Appendix G

Approaches to Monitoring Organic Environmental Contaminants in Food*

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INTRODUCTION

Trace quantities of potentially toxic organic compounds are frequently found in the environment. These compounds sometimes possess properties that may have teratogenic, mutagenic, and even carcinogenic effects on humans and animals. Some of the compounds, such as pesticides, have been intentionally released into the environment (1), while others have found their way into the environment by accident, through carelessness, or as byproducts of industrial processes. Many of these compounds are subject to breakdown in the environment as a result of both physiochemical and biological processes, such as chemical weathering, photodecomposition, metabolism, and biodegradation by micro-organisms (1,2). In many cases, it is not uncommon for the breakdown products to have greater health effects on humans and animals, than the parent compounds.

Because of the potentially toxic properties of so many of these compounds and their breakdown products, several pieces of Federal legislation have been enacted in recent years to monitor, evaluate, and control the amounts of the pollutants in order to protect our health and environment. As a result, monitoring[1](screening) programs have been established for many types of organic pollutants in the environment. All of these programs rely heavily on the ability of analysts to correctly identify and quantify these compounds at the parts per million (ppm) and parts per billion (ppb) levels in a variety of sample matrices (3). In order to successfully obtain useful information at these concentration levels, it is essential that methods which are sensitive and selective be developed and used. Whatever method is ultimately selected, it is imperative that it provide unequivocal results.

Most methods for monitoring and for the analysis for trace levels of organic pollutants in the environment necessarily consist of several steps. These include sample collection and storage, sample workup, and component identification and quantification.

The collection and storage of samples is an important phase of an analytical method if meaningful interpretation of the data obtained is to be achieved. The sample selected for analysis must be representative of the whole system being examined, and must be free of contamination due to improper collection and handling techniques. Once collected, the samples must be stored under conditions that will reduce or eliminate changes in their composition.

Another important phase in an analytical method is the sample workup. During this stage, the sample usually undergoes an extraction process whereby the compounds of interest are removed from the sample matrix. Organic solvents, and in some cases inert gases, are usually employed in the extraction process. Because the extraction process is seldom very selective, many organic compounds in addition to those of interest are also extracted. In order to reduce the amounts of these other compounds that may interfere with the analysis, a sample cleanup procedure usually follows the extraction process.

The last and most difficult phase of an analytical method is the identification and quantification stage. The identification process is often accomplished by comparison of the physical and chemical properties of the unknown compound against the same properties of an authentic standard compound. For complete unknowns, the identification process can be very difficult. The quantification process can only be accomplished after the unknown compound has been identified and usu-

* For purposes of this document, food represents all solid, semisolid, and liquid forms of food products, including bottled water, consumed by man.

[1]The terms "monitoring" and "screening" are used interchangeably in this document.

ally involves comparison of the detector response for the compound of interest against the detector response for known quantities of an authentic standard.

In the analysis for trace levels[2] of organic compounds in the environment, it is often very difficult to obtain accurate and reliable qualitative as well as quantitative results. This is evident by the countless examples in the literature of errors in both qualitative identifications and quantitative estimations of trace quantities of many organic compounds in the environment (5).

Of the many methods currently available for the qualitative identification and quantification of organic compounds, few are sensitive and specific enough for meaningful trace analysis. Table G-1 summarizes the techniques for organic analysis and some of their advantages and disadvantages (4,6).

The most common techniques in use for trace organic analysis are gas chromatography with the use of selective detectors (7) such as the electron capture (EC). Hall electrolytic conductivity, and flame photometric detectors; high performance liquid chromatography (HPLC) (8); and combined gas chromatography-mass spectrometry (GC-MS). Selective detectors for gas chromatography are necessary because environmental samples are often very complex and the selectivity of the detectors simplifies the analysis by allowing only certain compound classes to be detected at any one time. The most powerful of these techniques is the GC-MS technique (4,6,9-1 1), since it not only provides qualitative information of nanogram quantities of single compounds present in the sample, but also provides information which can be used for quantification of individual components in the sample.

A more recent technique involves the combination of liquid chromatography and mass spectrometry (12-14). This technique expands the area of trace organic analysis to the identification and quantification of compounds that are not suitable for analysis by gas chromatographic techniques.

[2] For purposes Of this document, "~ rare levels" is defined as a concentration below the low parts-per-million level. Detection at these concentrations is important because many organics are biologically active even at the parts-per-trillion level.

