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Chromatographic and Mass Spectrometric Characterization of a Landfill Leachate and an Industrial Wastewater

by

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(ABSTRACT)

The purpose of this research was to apply analytical techniques to identify and investigate specific organic compounds present in a municipal landfill leachate and an industrial wastewater. Accurate characterization of wastewaters can assist environmental engineers and scientists in the design of treatment systems. Several extraction and analytical techniques were utilized for the analysis of components in complex environmental samples focusing on nonvolatile or thermally labile compounds.

Of the extraction procedures evaluated, C_{18} solid phase extraction was found most useful in preparing the samples for analysis. Recoveries ranged from 48% for a benzenesulfonamide to 96% for 2,4-dinitrotoluene. Liquid chromatography/mass spectrometry (LC/MS) techniques were utilized in conjunction with gas chromatography/mass spectrometry (GC/MS) and liquid chromatography with a diode array detector (LC/DAD), to identify specific organic chemicals in the samples.

GC/MS analysis of the leachate confirmed the presence of two benzenesulfonamides and two phthalate esters. Several other components were detected, but not identified. A significant number of components were detected by LC/MS that were not detected by GC/MS. Thermospray LC/MS results provided positive and negative ionization spectra which were useful for identifying standards and providing molecular weight information.

GC/MS, LC/DAD and LC/MS analysis of the industrial wastewater confirmed the presence of 2,4-dinitrotoluene, 2,4,6-trinitrotoluene, diphenylamine and dibutyl phthalate. GC/MS analysis also confirmed the presence of 4-nitro-2-aminotoluene. Tentative identification of methylnitrobenzene, dinitrobenzene, aminonitrobenzaldehyde, and a dinitrotoluene isomer was made by GC/MS while two components remained unidentified. LC/DAD analysis also confirmed the presence of dioctyl phthalate, aminobiphenyl and a diphenylamine impurity while ten components were not identified. LC/MS results suggested the presence of a dinitrotoluene isomer, a diphenylamine dimer, N-nitrosodiphenylamine, methylnitrobenzenamine and dioctyl phthalate, while ten other components remained unidentified. Thermospray has severe limitations in its ability to identify unknown constituents. However, the application of the methods explored in this work to monitor the effectiveness of wastewater treatment is warranted.

Dedication

This thesis is a tribute to the love and support of the Danzig family. It is dedicated to the unity and pride that binds us as a family. May we remain together forever.

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Many people affected me during my seemingly interminable stay at Virginia Tech. I wish to acknowledge and thank all of them that made my stay here meaningful and memorable. I apologize in advance if I fail to mention anyone. Such omission can be attributed to the condition of my mind following completion of the rigors of this program (assuming I was more lucid when I embarked on my graduate career).

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One of my samples was supplied by an anonymous donor. I thank all those who provided assistance.

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I. INTRODUCTION

A. Background

The area of wastewater treatment is extremely large and varied. Environmental wastewaters may include contaminated ground and surface waters. Leachate from a landfill may be a potential source of pollution of both ground and surface waters. If it can be retained, leachate can be treated to mitigate deleterious effects on the environment. Industrial wastewaters are the effluents resulting from any type of industrial process. They may contain heavy metals, high organic loadings or low levels of extremely toxic substances.

The goal of engineers responsible for the treatment of wastewaters is to design treatment systems that produce desirable effluents. Since such treatment must be specific for each particular wastewater, the engineer must know what is to be treated. Accurate characterization of the waters is therefore essential. Information that is often obtained for design purposes include: pH, temperature, measurement of total organic loadings (total organic carbon; chemical and biochemical oxygen demands), and quantification of various inorganics such as nitrates, nitrites, phosphates, sulfates and metals. Analysis of specific organic chemicals is often overlooked.

If treated wastewater achieves a 95% reduction in chemical oxygen demand, but contains high levels of a priority pollutant, is that water sufficiently treated? Characterization of the organic fraction of wastewaters is very important, especially for regulatory purposes, permit compliance, etcetera. Proper identification of specific organic constituents not only highlights specific treatment needs, but may also alert one's attention to reaction products that may result from a certain treatment technique of a particular class of compounds. Or perhaps a group of constituents are amenable to treatment at one pH range, but remain untreated at another. In order to best know how to treat a waste, the composition of the wastewater needs to be known. A multitude of techniques are available for organics analysis. It is important for environmental engineers to know what information each analytical tool can provide and what are their limitations.

The techniques most commonly used for the analysis of organics in water rely on gas chromatography (GC) with various detectors. These techniques have developed into powerful analytical tools, yet they are limited to the detection of volatile or readily volatilized organic substances. It is estimated that approximately 80% of the total organic matter in water are of the nonvolatile fraction and therefore not amenable to GC analysis (Crathorne *et al.*, 1984). Much work has been conducted to develop techniques for the analysis of nonvolatile, generally polar organic compounds, utilizing liquid chromatography with mass spectrometry (LC/MS).

B. Research Objectives

This research served to continue in the endeavor to develop and apply methods for broad spectrum analysis of environmental samples that are capable of detecting and identifying both semivolatile and nonvolatile organic compounds. The objectives of this research may be stated as follows:

- to develop methods for the analysis of nonvolatile, primarily polar organic pollutants that are difficult to determine by previously utilized techniques;

- identification of specific organic compounds present in a landfill leachate using these techniques in conjunction with LC/MS analysis;

- to apply these methods to characterize other environmental samples, specifically an industrial wastewater;

- apply these techniques to monitor the effectiveness of wastewater treatment.

II. LITERATURE REVIEW

I. Developmental Perspective On Chromatographic Methods

A. General

Much of environmental research centers on man's desire for clean drinking water and natural waters suitable for wildlife and recreation. "Clean" is often determined/limited by the analyst's ability to detect and identify anthropogenic materials broadly classified as pollutants. Unless one has the technical capability to determine that a compound is in a sample, the compound is, for practical purposes, not there.

The existence of organic pollutants can be grossly measured by such techniques as Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Total Organic Carbon (TOC). However, these methods do not identify specific pollutants in the sample (Keith, 1976). In 1950 the need to separate constituents of mixtures for identification was recognized, and this lead to the development of chromatographic methods (Rosen, 1976). The separation of compounds is really a definition of chromatography. In the 1950's forms of chromatography

available included column adsorption, column partition, paper, electro, thin layer and gas chromatography (Rosen, 1976).

B. Gas Chromatography

When gas chromatography (GC) was developed in 1952, this method obtained phenomenal separation of organic compounds compared to other methods existing at the time (Miller, 1988). GC performance was greatly enhanced in the 1970's with the development of fused silica capillary columns. Common internal diameters of these columns are 0.25, 0.32 and 0.53 mm. The interior walls of fused silica columns are typically coated with a thin (0.1 - 1.0 μm) film of liquid stationary phase (Miller, 1988). Excellent sensitivities were obtained when GC was coupled with any of a number of detectors including electron capture, flame ionization, thermal conductivity and mass spectrometry. One of the first environmental applications of gas chromatography was the analysis of chlorinated pesticides (Rosen, 1976). An example of early uses of gas chromatography coupled with mass spectrometry (GC/MS) was the isolation and detection of geosmin, a source of musty odors in water and a major taste and odor problem for water utilities (Rosen, 1976).

C. Mass Spectrometry

Mass spectrometry provides high sensitivity (picogram level) and great versatility. MS is a universal detector, i.e. it can detect any compound that can be ionized. Ionization, a key event in mass spectrometry, can be acheived by a variety of modes, e.g. electron ionization (EI), chemical ionization (CI), fast atom bombardment (FAB), field desorption (FD), and other methods. MS provides a wealth of structural information for detection and identification of unknown analytes in samples.

EI typicaly uses 70 eV electrons to ionize molecules already in the gas phase and is the most common technique used with GC. The impact of high energy electrons results in extensive fragmentation of the molecules which often yields enough structural information to identify the molecule (Rose and Johnstone, 1982). Mass spectral libraries are generally of EI mass spectra at 70 eV, and can be applied to elucidate the identity of an unknown molecule. The NBS(EPA)NIH library contains approximately 45,000 compounds; the Wiley/NBS *Registry of Mass Spectral Data* includes approximately 112,000 different compounds. Library matching is not, however, always correct. Spectral libraries routinely check for errors, but cannot always compensate for sample impurities, spectrometer performance, meassurement inaccuracies, and data transcription (McLafferty and Stauffer, 1989). Therefore, techniques other than EI are needed.

Chemical ionization is a soft ionization technique that ionizes sample molecules with much less energy transfer than EI, and therefore less fragmentation. It results in the formation of molecular ions which yields primarily molecular weight information. The technique was pioneered by Frank Field and Barnaby Munson in the mid 1960's (Watson, 1985). CI complements EI; it utilizes different techniques to provide different information. CI forms even electron ions which have less tendency to fragment. The amount of energy transferred in a CI reaction can be controlled by knowing the proton affinity (P.A.) of the reagent gas compared with that of the analyte. The proton affinity of the analyte must be greater than that of the reagent gas for the analyte to be protonated by the reagent ion. For example, methane (P.A. = 5.9 eV) can ionize a broader range of compounds than ammonia (P.A. = 9.1 eV). As the amount of energy transferred increases, the amount of fragmentation increases.

