lawrence

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Chapter 1

General Considerations in Developing a Trace Analytical Technique Employing Liquid Chromatography

I. LIQUID CHROMATOGRAPHY VERSUS GAS CHROMATOGRAPHY

Most analysts entering the field of liquid chromatography (LC) probably have had previous experience with gas chromatography (GC). Although the concepts of “chromatography” remain the same, there are important differences between the two types. In GC, the two major variables used to achieve a separation are the type of stationary phase and the column temperature. The chromatographic separation is based on a vapor pressure phenomenon and an affinity for the stationary phase. In LC the separations may be described as solute-solvent interactions where the solute molecules establish an equilibrium between stationary and mobile phases. For LC analyses one has the choice of different stationary phases and mobile phases. Column temperature is usually ambient and any deviations from this are used for special cases. Figure 1.1 illustrates a typical LC equipment setup.

At present, over 150 commercially available stationary phases for gas chromatography are available. These range from very nonpolar types, such as OV-1 methyl gum where separations are essentially based on differences in boiling points, to polar columns, such as the ethylene glycol esters or cyanopropyl silicones. The characteristics of many of these phases for separating several types of compounds have been tabulated by McReynolds (1). In LC the choice of stationary phases is far more limited. For adsorption chromatography silica gel or alumina is usually used.

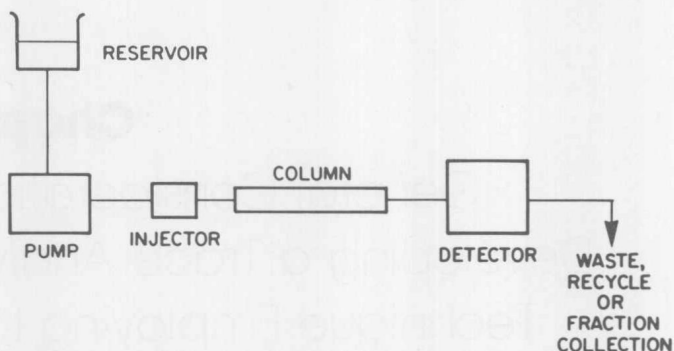


Fig. 1.1 Typical arrangement of LC equipment for organic trace analysis.

There are perhaps four or five commercially available types of polar bonded phases useful for normal-phase partition chromatography, and about seven or eight for reversed-phase partition chromatography. Research in this area is continuing at a good pace. However, at the moment, the vast majority of LC analyses are carried out on either silica gel or the C_8 or C_{18} reversed-phase materials.

From this it can be gathered that the mobile phase plays a far greater role in LC than in GC. It is this variable which gives LC its great flexibility. Such types of chromatography as adsorption, normal-phase partition, reversed-phase partition, ion-exchange, ion-pair, exclusion, ligand-exchange, and affinity chromatography make possible the separation of closely related species by a much wider variety of selective interactions. In effect, LC should be suitable for any type of compound that is soluble in a liquid suitable for a mobile phase. The choice of mobile phase is limited only by the compatibility of the solvents with the materials with which they come in contact (metal tubing, pump components, stationary phase material) and the type of detector. For example, halide salts or acids usually have to be avoided since they damage the stainless steel of most pumps and connecting tubing. If a UV detector is employed, then solvents such as benzene, acetone, or carbon disulfide cannot be used where absorbance is monitored below 280 nm, a region where most work with that detector is carried out. This illustrates the need for the analyst to understand the operation and limitations of his equipment.

In comparison to GC, LC is much less flexible in its detection systems. There are a number of very sensitive general and selective detectors for GC, including electron capture, flame ionization, and thermal conductivity as general detectors; the alkali-flame ionization detector is selective for nitrogen or phosphorus; the flame photometric selective to sulfur or phosphorus; and the electrolytic conductivity detector selective to ni-

trogen or halogens. Many attempts have been made to convert several of these for use with LC systems. However, since all of these operate by monitoring vapors, problems have been encountered in the phase change required. Transport arrangements have been designed where the eluant from an LC column flows into an oven or onto a moving wire, chain, or rotating disk, where the solvent is evaporated. The remaining solute molecules pass through either a detector using a flame or an electron capture cell. Because of the problems associated with the phase change, this approach to LC detection has not become popular. Thus, the most widely used detectors to date are those that carry out measurements in liquid streams. These include detectors based on absorbance, fluorescence, electrochemical oxidation or reduction, and refractive index differences, the last being the least sensitive or selective, thus generally unsuitable for trace analysis.

In GC, to analyze several compounds over a wide polarity or volatility range, temperature programming is employed. The equivalent in LC is gradient elution, where the mobile-phase composition is altered continuously during the chromatographic run. This normally requires two pumps and the necessary electronics to program them. However, ternary electronic mixing devices have been developed for solvent mixing and gradient elution with a single pump. Such an apparatus should become popular since it avoids the cost of a second pump.