Table G-1 .—Techniques Available for Qualitative and Quantitative Organic Analysis

Method	Approximate detection limit, gm	Specificity or common uses
Gas chromatography		
Retention indices	10^{-10} (H^2 flame)	Detects most compounds
Electron capture	10^{-12}	Halides, conjugated carbonyls. nit riles. di- and trisulfides
Flame photometer	10^{-9}(S), 10^{-11}(P)	Phosphorus, sulfur
Nitrogen/ phosphorus	10^{-12}	Nitrogen, phosphorus
Chemical methods		
Pyrolysis	10^{-9}	Compound type determination
Chemical reagents	10^{-6}	Classical functionality determination
Electrolytic systems	10^{-8}	Sulfur, nitrogen, halogens
Instrumentation		
Infrared-grating	10^{-6}	Compound category type identification
-interferometer	10^{-7}	Compound category type identification
Ultraviolet	Variable to 10^{-10}	Aromatics conjugated carbonyls
Proton magnetic resonance	10^{-5}	Excellent for function. some molecular weight data
Mass spectrometer		
Batch inlet	10^{-7}	Best for complete identification, molecular weight structure, and function Confirm any compound
GC-MS mode	10^{-11}	
Multiple ion detection	10^{-12}	

SOURCE: Adapted from W. McFadden, "Techniques of Combined Gas Chromatography/Mass Spectrometry," Wiley-Interscience, New York, N.Y. 1973, p. 4, and "Trace Organic Analysis," Environmental Science and Technology, *12*, 757 (1978).

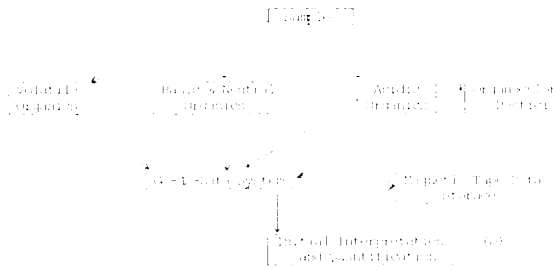
ANALYSIS FOR EPA PRIORITY POLLUTANTS IN FOODS AND WATER

The recent EPA/NRDC consent decree established an analytical procedure for the analysis of 129 priority pollutants (chemical indicators of organic pollution) in industrial waste water (1 5). Of these, 15 pollutants are metal, with the remainder being individual organic compounds and compound classes. The procedure for the analysis of the organics is strictly a GC-MS computer method designed to provide qualitative as well as quantitative information about the presence of the priority pollutants in waste waters.

Basically, the procedure requires that four separate analyses be performed on the sample. These are an analysis for the more volatile organics, an analysis for the basic and neutral extractable organics, an analysis of the acidic extractable organics, and an analysis for organochlorine pesticides. The analysis for volatile organics is usually accomplished by a vapor-stripping technique more commonly called the purge and trap technique, whereas all other analyses involve liquid-liquid extractions, and subsequent injection into a GC-MS system.

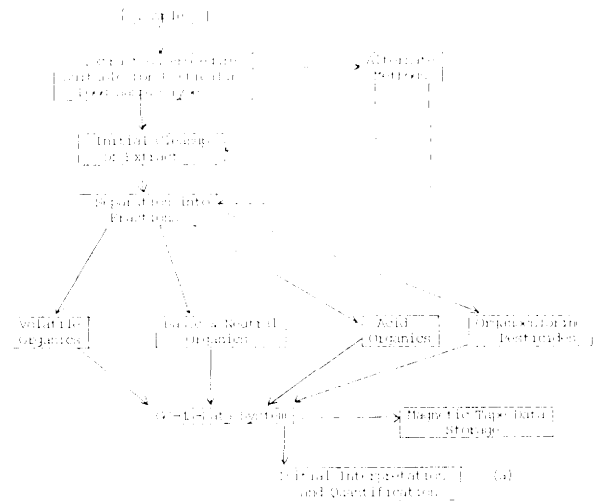
The procedure for waste water analysis can easily be applied to water and other liquids including foods; however, some modifications of the EPA procedure are necessary. This can be accomplished, since techniques similar to those developed for the analysis of tissues (16-18), can be adapted for foods. However, because of the greater complexity of the sample matrix, the resulting extracts usually contain large quantities of high-molecular weight organics that must be removed in order to achieve analysis of the individual trace organics. This removal process can be accomplished by one of several cleanup methods which are available, such as, liquid-liquid partitioning, chemical digestion, thin-layer chromatography (TLC), and liquid chromatography (LC). Whatever method is selected, it must be gentle enough to preserve the composition of the mixture of the trace organics, it must have a high recovery of the compounds of interest, and it must be quick and reproducible. Once the extraction and cleanup stages are complete, the remaining sample can be divided into different fractions and treated according to the methodology prescribed for the waste water analysis. The general analytical scheme is illustrated in figure G-1. Figure G-2 shows the general scheme modified for analysis of food samples. An estimate of the space, manpower, and cost associated with the analysis of the present 114 organic EPA priority pollutants in food and water samples is given in table G-2. It should be pointed out that considerable research and development effort must be expended to adopt the methods proposed to foods in general.