Samples can be introduced into a mass spectrometer in all phases. Gases and liquids can be injected directly into the ionization chamber while solids are introduced into the vacuum system by positioning a direct inlet probe at the edge of the ionization chamber. Samples are often applied to the probe tip in glycerol or some other matrix. The extent of vaporization off the tip is often affected by the probe tip temperature. If impurities or more than one compound

are on the probe tip, multiple spectra may be obtained and the analysis skewed. For mass analysis of complex samples, it is necessary to separate the constituents prior to introduction into the MS. This has resulted in the coupling of chromatographic systems with mass spectrometers.

This was first achieved in the 1950's with the coupling of gas chromatography to mass spectrometry (GC/MS). By combining the potent separation abilities of gas chromatography with the broad detection capabilities of mass spectrometry, GC/MS has developed into one of the most powerful tools in environmental analysis. This fact is recognized by the US Environmental Protection Agency (EPA) in the October 24, 1984 Federal Register (Extrel). GC/MS is the basis of many of the regulated EPA methods and literally thousands of GC/MS analyses are performed daily to meet monitoring and analysis requirements.

D. Liquid Chromatography

What, then, is the need for the development of a whole new analytical system of liquid chromatography coupled with mass spectrometry (LC/MS)? The answer lies in the perspective of recognizing the limitations of currently available techniques. When looking at complex environmental samples, one frequently has little prior knowledge of which compounds, especially potentially hazardous components, may be in the sample, and therefore would like to utilize analytical methods that will detect and identify as many compounds as possible. GC methods are limited to the analysis of volatile or readily volatilized organic chemicals. It is known that certain potential carcinogens, such as most nitrosamines, do not possess adequate thermal stablity to be detected by GC/MS (Beattie *et al.*, 1985). A current problem in environmental analysis is the inadequacy of broad spectrum methods to analyze nonvolatile, generally polar, organic components. It is estimated that approximately 80% of the total organic matter in aqueous environmental samples are of the nonvolatile fraction (Crathorne *et al.*, 1984). Although over 1500 organic compounds have been identified by GC in various types

of waters, over 2,000,000 organic compounds are known to exist (Keith, 1976). Similarly, metabolic studies of environmental pollutants usually require analysis of polar metabolites which are not amenable to GC/MS, but are more readily separated by liquid chromatography (Dietrich, 1987). Killops *et al.* in their 1985 study of humic and fulvic acid by-products stated, "techniques for investigating the non-volatile fraction (polar and high molecular weight compounds) need to be developed and applied". Successful analysis requires a method that combines good separation with high specificity and sensitive detection.

Liquid chromatography, with the wide variety of phases available, is ideally suited for the separation of polar, nonvolatile or thermally labile compounds. The only restriction is that the sample must be slightly soluble in the mobile phase. High performance liquid chromatography (LC or HPLC) has broad application to environmental problems and can compliment GC analysis. It can be used to detect sources of pollution, to test the effectiveness of treatment steps and to determine the ultimate environmental effects of process effluents (Pitt *et al.*, 1976). In one of the first environmental applications of HPLC in 1972, 77 constituents from a primary sewage were identified (Rosen, 1976). Pitt *et al.* (1976) identified 56 soluble organic compounds in primary effluent from a municipal sewage treatment plant, while 103 constituents remained unknown. They note that with HPLC, the "likelihood of altering the nature of the compounds in the samples, as occasionally occurs with other methods, is reduced".

Other advantages of LC over GC are that compounds are not exposed to excessive heat, less sample cleanup is required, and derivatization is usually not necessary (Covey *et al.*, 1986). The major disadvantages of LC are the reduced chromatographic efficiencey compared with capillary column GC and the inability of most HPLC detectors to differentiate unresolved peaks. The most common HPLC detector is the ultraviolet/visible (UV/VIS) spectroscopic detector. Single wavelength absorbance detection is not specific enough to allow qualitative identification of compounds present in complex samples with any degree of certainty (Vargo and Olson, 1985).

Different chemical classes of compounds may display overlapping retention times such that specific identifications are not readily made from a non-specific detector like UV. Therefore, a detector that gives more highly specific information than single wavelength UV is desirable for analyzing complex mixtures (Amateis, 1984). The introduction of the diode array detector (DAD) has alleviated many of these limitations. This variable wavelength detector simultaneously monitors absorbance over the ultraviolet (UV) i.e. 210 - 380 nm, and part (usually up to 600 nm) of the visible wavelength range. It is estimated that about 65% of the organic compounds analyzed by liquid chromatography absorb some light at 254 nm (where most single- wavelength UV detectors operate) while over 90% absorb light somewhere in the range of most variable wavelength detectors (Yost, Ettre and Conlon, 1980). UV/VIS detectors remain limited to detecting compounds that absorb in that wavelength range. They also provide limited structural, or other qualitative information that can aid in the identification of unknown constituents of a sample and cannot yield molecular weight information.

II. LC/MS

Mass spectrometric detection of analytes separated by liquid chromatography can certainly overcome the limitations of UV/VIS and other LC detectors by providing information that those detectors do not provide. Direct coupling of the two techniques can serve as a powerful analytical tool in much the same way that GC/MS methods enhanced the performance of gas chromatographic analysis. The ability to both separate compounds in a complex mixture and furnish specific structural and qualitative data about those compounds offers profound opportunities for all types of analysis, especially environmental analysis.

The benefits of on-line coupling of LC with MS have been known since the late 1970's. However, for many years such an on-line combination was regarded as an "unapproachable ideal" because the two techniques appeared fundamentally incompatible (Arpino and

Guiochon, 1979). One must link a relatively large volume of liquid effluent from the LC at atmospheric pressure to a MS detector that requires only a small amount of sample under high vacuum (10^{-6} torr). Experimental prototypes were worked on in Sweden and Russia in the 1960's, but the effective first chapter of LC/MS history came in 1973-74 with the results published by E. C. Horning, I. W. McLafferty and R. P. W. Scott (Arpino and Guiochon, 1979). While many subsequent chapters have been written, the problems associated with interfacing the two techniques in a practical way are considerable.

Arpino and Guiochon (1979) have identified many of the problems that must be overcome for LC/MS analysis to be successful. Primary among them is the need to reduce a large liquid volume at high pressure to a small volume under vacuum while retaining the analytes in sufficient quantity to be detected by the mass analyzer. This is compounded by the fact that 1 mL of water generates approximately 1200 mL of gas at STP (Cerruti, 1989). MS requires that molecules be in the vapor phase to be analyzed. The LC effluent must be heated rapidly from about 40°C to 250°C. The interface must also tolerate varied solvent systems and conditions, especially when gradient elutions are used. The interface should yield sufficient sample to ensure proper utilization by the MS without modifying the analytes in the sample. Conditions should be constant and independent of the chemical nature of the solutes and solvents yet be flexible enough to allow the MS to be operated in CI and EI modes (Arpino and Guiochon, 1979). Games (1981) identifies six additional criteria for the ideal LC/MS system: i. Maintain chromatographic performance; ii. No restriction on HPLC systems; iii. Sensitivity comparable to GC/MS; iv. System capable of working long periods; v. Mass spectral data with maximal structural information available from thermally labile and low volatility compounds; vi. Reasonable costs. To date, no single LC/MS system has been devised which meets all six of these criteria. Several systems have been designed, each with its own advantages and disadvantages. A discussion of current LC/MS techniques follows.

III LC/MS Techniques

A. OFFLINE

One way to link the two systems is to manually collect fractions of LC effluent, evaporate the solvent, and transfer the sample to a matrix suitable for mass spectrometry. This is called "off-line" linkage. Samples may be mass analyzed in any mode such as EI, CI and FAB. Off-line techniques offer the benefits of MS detection, but introduce a two-analysis system. However, most of the efforts to date have focused on direct linkages or "on-line" LC/MS interfaces. Some of these include Moving Belt, Atmospheric Pressure Ionization (API), Direct Liquid Introduction (DLI), Thermospray (TSP), and MAGIC or Particle Beam systems. Both on- and off-line methods were explored in this research.

B. MOVING BELT

The moving belt interface is a mechanical transport devise that continuously carries total effluent from the LC to the MS without splitting the flow. Solvent is evaporated as the sample passes beneath an infrared heater. The pressure is reduced from atmospheric at the chromatograph to high vacuum at the spectrometer where the solutes are thermally desorbed from the belt to an ionization chamber, ionized and analyzed. These devices offer the possibility of measurement of electron ionization, chemical ionization, fast atom bombardment, and other spectra independent of any influence of the mobile phase (Yergey *et al.*, 1990). This allows for comparison of spectra acquired by direct means, such as included in MS libraries and is a key advantage of the moving belt system. Another advantage is that some involatile or labile compounds which failed to yield abundant parent ion currents by conventional direct insertion methods, were observed to do so when measured from the belt. However, the

analysis of involatiles using the belt system is restricted to the smallest compounds in each class of involatiles (Yergey *et al.*, 1990). Since its introduction in 1976, the moving belt interface has been used in the analysis, by EI or CI, of a wide range of materials and compounds including pesticides, polynuclear aromatic hydrocarbons, drugs, oligosaccharides, and many other natural products and organic compounds (Watson, 1985).

A major limitation to moving belt systems lies in the difficulty and reliability of solvent evaporation. Efficiency of the operation of these systems is affected by the need to balance the rate of solvent deposition with the speed of the belt. Desolvation is also affected by solvent composition; solvents with large aqueous phases are much more difficult to evaporate (Yergey *et al.*, 1990). One solution is to increase the power of the heater lamp, but this risks damage to the Kapton polyimide belt.