The reproducibility of gradient runs and time required for reconditioning are somewhat superior in GC compared to LC. It is of course easier to make reproducible temperature-programming runs in GC since the stationary and mobile phases are not altered. However, in LC where the mobile-phase composition actually changes, the initial chromatographic conditions require a longer time to be reestablished. Also, retention values (k') are less accurately reproduced from one run to another in LC than in GC. Both systems are susceptible to detector interferences resulting from temperature (GC) or mobile-phase (LC) changes. These can be especially problematic when doing residue analysis where high detector sensitivities are required. In both types of chromatographic systems, isothermal (GC) and isocratic (LC) separations are to be preferred.

Start-up times are usually shorter for LC than for GC. Detector stability and chromatographic conditions are usually established in 30–60 min from complete shut-down. The time required for GC start-up is usually significantly longer. In some special cases in LC, such as ion-pair chromatography, start-up time also may be longer.

Maintenance requirements for both systems are of course different, but comparable in time required and frequency.

Since most analytical laboratories have more GC than LC equipment,

methodology is usually directed to GC. It is the opinion of this author that LC need be used only for those compounds that are not easily analyzed by GC. There is little value in developing LC methodology for compounds such as organochlorine pesticides, for example, since the GC methodology is so well developed. The area where LC has made great gains is in the pharmaceutical industry, where many drugs because of their size and nature are not suitable to GC analysis, whereas they are very easily analyzed by LC. In the final analysis, it is up to the individual to decide which technique would be more suited to his needs. This can only be done through experimentation.

Figures 1.2 and 1.3 compare GC-EC and LC-UV results of two pesticides in foods. The chromatograms each represent results from the same sample solutions. It can be seen in the case of terbacil, a uracil type of herbicide, that the LC results are far superior to the GC ones. However, at the same time, the opposite is true for the wild oat herbicide benzoyl-propethyl. Thus it cannot be said that one technique is better than the

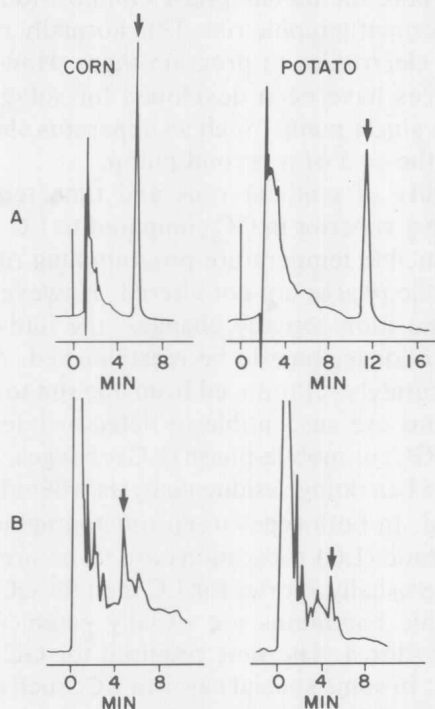


Fig. 1.2 Chromatograms of terbacil in corn (2.0 ppm) and potato (0.2 ppm) by LC-UV, 254 nm (A) and GC-EC (B). Injections were made from the same sample extracts. Arrow indicates terbacil peaks. From Lawrence (3), with permission from Preston Publications, Inc.

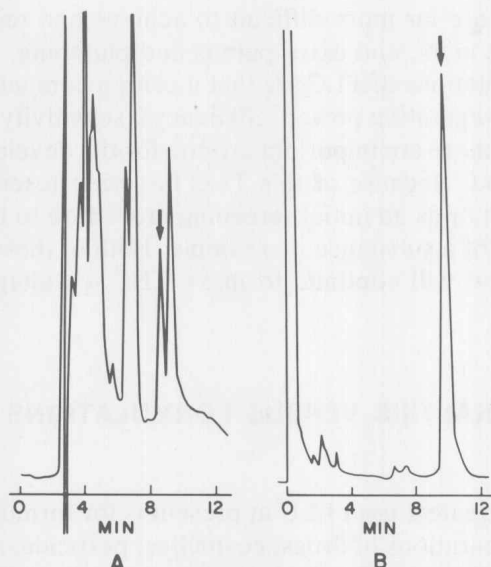


Fig. 1.3 Chromatograms of benzoylpropethyl in corn at 0.2 ppm. A, LC-UV (254 nm); B, GC-EC. Injections were made from the same sample extract (diluted in the case of the GC-EC results). Arrows indicate the peaks. From Lawrence (3), with permission from Preston Publications, Inc.

other. LC and GC complement one another and should be employed in that manner. Further discussion of the integration of LC methodology with that of GC is presented in Chapter 7.