Figure G-1 .—A Simplified Diagram for the Qualitative and Quantitative Analysis of the Organic EPA Priority Pollutants



NOTES
[a]For most foods, this procedure requires modification. See figure G-2.
[b]Identification is based on the presence of characteristic ion fragments and associated chromatographic retention time data. Absolute identification would require a detailed interpretation of the complete mass spectrum of the organic compound of interest.

Figure G-2.—A General Scheme for the Qualitative and Quantitative Analysis of the EPA Organic Pollutants in Semisolid Foods



NOTE: Identification is based on the presence of characteristic ion fragments and associated chromatographic retention time data. Absolute identification would require a detailed interpretation of the complete mass spectrum of the organic compound of interest.

As noted in the revised April 1977 EPA document.

Table G-2.—Estimated Space, Manpower, and Cost Associated With the Analysis of Priority Pollutants in Tissue and Water Samples According to EPA Analytical Protocol[a]

Category	Response and/or cost
A. Instrumentation	GC-MS data system with automated liquid injection device. -$200,000 each
B. Space	1,500 ft² equipped with typical[b] laboratory facilities and furniture including adequate air-conditioning.
C. Downtime for instrumentation	30% (this figure can vary from 20 to 50% as a function of staff experience and logistics support).
D. Minimum assignable manpower	Ph. D or equivalent - 1 M.S. or equivalent - 1 B.S. or equivalent - 2 Total 4[c]

Estimated operational cost per routine sample -$2,000 to $2,500 (Scheme One as illustrated in figure G-I).

Estimated operational cost per routine sample -$2,500 to $3,000 (Scheme Two as illustrated in figure G-2, as a function of cleanup difficulty).

[a] It is assumed that the laboratory has other ongoing activities and further that samples would be analyzed on ly once and In succession The ultimate size and cost of such a monitoring facility is based on processing only a few hundred samples per vear No capital costs are calculated into the operational cost per sample

[b] Typical laboratory facilities and furniture Include laboratory benches tables with sinks shelves for reagents and equipment. vented hoods, refrigerators, laboratory balances and scales, pH meters, hot plates, laboratory glassware, c hem ical, etc This includes special toxic chemical and carcinogen processing fac ilities Laboratory space of the type desc ribed above cost on the order of $100/ft²

[c] Manpower requirements are not Included for maintenance and logistic suPPort

SCREENING FOR UNSPECIFIED POTENTIALLY TOXIC COMPOUNDS AND CHEMICAL CLASSES IN FOOD AND WATER

There is a possibility that other compounds or classes of compounds not included in the EPA priority pollutants list will find their way into the environment. For this reason, it is essential that some form of monitoring system be established that will look for the appearance of particular compounds or classes of compounds in food and water over a period of time. A recent National Research Council report on environmental monitoring (20) recommends the establishment of new monitoring programs to anticipate pollution problems and to discover environmental pollutants in their early stages of development so that appropriate corrective measures can be implemented before the problem becomes unmanageable, or worse, irreversible. A typical monitoring program is the EPA Mussel Watch Program (2 I), the EPA National Pesticide Monitoring Program, and the National Pesticide Monitoring Network for Birds operated by the U.S. Fish and Wildlife Service.

In establishing a monitoring program, the type of compounds to be monitored would be selected as candidate compounds on the basis of their chemical class, their use, and their suspected toxicity, These candidate compounds might include steroids, phenols, amines, halogenated organics, polynuclear aromatic hydrocarbons, and any newly appearing organic that illustrates a marked increase in concentration over a period of time.