C. *ATMOSPHERIC PRESSURE IONIZATION*

Atmospheric pressure ionization (API) systems utilize mass spectrometers with no vacuum in the ion source. This obviates the need to pump the solvent vapor of the evaporated eluent, but the molecules or ions of interest must then be coupled into the MS. A summary of Covey *et al.*'s (1986) description of API methods follows: There are three types of API systems: the heated pneumatic nebulizer; liquid ion evaporation; and electrospray.

1. Heated pneumatic nebulizer

   This commercially available, probe-type interface is the most common one currently used on an API source system. It can operate at LC flows up to 2 mL/min. and tolerates volatile acids, salts, bases, and other mobile phase additives. The LC effluent is nebulized and desolvated as it passes through a heated nebulization region. Solvent molecules are ionized by a 6000 V corona discharge needle. Atmospheric pressure chemical ionization

(APCI) produce analyte ions from the solvent ions. These are focused through a dry nitrogen curtain gas before passing through a 100 μm orifice into the high-vacuum region of the MS where they are mass analyzed. This form of ionization is rather mild and fragmentation data is limited. This makes it very difficult to identify unknown compounds by MS analysis alone.

2. Liquid ion evaporation

A liquid ion evaporation interface was not commercially available until 1985. In this method, LC effluent is dispersed through a pneumatic nebulizer into air at atmospheric pressure. Ions are produced from small, charged droplets. A small, high-voltage electrode near the sprayer induces droplet charging. High temperatures are not required and conventional LC flow rates can be used. This system is limited to use with reversed-phase solvents and the analyte must be readily ionizable in the liquid phase. Liquid ion evaporation is well suited for polar, ionizable compounds. A unique advantage of ion evaporation over other methods, such as thermospray ionization (see below), is its very mild ionization at room temperature.

3. Electrospray

In electrospray systems, droplets are charged as they pass through a metal capillary tube that is at a potential of several kilovolts relative to the surrounding chamber walls. Ions discharged from the charged droplets are conveyed into the vacuum chamber of a mass spectrometer and are mass analyzed. This technique requires the use of micro-bore packed columns because best results are achieved with flows rates of 5 - 10 μL/min. Electrospray enables difficult compounds to be successfully analyzed. Other advantages include the lack of critical temperature control, good sensitivity, and the absence of a small orifice which can cause practical problems.

D. DIRECT LIQUID INTRODUCTION

Direct liquid introduction (DLI) is the simplest and least expensive interface used (Cerruti, 1989). LC effluent is introduced directly into the MS ion source region. Since this results in twenty times more gas than the system can handle, the sample is split so that only 1-5% of the total effluent enters the MS. This results in lower sensivity and detector limits of 0.1 - 1 μg (Covey *et al.*, 1986). DLI is conducive to chemical ionizaition, not electron ionization. Therefore, only molecular weight information is provided. DLI is good for thermally labile or fragile compounds. It is also condusive to use with microbore HPLC which typically uses flow rates of only 10-50 µL/min.(Covey *et al.*, 1986). In general, buffering salts such as ammonium acetate, are not used in DLI solvent systems due to the tendency of the capillaries to plug when heated (Yergey *et al.*, 1990).

One form of DLI is the open-tubular liquid chromatography (OTLC) interface. Large analytical efficiencies require very small sample sizes and long analysis times, but these conditions also result in poor sensitivity (Arpino and Guichon, 1979). This remains a controversial problem. Arpino and Guiochon in 1979 stated that open-tubular capillary columns in LC are very tempting but the OTLC/MS approach is unattractive for LC/MS.

Researchers at the Laboratory of Molecular Biophysics of the National Institute of Environmental Health Sciences, Research Triangle Park, NC, have developed an interface linking an open-tubular liquid chromatographic system with a quadrupole mass spectrometer. The system employs uncoated glass capillary columns (16 µm i.d.) or 10 µm i.d. fused silica columns coated with OV-17-V stationary phase (deWit *et al.*, 1987). Flow rates of less than 60 nL/min. provides a more efficient use of sample than effluent splitting where only 1-5% of the sample effluent enters the ion source (deWit *et al.*, 1987). The low flow rates allow the entire effluent to be introduced into the ion source and also permit this system to operate under both EI, CI and negative chemical ionization (NCI) MS conditions. Estimates of detection limits for metabolites of the herbicide trifluralin range from 20 pg to 2 ng.

"The linear dynamic range of the OTLC/mass spectrometric system is relatively narrow as the upper limit is determined by the capacity of the OTLC column (approximately 50 ng total sample) and the lower limit by the sensitivity of the mass spectrometer. This may limit the utility of this technique for non-target analysis."

"While part of this sensitivity results from the inherent electron affinities of these nitro compounds (e.g. trifluralin), part also appears to result from the fact that the exit of the column is well within the ion source, in close proximity to the filament. We have observed in EI experiments on these compounds using this interface that while EI sensitivities are substantially lower than NCI sensitivities, the EI mass spectrometric sensitivities are significantly better for OTLC introduction than for introduction by conventional direct probe EI analysis" (deWit *et al.*, 1988).

Advantages of the OTLC/MS interface over other LC/MS interfaces include: its simple construction and operation; it does not require dedicated use of a mass spectrometer; EI mass spectra are readily obtainable; and CI mass spectra can be easily obtained by use of a reagant gas such as methane as in conventional CI (deWit *et al.*, 1987). Escoffier *et al.* (1989) describe other advantages and disadvantages: the very small amount of sample required per analysis; better detection limits due to the injection of the total sample into the ion source; better chromatographic resolution; and longer filament life times since less mobile phase enters the MS source . OTLC is limited by problems of column plugging, possibly due to high concentrations of inorganic salts in environmental samples, sensitivity to matrix effects, low capacity of OTLC columns, and the lack of a commercial source of columns or interface probes . Solubility of the analyte can also be a limiting factor (Escoffier *et al.*, 1989).

E. THERMOSPRAY

Thermospray (TSP) is the most widely used LC/MS interface commercially available. Due to its popularity, it is most responsible for bringing LC/MS into the lab (Cerruti, 1989).

Thermospray is applicable to a wide range of compounds including volatile and nonvolatile, polar and nonpolar, labile and stable. Although it has a few areas that must be critically timed, it is relatively simple to operate (Covey *et al.*, 1986). Most TSP LC/MS use quadrupoles, but magnetic mass analyzers may also be employed.

"It is not true that the term comes from early efforts which involved equal applications of heat and prayer that were occasionally successful" (Yergey *et al.*, 1990). CI-like interactions form the basis for certain LC/MS techniques such as thermospray. Thermospray ionization is the formation of ions without the use of an external source of ionizing electrons (Covey *et al.*, 1986). Ammonium acetate in the mobile phase is a good general purpose electrolyte for ionizing samples. One can identify five distinct steps in the process whereby liquid effluent at atmospheric pressure become ions in the low pressure (10^{-6} torr) gas required by the mass spectrometer:

Nebulization; Droplet Charging; Vaporization; Ionization; and Ion Transport (Vestal, 1989).

1. Nebulization

   Eluant is passed through an electrically heated capillary resulting in the production of a supersonic jet of vapor. Partial vaporization of the liquid generates the nebulizing gas in the capillary (Yergey *et al.*, 1990). This provides very efficient nebulization into relatively small droplets and furnishes a convenient heat source for vaporizing large liquid flows.

2. Droplet Charging

   "The major charging mechanism is the statistical charging resulting from violent disruption of the liquid containing ions in solution. This technique produces essentially equal populations of positive and negatively charged droplets." (Vestal, 1989).

3. Vaporization

The volatility of mobile phase is very important. Production of molecular ions from the charged droplets requires nearly complete vaporization of the mobile phase. The high latent heat of vaporization of water limits many LC/MS interfaces (Slivan *et al.*, 1989). In thermospray the heat of vaporization is supplied in the capillary and at the source block at reduced pressure. Vestal and Fergusson (1985) report that the premise that very rapid heating over a short length of the capillary was required to vaporize the liquid without pyrolizing the sample, is false. Direct electrical heating of the capillary, longer heated lengths and lower surface temperatures were found to increase performance and stability (Vestal and Fergusson, 1985). The liquid velocity in the capillary determines the maximum temperature for vaporization without causing premature vaporization within the capillary (Osterman *et al.*, 1987). Voyksner (1983) reported an optimal vaporizer temperature of 115°C and an optimal jet temperature of 300°C. Other thermospray operating temperatures have been reported (e.g. in Ballard and Betowski, 1986). In any event, it is vital that the heat input be properly controlled so that complete vaporization does not occur prematurely inside the capillary (Yergey *et al.*, 1990). Source block and tip temperatures must also be controlled for reproducible results.

4. Ionization

Molecular ions are produced from the highly charged liquid droplets after the solvent has been nearly totally vaporized. This is typically referred to as thermospray or filament-off mode. A filament can be used to generate ions under CI conditions in thermospray MS. This is called "filament-on" mode (Covey *et al.*, 1986). A filament at the vaporizer tip is typically operated at an electron energy of 200 eV and emission current of 0.05 mA (Voyksner, 1985). Ammonium acetate in the mobile phase is a good general purpose electrolyte for ionizing samples in filament-on or off modes. Concentrations of the buffer in water ranging from 0.01M to 0.1M have been successfully used (Bellar and Budde, 1987; Joyce *et al.*, 1985; King *et al.*, 1987). Joyce *et al.* (1985) report that ionic samples are best analyzed without ammonium acetate.