Thin-layer chromatography (TLC) is not really a technique competitive with GC or LC. The necessary instrumentation cost far less than the other two but it is usually employed for qualitative or semiquantitative analysis. The major advantage of TLC is its simplicity and low expense. Most TLC plates are disposable and are used for only one chromatographic run, which for a 20×20 cm plate means that about 10–15 samples can be analyzed each time. Since the plates are used only once, the analyst can afford to try methods that include very little cleanup, and it is not necessary to worry about irreversible contamination of the stationary phase (the TLC layer). If a sample proves to be too dirty, the TLC plate is discarded and more cleanup of the sample extract is carried out. In LC, such a procedure could prove costly in ruined columns, especially in adsorption chromatography.

Another advantage of TLC is that selective post-chromatographic reactions can be carried out with relative ease. This simply involves spraying, dipping and/or heating the developed TLC plate to produce a colored or fluorescent spot sometimes detectable in low nanogram quantities. Such

reactions in LC are far more difficult to achieve and require specialized reagents, mixing units, and extra pumps and plumbing.

The major limitations of TLC are that it cannot compete with LC or GC in quantitation, separating power (efficiency), sensitivity, and ease of automation. All of these are important criteria for the development of a trace analytical method. Because of this TLC has been reserved for use as a confirmatory test or as an initial screening procedure to indicate the presence or absence of a substance in a sample. Both of these uses, however, are important and will continue to make TLC a widely used analytical technique.

II. RESIDUE ANALYSIS VERSUS FORMULATIONS ANALYSIS

Probably the greatest use of LC at present is for formulations analyses. Commercial preparations of drugs, cosmetics, pesticides, coloring agents, etc., are often monitored for quality control by direct LC analysis with little or no cleanup. The reason is that the solutes are present at concentrated levels in relatively pure form. Even the analysis of trace impurities in such samples without cleanup is possible since these are usually in the order of 100 ppm to 0.1%.

Residue analysis, on the other hand, is far more difficult to carry out because of the nature of the samples and low concentrations of solute. Materials such as food, biological tissue and fluids, soil, and natural waters create great problems with LC analyses. The removal of a drug or pollutant from such matrices inevitably brings with it a host of compounds with many similar properties (solubility, polarity, etc.). The analyst's problem becomes one of how to remove as many of these as possible for successful LC analysis.

How this is approached first depends on the nature of the compound of interest. For example, if the compound were strongly fluorescent, one would try to employ a fluorescence detector for the analysis. The cleanup would involve removal of interfering *fluorescent* materials. It is possible to have many coextractives present in the final sample solution but if they do not fluoresce, they will not interfere in the analysis. However, it must also be kept in mind that coextracted material can interfere in the chromatography by distorting peaks or altering retention values, when too much is present. Even though chromatograms may be relatively clean, the repeated injection of "dirty" samples can lead to contamination and eventually to decreased column life. To keep the chromatographic system functioning well for as long as possible, it is good practice to inject as little

sample as necessary to obtain a reliable result. This is especially important for trace analysis and of lesser importance for formulations analysis.

The major difference in trace analytical methodology and methodology for formulations as well as other commercial preparations is the concentration of the analyte in the sample and the extent of cleanup required. These differences are large ones and have been the main reason why trace organic analysis by LC has been slower to develop.

III. PUMP AND INJECTOR REQUIREMENTS

The minimum requirements for pumps are that they deliver essentially pulseless flow over the range 0.5–2.0 ml/min at high detector sensitivity. The noise of course depends upon the type of detector, but generally a satisfactory pump produces less than 1% noise at 0.01 absorbance units full scale (AUFS) on a UV detector above the detector noise itself. Most popular pumps in use today, including the dual- and single-piston reciprocating pumps, easily meet this requirement. Fluorescence detectors are less affected by flow fluctuations than are electrochemical detectors.

The most useful injection ports are those incorporating a syringe-loop configuration. These permit injection of different volumes up to the volume of the loop. They are easy to use and are preferred to stopped-flow injection or injection via a septum. Although all syringe-loop injectors are rated for operation at 3000 psi or more (which is more than adequate for most LC applications), those capable of operating at 6000 psi permit the use of higher flow rates for rapid mobile phase changes, or column conditioning or cleaning.

IV. DETECTORS

The prime requirement for LC detectors that are to be used for organic trace analysis is sensitivity. This is very important when considering the quantities of sample that must be injected to produce a reliable peak. If detector sensitivity is poor, then more sample must be injected, and if done on a regular basis this will lead to shortened column life. Sensitivity becomes increasingly important if ultralow levels (e.g., parts per billion) are to be determined. For most applications, detectors sensitive to low nanogram quantities of substance (1–50 ng, for example) are required. This of course eliminates the refractive index detector for application to trace analysis.

Selectivity is another important factor in detection. It can be consid-