The monitoring program design would involve the establishment of appropriate sampling and sample-handling guidelines, together with the modification of currently used analytical procedures to include the new compounds or classes of compounds. A typical monitoring program would involve the use of high-resolution gas chromatographic techniques using the universal flame ionization detector (FID) capable of detecting traces of known candidate compounds, and computer techniques that allow for rapid comparisons of samples to establish trends. With the use of high-resolution gas chromatography (employing glass capillary columns"), one can readily separate in an environmentally derived sample,

"Glass capillary columns reflect the state-of-the-art of gas chromatography, and are superior to the conventional packed columns of years past. For example, glass capillary columns are generally about 10 to 12 times more effective in separating complex mixtures.

several hundred organic species, the vast majority of which have not been characterized.

The analytical program would involve a preliminary screening to establish baseline levels of the candidate compounds in samples of foods and water, or selected indicator species over a given period of time, followed by periodic screening of similar samples to determine changes or trends with time. If alarming trends are observed, corrective actions and measures can be implemented.

Of the techniques available for analysis, the methods best suited for the class or classes of compounds under consideration would be selected for routine monitoring. These would include high-resolution gas chromatography or high-performance liquid chromatography and supporting computer methods which would include the use of internal and external standards, and use of pattern recognition techniques.[5] The monitoring would be set at a concentration level below the actual legal accepted 1evel (for example, 0.01 ppm or one-tenth of the action level if it is known),

Should the screening result in an observation of an increase in levels of either unknown or selected compounds or classes of compounds, suggesting the entry into the food or water of new materials, additional analytical efforts would be employed to attempt characterization of the new compound(s) observed. Preliminary information would be transmitted to associated toxicologists for evaluation and comparison with information available on known toxic compounds.

[5]Pattern recognition techniques are "data-interpretation processes developed by empirical examination of data from known sources, to elucidate relations from unknown data." (22)

LABORATORY REQUIREMENTS

Because organic analysis at the submicrogram (ppm and ppb) levels is a complicated and difficult process requiring sophisticated instrumentation and expertise, the laboratory selected to perform analyses for trace organics in foods and water must be equipped with state-of-the art technology and experienced personnel. The best setting for such a laboratory is in a location where it can establish ties with the R&D community where experts in a variety of disciplines can be found who can serve and participate as consultants at various levels. It should be pointed out that analytical techniques may require modifications to upgrade the technology and a substantial expenditure of funds to keep the program operating at maximum efficiency and information output.

The typical nationwide monitoring program would have several regional centers, each well-equipped with the appropriate instrumentation and personnel to perform the analyses and conduct the program. In addition each center would have a review board composed of senior scientific personnel in such fields as toxicology, analytical chemistry, environmental chemistry, etc., to assess and interpret the data developed. To facilitate the handling of all the data acquired, each center would be equipped with data-archiving facilities for both GC, LC, and GC-MS data and data-processing methods that would allow for rapid retrieval, comparison, and evaluation of these data, Some of these data-handling techniques are now available and others are under development [23-26] for such a central data management system (see table G-3). Figure G-3 illustrates a proposed analytical scheme to monitor for the appearance of unknown organics in addition to the current EPA priority pollutants in food and water samples.

In support of the nationwide monitoring program would be a quality control laboratory which would coordinate intercenter calibrations, and would spot-check and confirm selected data developed by the regional laboratories. Of necessity, the quality control laboratory would be better equipped than the regional centers, so that it could resolve the problems and issues that can occur during routine analyses. A minimum of 10 percent of all samples analyzed by the regional centers would be confirmed by the quality control laboratory, Additionally, a library of samples used in the actual analytical studies would be stored for future reference.