A discharge ionization mode, "discharge-on", produced by a low current Townsend discharge, is used to produce an intense negative ion, $(M-H)^-$, and siginficant fragmentation in most cases (Jones *et al.*, 1989). "These three different ionization modes [filament off, filament on, discharge on] can accommodate most HPLC eluent conditions" (Covey *et al.*, 1986). Since most mass spectrometers are capable of detecting and analyzing both positive and negative ions, there exist, in effect, six possible operating modes for any particular analysis. Properties of both the sample and the mobile phase affect the results. "In general, for positive ion detection, samples must be more basic than the mobile phase (to form MH^+) or be sufficiently polar to form stable adducts, e.g. $(M + NH_4)^+$. For negative ion detection, samples must be more acidic than the mobile phase [to form $(M - H)^-$] or have a higher electron affinity (to form M^-). Use of either the filament or discharge is required to form M^-" (Yergey *et al.*, 1990).

5. Ion Transport

The most difficult part of the thermospray process appears to be transporting the ions through a conical exit aperature into the vacuum of the mass spectrometer (Vestal, 1989). High efficiencies are capable for compounds significantly more polar than the solvent. Effects of condensable vapor are mitigated by applying heat and sampling from a lower pressure (Vestal, 1989). At high ion source pressures, thermospray-produced ions are not affected by electric fields (Kidwell *et al.*, 1987). Ions escape from the source primarily by mass transfer with the solvent molecules which is governed more by the exit hole diameter than by the source pumping (Kidwell *et al.*, 1987).

Niessen *et al.* (1989), in their study of optimization of sensitivity and information in thermospray, identified two important aspects for TSP qualitative analysis: sensitivity, because people are interested in analyzing small amounts of sample; and information content, that is, to identify structures from molecular weight and fragmentation data.

"The most important parameter determining the sensitivity is the analyte itself"; differences of 5 orders of magnitude were observed with various compounds (Niessen *et al.*, 1989). Thermospray ionization is sensitive to certain compounds, but insensitive to others. Wire repellers connected to an external power source and attached to the commercial ion source block are used to enhance thermospray sensitivity 10 to 400 times (Jones *et al.*, 1989; Niessen *et al.*, 1989; Voyksner, 1985; Yinon *et al.*, 1989). Kidwell *et al.* (1987), on the other hand, report that a repeller significantly decreases sensitivity when fragmentation does occur. In discharge-on mode, intense protonated molecules are observed at low repeller potentials and fragmentation at high repeller potentials (Niessen *et al.*, 1989).

Thermospray is generally favored with ionic, polar or nonvolatile samples (Yergey *et al.*, 1990). Kidwell *et al.* (1987) claim thermospray LC/MS is the only viable method of analyzing polar compounds. This method has been used to characterize dyes in environmental samples (Ballard and Betowski, 1986; Voyksner, 1985), nitrobenzene decomposition products (Solsten *et al.*, 1987), and nonvolatile pesticides (Bellar and Budde, 1988) as well as many other samples.

Thermospray is compatible with HPLC flow rates of 0.4 to 2 mL/min using a cryopump to remove excess vapor and is useful for analysis of nonvolatiles such as carbohydrates, peptides and antibiotics (Cerruti, 1989). Filament on chemical ionization is effective when the mobile phase contains a large organic fraction and is almost essential for normal phase chromatography (Yergey *et al.*, 1990).

Eluents with a high percentage of water are most commonly used in reversed-phase HPLC. Discharge ionization is most useful with largely aqueous mobile phases. Carbon deposits can build up on and short out the discharge electrode if used with organic fractions greater than about 60% (Yergey *et al.*, 1990).

Solvent conditions for optimal HPLC are not always congruous with those for optimal thermospray ionization. To succeed with optimal HPLC separation as well as TSP ionization, Bean *et al.* (1987) introduced a third pump and a micro needle valve/T post-column because polar compounds were not retained in the column with a high amount of organic solvent. Water is the preferred mobile phase with either methanol or acetonitrile and a volatile buffer (Yergey *et al.*, 1990).

In summary, thermospray is the most widely used spray technique because of its ability to handle normal LC flows and to provide sensitive detection of a wide range of compounds under a variety of LC conditions, independent of UV chromophoric moieties (Vestal, 1989). The technique is limited by poor quantitative capabilities and a strong dependence on experimental parameters (Kaiser *et al.*, 1989). Structural identification of unknown compounds is extremely difficult with TSP LC/MS due to insufficient fragmentation. Other problems with the technique relate to the complexity and expense of the equipment, and the difficulty to operate and maintain it.

F. MAGIC and PARTICLE BEAM INTERFACES

A major disadvantage of thermospray is its inability to achieve electron ionization spectra. One of the few systems available that provides EI mass spectral data is the ***m***onodisperse ***a***erosol ***g***enerator for ***i***ntroduction of liquid ***c***hromatographic effluents (MAGIC). Highly uniform-sized droplets are generated, desolvated and directed to the high vacuum region of the MS with an aerosol beam generator. "Flow rates of 0.1 to 0.5 mL/min. are optimal and the complete separation of the sample from the HPLC eluent provides a free choice between CI and EI mass spectra" (Covey *et al.*, 1986). Up to 1 liter/min of dispersion gas (usually He) is required. Recently, a particle beam interface was developed from the MAGIC system (Cerruti, 1989).

Two particle beam systems (PB LC/MS) are currently commercially available: one from Hewlett Packard (HP 5988UA) and the Thermabeam™ system from Extrel Corporation. The "Thermabeam" interface uses a thermospray vaporizer as a nebulizer, which appears to produce smaller initial droplets at higher temperatures (Yergey *et al.*, 1990). This permits improved desolvation and reduces the likelihood of plugging; both are distinct advantages over the MAGIC system. These systems offer greater control over nebulizer temperatures than thermospray as well as the ability to run a substantially "wet aerosol" in order to deliver thermally labile or higher vapor pressure compounds into the ion source (Extrel). They are sensitive to low nanogram levels with full scan EI (Extrel).

Factors which affect the overall response of an analyte are the operating parameters of the interface and the analyte itself (Behymer *et al.*, 1989). Operating parameters for particle-beam interfaces include: the position of the capillary transfer line with respect to the entrance to the desolvation chamber; the temperature of the desolvation chamber; the temperature and flow rate of the nebulization gas; and the composition of the mobile phase (Behymer *et al.*, 1989).

Smith *et al.* (1989) recently used PB LC/MS to study nine pesticides and related compounds (concentrations not provided; calibration done with 20 - 500 ng of aldicarb sulfone) with poor GC performance. They found that most interface parameters were mobile phase dependent, not analyte dependent. The best response was with methanol as the mobile phase. Particle beam sensitivity varies across an LC gradient; as the percentage of water in methanol increases, detected ion current and sensitivity decreases. Different buffers also affect the signal intensity. This same group found the optimum HPLC flow to be 0.6 mL/min, the best signal intensity with a helium flow at approximately 30 psi and desolvation chamber temperature at approximately 55°C. Optimal nebulizer settings and MS source temperatures are compound dependent (Smith *et al.*, 1989). The main variables cited were nebulizer position, nebulizer helium flow rate, and desolvation temperature.

A capillary LC/MS interface is being developed as a non-aerosol vaporizer, efficient at conventional LC flow rates, for use with a thermospray ion source as well as with a particle beam system (Slivon *et al.*, 1989). It operates at a constant temperature of 160°C for isocratic and gradient elution so as not to expose the vapor to excess temperature (Slivon *et al.*, 1989).

G. SUMMARY

The limiting factor for many MS methods is the inability to vaporize the sample in a way to adequately represent its original structure (Harris and Browner, 1989). Samples must be vaporized in the ion source. Volatility problems arise when the energy added causes decomposition reactions before vaporization, that is, kinetic competition between breaking intramolecular bonds (decomposition) and intermolecular bonds (volatilization). The activation energy of the former is less than that of the latter (Harris and Browner, 1989). Decomposition can be limited by changing the relative rates of the two competing processes. This is effected by temperature and sample concentration (Harris and Browner, 1989). Compounds of high volatility typically have boiling points less than 125°C while those of intermediate volatility have boiling points between 125 and 155°C. Compounds from HPLC effluents too involatile to produce great enough vapor pressures for EI and CI processes require special ionization techniques such as laser desorption and FAB (Kirk and Browner, 1989).

On-line LC/MS requires consideration of the needs of both chromatographic separation and MS ionization techniques. Development of broad spectrum techniques for analysis of non-volatile pollutants and polar metabolites using LC/MS will allow for a greater understanding of complex environmental samples such as landfill leachates and industrial wastes. As techniques improve, LC/MS will prove to be an even greater compliment to gas chromatography in environmental analysis.

IV. Applications of LC/MS

As previously stated, in a broad sense any of the 80 - 90 percent of the compounds not amenable to GC/MS analysis are candidates for LC/MS analysis. "HPLC is capable of analyzing many organic substances which are not volatile enough to undergo elution through a GC column but which nevertheless have a sufficient vapor pressure to be analyzed by mass spectrometry in the conventional EI and CI modes." (Arpino and Guiochon, 1979)

LC/MS techniques are used to identify and quantify numerous natural and synthetic chemicals present at low levels in complex matrices including: additives and contaminants in food stuffs; drugs, their metabolites and other physiologically important compounds in biological fluids; contaminants in water supplies; confirmation of newly discovered natural products such as plant extracts with desirable properties; peptide sequencing studies; and analysis of sugars and nucleosides (Games, 1981). Applications of particular environmental interest include analysis of carbamate insecticides (Games, 1981), sulfonated azo dyes and phenoxyacetic acid herbicides (Henion and Edlund, 1989), azo dyes (Budde, 1989), and substituted urea herbicide analogues (Shalaby, 1984; Wells and Cowan, 1982).

Concern for the presence of organics in drinking water has been chiefly confined to volatiles. Many researchers recognize the need to detect nonvolatiles compounds responsible for adverse health effects. For example, N-Nitroso compounds, a major class of carcinogens, have been found in sewage effluent (Pitt *et al.*, 1976). Crathorne *et al.* (1979) cite a World Health Organization report supporting the need to detect and identify the 80-90 percent of organics in drinking water not detectable by GC/MS. While a large portion of organics in water are humic, fulvic and hymatomelanic acids, the remaining discrete organic compounds that are nonvolatile by virtue of polarity, thermal instability or high molecular weight, are largely unknown (Crathorne *et al.*, 1984). LC/MS techniques can provide needed information on the nature of the organics in water (Crathorne *et al.*, 1984).

V. Sample Preparation

Introduction: In environmental analyses, one frequently encounters dilute samples which require that the analytes of interest be concentrated and/or extracted into a matrix suitable for analysis. Suitable methods to achieve desired results are not always readily apparent and are generally dependant on many factors including the sample matrix and properties and concentration of the analytes of interest. For example, what analytical procedures are most appropriate for the trace analysis of constituents in an untreated industrial wastewater that contains a major component? Extraction and concentration activities will serve to magnify the presence of the major component and may therefore serve to inhibit the sensitivity of the analysis on the desired compounds. If techniques are employed that selectively exclude the interfering factor, compounds of interest which possess similar chemistry may also be excluded from the analysis. Thus, the right combination of concentration, extraction, separation and analytical techniques greatly enhances one's ability to qualitatively and quantitatively identify environmental pollutants. From an engineering point of view, proper identification can assist in both the choice of treatment as well as the monitoring of the efficacy of treatment. Procedures used to prepare environmental samples for analysis range from very simple to complex, multi-step endeavors.

Ballard and Betowski (1986) report that direct analysis of dye manufacturing waste, without pretreatment, using flow injection thermospray and tandem mass spectrometry, provides rapid screening of complex environmental samples containing nonvolatile analytes. The number of samples that could be analyzed without fouling the system was not, however, stated. Some of the problems associated with direct mass spectrometry only methods of analysis are: matrix and salt interferences from artifacts in the mixture; loss of sensitivity; inability to resolve isomers; and suppression of ionization of components present in low relative concentrations (Games, 1981; Voyksner and Williams, 1987).

Extensive cleanup procedures are usually followed to prepare samples for either HPLC or MS analysis. Sample preparation is ordinarily the most time-consuming process in environmental analysis of organics (Wells and Michael, 1987). Extraction methods can be tailored to the chemical and physical properties of specific compounds, but techniques to isolate and concentrate a broad range of compounds are problematic (Watts *et al.*, 1982). Methods developed for extraction of nonvolatile organics from water include adsorption, precipitation and liquid-liquid extraction (LLE).

A. Liquid-liquid Extraction

Liquid-liquid extraction is one way to draw organic compounds of interest from a sample matrix into a matrix suitable for analysis. Classes of compounds can be selected by their solubilities in different solvents. For example, nonpolar compounds will be extracted into hexane easier than methanol while the reverse is true for polar compounds. However, in order for LLE to be effective, the extraction solvent must be relatively insoluble in the sample matrix to facilitate phase separation. In the example just given, methanol is completely soluble in water so separation will not exist. A widely used extraction solvent is methylene chloride (perhaps due to its codification in EPA Method 625). This solvent extracts relatively nonpolar compounds. To extract more polar compounds, ethyl acetate can be used as the LLE solvent. However, due to its high solubility in water (appr. 10%), good phase separation does not always occur. A major problem with LLE procedures is the formation of emulsions and the resultant difficulties they provide. LLE is also a rather burdensome process especially when handling hazardous solvents like methylene chloride. Another disadvantage of LLE is that impurities in solvents, such as cyclohexane in methylene chloride, will be concentrated during the procedure (Ibrahim, *et al.*, 1987; Dietrich, *et al.*, 1986; Jolly, 1981).

B. Lyophilization

Lyophilization is a freeze drying technique that permits concentration of nonvolatile organics by a factor of several thousand. Watts *et al.* (1982) demonstrated an environmental application of lyophilization. Concern over the possible mutagenic effects of by-products of chlorination and ozonation has emphasized the need for suitable analysis of nonvolatile organics in water. Freeze drying followed by methanol extraction appeared to be the most suitable method of sample preparation (Watts *et al.*, 1982).

The degree of concentration is limited by the recovery of organics from the residue which is composed largely of inorganic salts (Jolly, 1981). Inorganic salts can result in peak broadening on reversed phase HPLC and interferences in mass spectrometry (Watts *et al.*, 1982). Filtration, sonication and centrifugation of the lyophilized samples are techniques available that may resolve these problems. Ion exchange resins have been shown to be very efficient at removing salts from both the aqueous phase and the methanol extract of freeze-dried water samples (Watts *et al.*, 1981). However, organics in solution, especially organic acids were also effectively removed by ion exchange columns.

Crathorne *et al.* (1979) lyophilized 15-60 liters of water and extracted the solid residue with three aliquots of methanol in a sonicating bath before centrifugation. Extracts were combined and concentrated by rotary evaporation. Analysis of total organic carbon (TOC) before and after freeze drying indicated high TOC recovery (Crathorne *et al.*, 1979).

C. Solid Phase Extraction

"Solid phase extraction (SPE) [also called liquid-solid extraction] is rapidly eliminating the need for liquid-liquid extraction in many procedures" (Hoke *et al.*, 1986). Introduced in the 1970's, it is not a new technique, but with the innovation of many stable adsorbents covalently

bonded to porous silica, SPE has become a more convenient method for many procedures. It has found extensive use in pharmacological and clinical applications. SPE has been applied to environmental samples for the analysis of pesticides, priority pollutants, aliphatic hydrocarbons, benzene and alkyl benzenes, polycyclic aromatic materials, chlorinated phenols, PCB's, chloroanilines and tributylin chlorides (Junk and Richard, 1988). SPE preparation of samples for chromatographic analysis consumes less time, costs, labor and solvent use compared to traditional alternatives (Wells and Michael, 1987). Formation of emulsions associated with LLE is avoided also.

The technique of solid phase extraction is based on the separation mechanisms of liquid chromatography, and in fact, SPE cartridge packings are analagous to LC column packings. Specific interaction between a solid sorbent and analytes from a sample matrix can selectively retain and concentrate either the analyte itself or interfering components from the sample matrix (Zief and Kiser, 1988). The separation mechanism is due to the interactions between analyte molecules and sorbent functional groups.

Octadecylsilyl bonded phases have 18-carbon alkyl chains bonded to the silica support material as follows:

$$\text{-Si-OH} + C_{18}H_{37}\text{Si-Cl} \rightarrow \text{Si-O-Si-CH}_2\text{-(CH}_2)_{16}\text{-CH}_3$$

When an aqueous sample is passed through the column, hydrophobic compounds are adsorbed onto the packing. These compounds will be desorbed from the column if they have a greater affinity for an elution solvent passed through the column than for the packing.

Ion exchange can be a useful mechanism to select for nonvolatile polar organic molecules. It works according to the relative ionic strengths of both the sample analytes and the ion exchange resin. Ion exchange packings carry surplus positively or negatively charged materials. These can be displaced by stoichiometrically equivalent counterions introduced into the stream (Yost *et al.*, 1980).

The equilibrium dissociation expression for an organic acid, according to the Le Chatelier Principle, is:

$$RCOOH \rightleftarrows RCOO^- + H^+$$

or the more general form:

$$HA \rightleftarrows H^+ + A^-$$

Ionic activity or strength of the acid is expressed by the negative logarithm of the dissociation constant or pKa. The stronger the acid, the smaller the pKa. The Henderson-Hasselbach equation correlates the pKa with pH as follows (Yost *et al*, 1980):

$$pH = pKa + \log\left(\frac{[A^-]\{ionized\}}{[HA]\{unionized\}}\right)$$

Ionization is suppressed at pH < pKa while it is increased at pH > pKa.

Ion exchange packings have ionizable functional groups bonded to a solid support which may be displaced by equivalent analyte ions. An anion exchange column functions as follows:

$$Resin^+Y^- + X^- \rightleftarrows Resin^+X^- + Y^-$$

where Y = mobile phase buffer anion; X = sample anion.

If the column is buffered to a pH at least 2 units below the pKa of the sorbent and at least 2 pH units above the pKa of the analytes of interest, the analytes will adsorb onto the column. The sample must be in an environment which ensures total ionization of the compounds of interest. At pH 7, compounds with a pKa less than 5 will be completely ionized and retained by the column. Compounds with pKa's greater than 5 will be poorly retained.