Table G-3.—Estimated Space, Manpower, and Cost Associated With the Monitoring of Unknown Potentially Toxic Compounds in Addition to the EPA Priority Pollutants

Category	Response and/or cost
A Instrumentation	a Small, high throughput GC-MS data-system with automated liquid injected device -$50,000. b EI/CI-equipped high-resolution MS-data system with automated injection device -$200,000 to $300,000 c GC-FID-EC system $15,000 (additional chromatographic systems may be required). d LC Interfaced into MS system -$20,000 e Central data management system $750,000 to $1,000,000. f. Cold storage and processing facilities $200,000.
B Space	~ 5,000 to 6,000 ft² equipped with typical laboratory facilities[a] and furniture including adequate air-conditioning.
C Downtime for instrumentation	30% (this figure can vary from 20 to 50% as a function of staff experience and logistics support).
D Minimum assignable manpower	a. *Direct* Ph.D or equivalent - 2 M.S. or equivalent - 3 B.S. or equivalent - 3 Total 8 b. *Indirect support* Ph.D. or equivalent - 2 B.S. or equivalent - 2 Total 4[b]
E Estimated operational cost per routine sample	~ $3,000 to $4,000 per sample if data are routine. The cost per sample which has new components and requires extensive characterization is difficult to estimate, but would certainly be greater than the cost per routine sample. For example, cost may exceed $10,000 per individual compound.

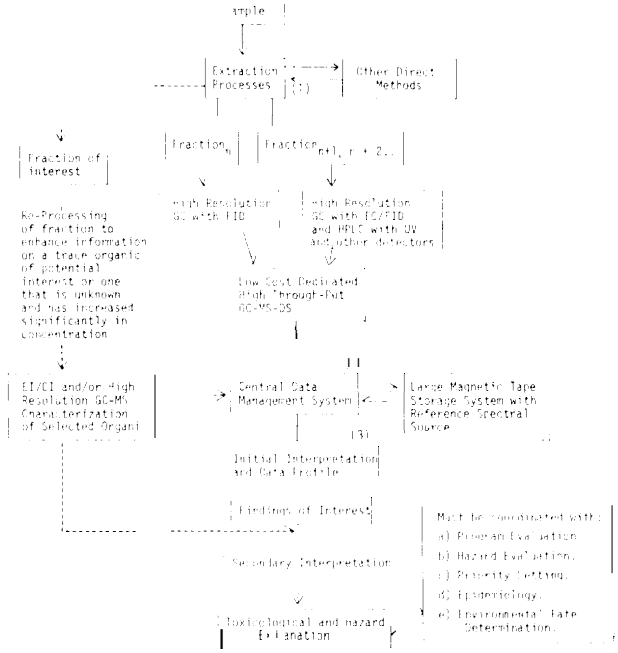
[a]Typical laboratory facilities and furniture include laboratory benches, tables with sinks, shelves for reagents and equipment, vented hoods, refrigerators, laboratory balances and scales, pH meters, hot plates, lanoratory glassware, chemicals, etc. This includes special toxic chemical and carcinogen processing facilities.

[b]Manpower requirements are not included for maintenance and logistic support.

NOTE 1: It is assumed that the laboratory is not involved in toxicological and human hazard evaluation and the general data system hardware is available. However, only a portion of the required software can presently be employed and R&D work remains to be done to make the entire proposed system functional (22-25).

NOTE 2: The size and estimated cost of such a monitoring facility is a function of the numbers of samples to be processed per year. At this time it is impossible to estimate the number of indepth characterizations that may be necessary per year. The above described facility is designed to process only a few hundred samples per year. No capital costs are calculated into operation cost per sample.

Figure G-3.—General Analytical Scheme To Detect and Monitor New Trace Organics and Priority Pollutants in Food and Water Samples



NOTES

1 A variety of fractionation and isolation procedures may be employed in addition to liquid/liquid extraction

2 Central data system would conduct the following activities:
- index and store all raw data on magnetic tape or disc
- calculate a retention index for each unresolved component
- compute changes with time for each resolved component and estimate normal concentration variations per unit time
- flag variations outside expected threshold values and/or the appearance of a newly resolved component
- conduct pattern recognition techniques for new groups or species of organics
- store all primary and secondary interpretations of finds, qualitative and quantitative findings, and information for toxicological and hazard evaluation
- cross correlate mass spectral data of known compounds with gas chromatographic data for unresolved unknowns

3 A complete mass spectrometric evaluation of each fraction from a given sample type or location would be accomplished initially to establish the identity of resolved components and followed by analytical exercises designed to provide information on specific components

4 Preliminary interpretations of data would be designed to pick out the presence of new compounds, or an increase in concentration of those already present. Confirmation would require a re-analysis of a given sample

DEVELOPMENTS THAT WILL IMPACT LOW CONCENTRATION ORGANIC MONITORING

Of all the developments in technology for chemical analysis, those which will have a greater impact on monitoring programs are the following: 1) developments in mass spectrometry instrumentation, such as pulsed positive-negative chemical ionization and detection techniques; 2) developments in selective detectors for gas chromatography and liquid chromatography, such as atomic absorption (AA) and atomic fluorescence (AF) spectrometry, and Fourier transform-infrared (FT-IR) detectors; and 3) developments in computer system software capable of handling massive volumes of chromatographic as well as mass spectrometric data of the type obtained in a monitoring program. Developments in other methods such as electrochemical techniques and plasma chromatography show some promise for trace organic monitoring, but will only be useful if they can be coupled to GC or LC systems.