There are four simple steps to SPE:

i. Column conditioning - solvate functional groups

ii. Sample loading - analyte retention

iii. Column postwash - remove impurities

iv. Elution - extract analytes

Solvent selection depends on the extraction mechanism (normal phase, reversed phase, cation or anion exchange). Prior to application of the sample, the column must be conditioned. Protocols suggest that one to two column volumes of methanol be passed through the column followed by 2 to 3 column volumes of the same aqueous buffer solution which was used to buffer the sample. This is necessary to lower the surface tension within the SPE and allow the alkyl chains of the stationary phase to stick out rather than lie flush against the solid adsorbent. The buffered sample may then be applied. At no point during the conditioning steps should the column be allowed to dry out before sample application. Column drying has been associated with irreproducibility in column performance. Usually a column is considered to be dry if it is exposed to the vacuum for 20 - 30 seconds with no liquid on the cartridge. It is recommended to apply one column volume of the same aqueous buffer solution used to condition the column as a wash step following sample application. This is to remove any endogenous interferences from the column. The column is then dried by running the vacuum for at least 10 minutes.

The final step is to elute the analytes off the column. For a reversed phase column, analytes are eluted with an organic solvent such as methanol, acetonitrile or methylene chloride. For an ion exchange sorbent, elution can be achieved with three types of solutions:

1. A solution of high ionic strength such as citrate ions will act as counterions and replace the analytes on the column.

2. A high pH solution will decrease the ionization of bases while increasing ionization of acids, that is, the sorbent will be neutral so the ionized compounds will elute.

3. A low pH solution will neutralize the acids while ionizing the bases, that is, the sorbent will be ionized and the unionized compounds will elute.

Hoke *et al.* (1986) utilized a cleanup prodedure that enabled them to reuse their columns. Specific procedures are more or less defined by the characteristics of analytes and impurities in the sample. Selection of the proper extraction column and the proper elution solvent are two important factors that need to be optimized. But what does one do with complex samples containing unknown constituents? "It is difficult to find a single adsorbent that will extract all of the compounds of interest. To optimize the extraction for all compounds of interest one has to use mixed phases and different solvents that cover most solubilities" (Ghaoui, 1987).

Various procedures are reported in the literature utilizing a wide range of sample size (20 to 2000 mL), flow rate (2 to 200 mL/min.), sorbent mass (100 to 1200 mg), and volume of eluting solvent (0.1 to 5 mL) (Junk and Richard, 1988). Large sample size associated with environmental samples may have inhibited the development of SPE in this area. However, recent literature suggests SPE of small volumes of environmental samples yield results sufficient for trace organics analysis (Bellar and Budde, 1988; Junk and Richard, 1988; Wells and Michael, 1987). "Adjusting the sample size and sorbent mass in relation to the strength of the interaction (van der Waals, electrostatic, hydrophobic, etc.) between the sorbent and the solute produces successful results for environmental samples" (Wells and Michael, 1987).

Methanol (0.5 - 5%) may be added to aqueous samples prior to extraction. This provides further conditioning of the SPE column by promoting interaction of the highly hydrophobic C_{18}

with the aqueous sample and is necessary for large sample volumes since water will wash off the methanol added during conditioning. Methanol addition may also enhance the solubility of organics in the sample (Benjamin).

Results indicate that flow rates don't have to be closely controlled (Junk and Richard, 1988). Optimal sorbent mass is determined by the capacity factor of the solutes in the sample. Breakthrough can be determined by applying variable concentrations of sample to a constant mass of sorbent (Wells and Michael, 1987). For reversed-phase columns, breakthrough is a function of the hydrophobicity of the solutes.

Results for elution parameters are not clear cut. Junk and Richard (1988) found ethyl acetate to be superior to methanol and acetonitrile for eluting hydrophobic compounds. They collected 100 µL of the eluant of which 1 µL was gas chromatographed. Average recovery of >85% was reported. Hoke *et al.* (1986) eluted with two 1 mL aliquots of methanol which was diluted to 5 mL with water; 200 µL was analyzed on an HPLC under isocratic conditions using methanol:1% acetic acid (68:32). Recoveries were 29-74% for their environmental samples, 80-105% for the controls. Bellar and Budde (1988) selected methanol as the ideal solvent for the final extract. Columns were eluted with three 1 mL aliquots of methanol, concentrated to 1 mL, 20 µL of which were analyzed by LC/MS. An acetonitrile/0.1M ammonium acetate in water HPLC gradient was used because it gave shorter retention times with adequate resolution than a similar gradient with methanol as well as better results with the thermospray. They report a grand mean recovery of 76% for 29 compounds.

Wells and Michael (1987) selected different elution solvents and volumes for different analytes (25% acetic acid for picloram; methanol for 2,4-D). Environmental samples were eluted with 4.5 or 9.5 mL of methanol, evaporated to dryness and the residue reconstituted with 5.0 mL of the LC mobile phase (in this case acetonitrile). This solution was refrigerated overnight for equilibration because "spurious results were obtained if the samples were transferred immediately after reconstitution."

Wells and Michael (1987) recommend a series of steps to follow to develop a protocol for SPE. Start with 200 mL of sample at a concentration of 100 ppb and 1.0 g of sorbent. Acidify samples to pH 2 with sulfuric acid to suppress ionization. Screen solvents for best elution and optimize solvent volume by plotting percent recovery versus elution volume. Optimize retention by determining the best sample pH, concentration and volume as well as optimal sorbent mass. The method is verified when constant recoveries are obtained from different concentrations of the sample.

In trace organic analysis, the presence of impurities are very important. A number of possible interference compounds from SPE cartridges have been identified by GC/MS, among them, alkanes, alkenes, plasticizers (phthalates), antioxidants and silanols (Junk *et al.*, 1988). Silanols are probably formed from hydrolysis of the bonded porous silica. The levels of impurities varied with the lot number of the cartridges (Junk *et al.*, 1988).

Another advantage of SPE is the ability to process water samples on-site. This obviates the need for transportation, cold storage and possible losses from breakage. Richard and Junk (1986) forced 100 mL samples of surface water through SPE cartridges using a 50 mL glass syringe. The biggest problem with this technique was reduced flow from suspended sediments until a 0.7 µm pore size glass fiber filter was placed between the syringe and cartridge. The cartridges were dried and eluted in the lab.

VI. Leachate

One of the author's areas of interest lies in the identity and characteristic of leachate from Dixie Caverns landfill in Roanoke County, VA. GC/MS analysis has detected only a small fraction of the compounds constituting the 75-100 mg/L chemical oxygen demand and/or 4-10 mg/L biochemical oxygen demand measured for this leachate (Freedman, 1989; Marickovich,

1989). Thus, other analytical methods are required to identify the remaining constituents of the organics in this leachate.

Thousands of landfills, active or abandoned, have been operated with little concern for dangers of water contamination by leachates. Few studies have addressed the occurrence of potentially hazardous organic compounds in landfill leachates (Reinhard *et al.*, 1984). Recent public concern has focused on the potentially hazardous organic chemicals that may leach from the large quantities of commercial and household waste chemicals being disposed of in landfills (Shi-LiLiu *et al.*, 1987). Leachate containing these chemicals may contaminate surrounding surface and ground waters. Dunlap *et al.* (1976) citing Miller *et al.* (1974), noted 60 cases where landfills were identified as sources of groundwater pollution. They also state that the probability that most of the compounds were leached very slowly from the landfills implies the potential for long term subtle pollution by organics from landfills: "Slowly decaying domestic and commercial products in landfills would appear likely to serve as reservoirs feeding low levels of industrial organic pollutants into aquifers for many years after the landfills have been closed and forgotten."

Tinsley (1979) asserts that the leaching process is determined by the water solubility of the chemicals, hydrological characteristics and a Freundlich adsorption relation. Consistent relationships between water solubility and leaching rates are not always observed with different classes of compounds, therefore a more reliable criterion for predicting the tendency to leach is the adsorption coefficient with the soil under consideration (Tinsley, 1979). Other factors include ionization state of the molecules, soil composition, pH and porosity , and the rate of water movement through that soil (Tinsley, 1979).

The realization that leachates can contaminate surface and ground water resources has affected management practices and regulatory requirements concerning proper disposal of hazardous wastes (Jackson *et al.*, 1984). Accurate characterization of leachate contaminants in solid hazardous waste prior to landfilling is becoming part of an overall waste management

strategy (Jackson *et al.*, 1984). Due to the complex nature of leachate, as broad an analytical approach as possible is desired if an accurate characterization is to be achieved. GC/MS and LC/MS complement each other in this regard.

Many different compounds have been discovered in landfill leachates by both techniques. Some of these include pesticides (Foster *et al.*, 1983), organic acids, fatty acids, alcohols, high molecular-weight humics, benzene and other aromatics (DeWalle and Chian, 1981; Sawhney and Kozloski, 1984), phthalates, chlorinated and aromatic hydrocarbons, phenols, alkyl phosphates, aliphatic and aromatic acids, nitrogen-containing aromatics, terpenes, alkyl phenol ethoxylates and many other compounds still to be determined (Richard *et al.*, 1984; Shi-LiLiu *et al.*, 1987). The presence of some of these compounds may be due to degradation of organic waste under both aerobic and anaerobic conditions of landfills (Sawhney and Kozloski, 1984). Other compounds indicate that industrial wastes were buried with domestic wastes (Reinhard *et al.*, 1984).

Anaerobic environments were found to enhance the transport of phenols and possibly other organic pollutants (Sawhney and Kozloski, 1984). Polymerization, and hence adsorption, of phenols appears to be inhibited in anaerobic conditions (Sawhney and Kozloski, 1984). Methanogenesis appears to be the major removal process for dissolved organic carbon (Reinhard *et al.*, 1984). Many of the organic acids found in leachate are anaerobic degradation products (Reinhard *et al.*, 1984). Microbial degradation may enhance the leachability of compounds deemed to be water insoluble and hence immobile, by producing soluble products such as chlorinated aromatic acids and chlorinated phenols (Reinhard *et al.*, 1984).

This information indicates that impacts of landfill leachate may be addresed in several ways:

- What are the potentially harmful constituents in the leachate?

- What are the local hydrogeologic conditions?

- What is the significance of biological and/or chemical degradation, adsorption and other processes? (Reinhard *et al.*, 1984)

VII. Industrial Waste

The analysis of industrial wastes in water has become of major interest in the environmental field. The discharge of untreated or poorly treated waste waters from manufacturing processes into nearby waterways creates major pollution problems. The analysis of trace levels of contaminants in polluted water is complicated by interferences from a variety of other organic compounds present in the water (Parker *et al.*, 1982). Methods sensitive to the analysis of industrial contaminants can be used to characterize specific waste effluents and to monitor the effectiveness of waste treatment.

An object of this research was to develop methods for the characterization of environmental samples and to apply those methods to characterize the organic component of a leachate and an industrial waste or other organic samples.

III. METHODS AND MATERIALS

A. Overview

There does not exist one distinct procedure for the analysis of organics in environmental samples. In fact, the term "organics analysis" is a misnomer in that it describes dozens of procedures and methods and depends upon which "organics" and which type of "analysis". Many procedures are designed to analyze very specific compounds or classes of compounds or classes of compounds. For example, EPA Procedures 601 and 608 for the analysis of volatiles and PCB's respectively. When one encounters samples of unknown composition, however, a broad spectrum, non-target type of analysis is desired, that is, one that will yield the most information about the constituents of a sample with the fewest procedures. EPA Method 625 is a widely used prodedure for the analysis of many volatile and semi-volatile organic acids and base/neutral compounds. No comparable standardized method exists for the analysis of nonvolatile organics.

The emphasis of this research was to investigate methods for the analysis of nonvolatile organic pollutants, particularly the polar fraction, that are difficult to determine by previously

utilized techniques. This involved using a number of existing procedures, with or without modifications, and applying them in the appropriate sequence to achieve the desired result.

There are two major categories of procedures that must be performed for sample analysis: sample preparation and instrumental analysis. The former group involves procedures that extract the desired orgainc fraction into a matrix suitable for qualitative and/or quantitative analysis free of interferences. Instrumental analysis can utilize a number of chromatographic techniques and detectors resulting in numerous possible combinations of procedures.

This chapter will provide details of all the methods investigated for both sample preparation and analysis. Experiments that tested the validity of certain procedures are also described. Extraction procedures tested include: liquid-liquid extraction with ethyl acetate, lyophilization, and solid phase extraction. Extracts were analyzed by LC with a diode array detector (LC/DAD), mass spectrometry and liquid chromatography/mass spectrometry. GC/MS analysis was also performed for comparative purposes.

Two types of environmental samples were analyzed: 1) a landfill leachate; 2) an industrial waste.

B. Site Selection/Sample Collection

The methods described herein were developed using leachate from the Dixie Caverns Landfill in Roanoke County, VA. Samples from this site were chosen for several reasons: this research served to compliment work previously conducted by others (Freedman; Marickovich, 1989); samples were accessible, available and of close proximity to the primary research facility. Also, the leachate is representative of a potentially significant source of pollution in Virginia. There are numerous municipal landfills in the State, both operating and abandoned, that produce leachates of unidentified composition. Leachate samples were obtained from the

holding pond at the landfill on August 19 and October 2, 1989. All samples were stored at 7°C prior to analysis.

One of the objectives of this research was to apply these developed methods to characterize other environmental samples. To this end, an industrial wastewater was obtained that was expected to contain certain components that are not thermally stable and would therefore be amenable to analysis by LC/MS. A raw wastewater sample was taken on October 24, 1989 and received in the lab one week later. This was also refrigerated at 7°C.

C. Research Chemicals and Materials

Solvents: HPLC grade methanol, Burdick & Jackson (Muskegon, MI) was used for all applications of methanol. Water used in the HPLC was distilled and deionized prior to filtration in a Milli-Q Reagent Water System (Millford, MA) consisting of a Super C Carbon, two Ion-Ex, and Organex-Q cartridges in series. Other solvents used were high purity Ethyl Acetate from Burdick & Jackson and pesticide grade Acetone from Fisher Scientific (Springfield, NJ). Eighteen standards were used at one time or another. They are identified alphabetically with supplier, purity, molecular weight and abbreviations used in this text in Table 1.

All glassware used was cleaned with Sparkleen detergent (Fisher Scientific), rinsed with tap water, distilled water and methanol and allowed to stand dry. Standards were weighed on a Mettler H 10 Balance (Hightown, NJ) accurate to 0.0001 gram, in preweighed 10 mL volumetric flasks. All samples were transferred into two mL Kimble Opticlear® vials from Fisher with 11 mm crimp top teflon seals (Alltech, Deerfield, MI) prior to LC/DAD analysis. All standard solutions and extracted samples were stored at 10°C in a Fisher Flammable Material Storage refrigerator.

Table 1. Standards Used

Chemical Names	Abbrev. Used	Source	Purity	MW
4-Aminobiphenyl	ABP	Ultra Scientific	99%	169
1-Aminopyrene	APY	Aldrich	97%	217
Atrazine	ATZ	Chem Service	99.7%	215 (217)
Benzamide, N,N-diethyl-3-methyl (N,N-Diethyl-m-toluamide; DEET)	BZD	Chem Service	99.5%	191
Benzenesulfonamide-N-ethyl-4-methyl (N-ethyl-p-toluenesulfonamide)	BSF1	Chem Service	NA	199
Benzenesulfonamide-N,N,4-trimethyl- (N,N-dimethyl-p-toluenesulfonamide)	BSF2	Chem Service	98%	199
Benzothiazole	BZL	Chem Service	99%	135
m-Cresol	Cresol	Chem Service	98%	108
2,4-Dinitrotoluene	2,4-DNT	Chem Service	99%	182
2,6-Dinitrotoluene	2,6-DNT	Chem Service	99%	182
Diphenylamine	DPA	Aldrich	99+ %	169
Nitrobenzene	NBZ	Chem Service	99%	123
4-Nitro-2-aminotoluene (Benzamine-2-methyl-5-nitro)	4N2AM	Chem Service	NA	152
N-Nitrosodiphenylamine	NNDPA	Supelco	NA	198
Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl (2,6-Di-tertbutyl-p-cresol)	BHT	Chem Service	99%	220
Phthalate, dibutyl (1,2-Benzenedicarboxylic acid, n-dibutyl ester)	DBP	Chem Service	98%	278
Phthalate, dioctyl (1,2-Benzenedicarboxylic acid, n-dioctyl ester)	DOP	Chem Service	98%	390
2,4,6-Trinitrotoluene	TNT	Gift	NA	227

C. Extraction Experiments

1. Ethyl Acetate Liquid-Liquid Extraction

Liquid-liquid extraction entails the addition of an organic solvent to a liquid sample (in this case, aqueous) in order to partition compounds of interest into the organic phase. This phase is then separated, collected, cleaned up, concentrated and finally analyzed. Ethyl acetate was used to extract compounds more polar than would normally be extracted with methylene chloride, the solvent specified in EPA Method 625 for environmental analysis of aqueous samples. The ethyl acetate extraction procedure was only applied to a synthetic aqueous waste sample consisting of distilled water and three standards of varying polarity.

Duplicate synthetic samples were made by adding standards to one liter (L) distilled water. In order of increasing polarity, the compounds added were: 240 μg aminopyrene, 250 μg atrazine and 260 mg cresol. The samples were filtered by vacuum with Whatman 934-H (Maidstone, England) glass fibre filters. These 1.5 μm pore size filters were rinsed with approximately 500 mL distilled water prior to application of the sample. A serial extraction was performed by the addition of 60 mL of ethyl acetate to samples in 2 L separatory funnels. Samples were agitated for 2 minutes and allowed to stand for 10 minutes. The ethyl acetate fraction, being less dense than water, was collected after the water fraction was withdrawn. Distinct phase separation was difficult to determine with the first extraction, but improved with the second and third repetitions. The ethyl acetate fractions were combined in an evaporation flask and evaporated with a Buchi Rotavapor R110 (Flawil, Switzerland) to approximately 3 mL. This was concentrated further to approximately 1 mL in a 3 mL conical vial in a heated water bath under a ventilation hood. Two layers were evident in this sample. The top layer, presumably the concentrated ethyl acetate fraction ($\simeq$ 0.9 mL) was transferred to an HPLC sample vial. Approximately 900

μL methanol were added to the bottom fraction, a water/emulsion layer ($\simeq$ 0.1 mL). Both fractions underwent LC/DAD analysis as explained in section E 1.

2. Lyophilization

As noted earlier, lyophilization is a freeze drying technique that permits concentration of samples by a factor of up to several thousand. Sample volume and matrix interferences are two factors to consider with this technique. Larger volumes yield greater concentration factors, although the capacity of the lyophilizer can be limiting. Large amounts of solid residue, primarily salts, may cause matrix interferences with post-lyophilization analysis.

To test the utility of this technique, two experiments were conducted, one on synthetic samples, the second on leachate. Two sample volumes were tested, 500 mL and 1 L, which utilized the full 1.5 L capacity of the Labconco Bench Top Freeze Dryer Model 75034 (Kansas City, MO), used in this research. The 1 L samples were split into two 500 mL portions, and the extracts were combined after freeze drying.