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GLOSSARY OF ABBREVIATIONS AND TERMS USED

Abbreviations

AA—atomic absorption
AF—atomic fluorescence
CI—chemical ionization
DS—data system
EC—electron capture
EI—electron impact
FID—flame ionization detector
FT-IR-Fourier transform infrared
GC—gas chromatography
GC-MS--combined gas chromatography-mass spectrometry
HPLC--high performance liquid chromatography
LC—liquid chromatography
LC-MS--combined liquid chromatography-mass spectrometry
MS—mass spectrometry
TLC--thin-layer chromatography
UV—ultraviolet

Terms

Atomic Absorption Spectrometry—a form of spectrochemical analysis usually applied to the determination of the elements. The sample is heated to a relatively high temperature to cause dissociation of the chemical compounds into atoms. A source of radiation characteristic of the element to be determined is passed through the sample. If the sample contains the element, absorption of the radiation by the sample atoms occurs and the amount of absorption can be measured for quantitative determinations.

Atomic Fluorescence Spectrometry—a form of spectrochemical analysis usually applied to many organic compounds and some inorganic compounds which emit radiation energy after they have first absorbed radiant energy of a particular frequency. The simplest form of AF is the fluorescence provided by a monoatomic vapor, such as sodium.

Chemical Ionization—a method for ionizing samples for mass spectrometric analysis. The ionization of the sample results from the reaction between the sample molecules and low-velocity reagent ions which results in the transfer of a charged species other than an electron.

Electron Capture Detector—a very sensitive and selective detector for gas chromatography. This detector responds to the presence of a variety of compounds containing atoms with an affinity for electrons, such as the halogens--chlorine, bromine, and fluorine—and o t her atoms, such as oxygen, and sometimes. even sulfur.

Electron Impact—a method for ionizing samples for mass spectrometric analysis. The ionization of the sample results from the bombardment under high vacuum of the sample molecules by a beam of electrons, usually at an energy of 70 electron volts (eV).

Flame Ionization Detector—a universal detector for gas chromatography. This detector is fairly sensitive and very linear, and responds well to most organic compounds.

Fourier Transform-Infrared Spectrometry—this is the state-of-the art of infrared spectrometry. This technique employs minicomputers and Fourier transform methods in the acquisition of infrared spectra. Advantages of this technique include the making of measurements in a fraction of the time required for the more conventional methods, and increased sensitivity .

Gas Chromatography—one of the most widely used analytical techniques for trace organic analysis. The technique is simple and very rapid to use, is extremely sensitive, allowing the use of minute amounts of samples, and can be very useful for preliminary screening of environmental samples.

Gas Chromatography-Mass Spectrometry—a very powerful analytical technique that combines the features of gas chromatography and mass spectrometry.

High-Performance Liquid Chromatography—high-resolution, high-speed, and high-sensitivity liquid chromatography.

Liquid Chromatography—a separation technique that allows the partition of the sample between two phases, a liquid and a solid, or two liquid phases.

Liquid Chromatography-Mass Spectrometry—a recently developed analytical technique that combines the features of liquid chromatography and mass spectrometry.

Mass Spectrometry—a very powerful tool for providing the structural identity of complex organic molecules. Mass spectra furnish information about the arrangement of atoms within a molecule on the basis of the fragmentation pattern of the compound as a radical ion, which is usually produced by electron bombardment in the ion source of the mass spectrometry.

Thin-Layer Chromatography—a form of liquid-solid chromatography conducted on the surface of specially prepared plates. This technique is generally very quick and simple to use and is effective in performing separations of small amounts of sample.

Ultraviolet Spectrometry—a form of absorption spectrometry generally suited for analysis of compounds that are capable of absorbing ultraviolet radiation. These include aromatic compounds, conjugated ketones, and other conjugated compounds, such as polyolefins.