Synthetic samples were prepared with 240 μg/L aminopyrene, 250 μg/L atrazine and 260 mg/L cresol. Samples of 500 mL were lyophilized in three 1200 mL flasks for 48 hours. After lyophilization was complete, any dry residue on the rubber flask lid was washed into the flask with methanol. Each fraction was extracted by agitating the dry residues with three 25 mL portions of methanol for 5 minutes. The extracts were pipetted into evaporation flasks and rotoevaporated at 40° C to approximately 1 mL. The concentrate was pipetted into a centrifuge tube with about 2 mL of methanol used to rinse the flask. These 3 mL samples were centrifuged to try to separate any remaining solid residue from the sample. The residue was assumed to be salts insoluble in methanol. The supernatant was decanted into 3 mL conical vials and concentrated under nitrogen gas (N_2) to approximately 1 mL. Final volumes were measured with a 2.5 mL Hamilton syringe (Reno,

NV) and samples were filtered through a 0.45 μm filter in a leur lock syringe prior to LC/DAD analysis.

The results from the synthetic samples, particularly the matrix interferences from the residues, prompted certain modifications when leachate samples were lyophilized. Many steps were taken to limit the chance of introducing solids onto the LC column. Two samples of leachate, 500 mL and 1 L, were spiked with 240 μg/L aminopyrene, 250 μg/L atrazine and 260 mg/L cresol and filtered twice, first with the 1.5 μm pore size followed by a 0.45 μm Magna Nylon 66 membrane filter (Honeoye Falls, NY). The samples were refrigerated prior to lyophilization. Labconco flask filters were used in the freeze drying flasks to prevent possible sample loss through the vacuum tube, but they were often ineffective. The large sample volume approached the capacity of the lyophilizer. As ice built up, the lyophilizer core became less efficient. Therefore, after 24 hours, the core was thawed to remove the ice. Also at this time the two 500 mL samples that constituted 1 L sample were combined into one flask, refrozen and lyophilized until completion. The dry residues were extracted with three 25 mL aliquots of methanol in a sonicator for 5 minutes because of concern that organics trapped in the residue were poorly extracted in previous experiments. Each 25 mL fraction was centrifuged separately at 2500 rpm for 5 minutes in a Sorvall Superspeed RC2-B Automatic Refrigerated Centrifuge (Wilmington, DE) and combined when transferred into an evaporation flask. The extracts were then rotoevaporated to less than 3 mL, pipetted to 3 mL conical vials and concentrated under N_2 to about 1 mL. Samples were filtered and a final volume was measured prior to LC/DAD analysis.

3. Solid Phase Extraction

Solid phase extraction (SPE) procedures were pursued next as a simpler, less expensive and quicker alternative to the other two methods tried. Since there are numerous solid phase sorbents and elution solvents available, the question arose as to which would be most appli-

cable to these environmental samples. It was important to recall Ghoui's (1987) admonition that no one sorbent will likely extract all of the compounds of interest.

Thus, for the analysis of the leachate, two sorbents were utilized. Octadecylsilyl (C_{18}) bonded-phase columns were selected because they appear to be the type most often cited in the literature for aquatic samples and have broad applications including extraction of nonvolatile aqueous organics. Analytes that are nonpolar or which can be made nonpolar by adjusting the pH (e.g. acids and bases) can be adsorbed onto C_{18} sorbents. One of the objectives was to select for nonvolatile polar organic molecules. Aminopropyl (NH_2) columns were selected as weak anion exchangers in an attempt to selectively extract polar organic analytes from the leachate.

For the solid phase extraction of the industrial wastewater, only C_{18} columns were used. This was felt to be sufficient for the extraction of the nitroaromatics and nitroamines thought to be present in the wastewater.

All extractions utilized Bond Elut® sorbent cartridges purchased from Analytichem International (Harbor City, CA). All cartridges contained 500 mg of sorbent. Adapters to fit cartridges together were not available at the time the leachate was analyzed. Large reservoir (10 mL) cartridges were found suitable for attaching two units together during the serial extraction of the leachate. Regular (3 mL) cartridges were used with the wastewater samples.

As noted in Chapter II, SPE sorbents are analogous to liquid chromatography column packings. Adsorption and elution of analytes follow the same basic principles of LC, that is, the interaction between column packing and mobile phase. Once sorbent and elution solvent are chosen, these interactions are maximized in SPE by following the four-step procedure outlined in the previous chapter: column conditioning; analyte adsorption; column postwash; and analyte elution. Specific details of the procedures used for each sample in this research follow.

D. Solid Phase Extraction Experiments

1. Leachate Samples

a. Analysis With C_{18} Cartridges

Several experiments were conducted to determine an optimal SPE method for the samples used.

Elution Solvent and Volume: The first task was to determine an appropriate elution solvent and volume. Samples of 500 mL leachate or distilled water were spiked with identical amounts of Cresol, ATZ and APY. One percent or 5 mL of methanol was also added to the samples prior to filtration. The columns were conditioned with one to two column volumes of the solvents being tested, in this case methanol followed by methylene chloride. Distilled water was the final conditioning agent applied prior to introduction of the samples onto the columns. Solvents and samples were pulled through the columns by vacuum at approximately 25 psi which gave a flow rate of 5 - 10 mL/min. The columns were subsequently washed with approximately 10 mL of distilled water and allowed to dry for 10 minutes. A homemade device utilizing paper clips and thin flexible wire was created to permit elution of the samples directly into the 2 mL LC/DAD vials obviating the need for purchase of a specialized vacuum elution system. Three 500 μL aliquots of methanol followed by 3 500 μL aliquots of methylene chloride were drawn through the columns at 8 - 10 psi. Each fraction was collected separately for LC analysis. Final volumes of the methanol samples ranged from 380 - 420 μL, and from 170 - 320 μL for the methylene chloride fractions. Recovery of standards was determined by single point comparison of integrated areas of a mixture of the standards with the sample areas. While recoveries of each standard varied, it was determined that the three 500 μL aliquots of methanol were sufficient to elute the analytes retained on the SPE sorbent. In subsequent

extractions, the three aliquots of methanol were collected in one 3 mL conical vial, concentrated under N_2 with a Supelco 6-port Mini-Vap (Bellefonte, PA) and the final volume measured before being transferred to the LC vials for LC/DAD analysis.

b. Analysis With NH_2 Cartridges

Aminopropyl (NH_2) columns were selected as weak anion exchangers. A sample was adjusted to pH 7.4 in order to be at a pH that was apprximately two pH units above the pKa's of the target analytes in the sample. A phosphate buffer solution was prepared according to section 433 of *Standard Methods for the Examination of Water and Wastewater* (1985). To achieve a pH of 7.4, 1.18 grams of KH_2PO_4 and 4.30 grams of Na_2HPO_4 were dissolved in 1 L distilled water. This solution was used to condition (following application of methanol) and wash the anion exchange columns. Addition of the buffer to the leachate caused formation of a white precipitate which clogged the SPE columns. It was also feared that the precipitate would remove some compounds of interest by enmeshment in the floc. The precipitate was likely formed by phosphate salts of Ca, Mn, Mg and Fe which are present in large amounts (1 -500 mg/L) in the leachate (determined by Atomic Absorption). Therefore, the leachate, of pH 6.5, was not buffered prior to extraction. Thus the effectiveness of the extraction was limited to those compounds with a pKa less than 4.5. Even though the leachate was not buffered, the phosphate buffer was used to condition and wash the NH_2 columns. Three types of elution solvents were tried: i) a high ionic strength solution; ii) a high pH solution; and iii) a low pH solution. A 0.5 M citrate solution in 1% methanol was prepared by dissolving 15 grams sodium citrate (J. T. Baker, Phillipsburg, NJ) in 99 mL distilled water plus 1 mL methanol. The high pH solution was made to 0.1 N NaOH in methanol while the low pH solution was prepared to 0.1 N HCl in methanol.

c. Breakthrough

The manufacturer of Bond Elut cartridges suggest that the amount of analyte capable of being retained is equal to 5% of the sorbent mass. This would allow retention of 25 mg of substance on the 500 mg columns. Since the COD of the leachate was reported to be 75 -100 mg/L, 35 - 50 mg of material may be available per sample. Results from the first experiment suggested that not nearly that amount is extractable.

To test for breakthrough, two C_{18} cartridges were attached with tape and parafilm so that the sample would be applied serially. Leachate and control samples of 500 mL were spiked with Cresol, ATZ and APY. Ten percent or 50 mL of methanol were added to the samples prior to filtration. This experiment was also repeated using NH_2 columns conditioned with phosphate buffer and eluted with three 500 μL aliquots of a strong counterion, 0.5 M citrate in 1% methanol.

The capacity of the C_{18} bonded phase packing was measured for the most polar of the standards used, Cresol. The sorbent was removed from a SPE cartridge and packed into an empty LC guard column which was installed onto the HPLC. A 50 mg/mL solution (90/10, water/methanol) of cresol was prepared and pumped through the column as the mobile phase at 0.5 mL/min. The automatic delivery system of the LC permitted conditioning of the column with methanol and water prior to cresol application. Breakthrough time was taken at the inflection point of the Cresol chromatogram. Breakthrough was determined by dividing the product of breakthrough time, flow rate and cresol concentration by the sorbent mass. This experiment was repeated with aminopropyl packing and cresol. Phosphate buffer replaced water as a conditioning solvent.

d. C_{18}/NH_2 Serial SPE of Leachate

It was resolved that 500 mg of SPE sorbent would be sufficient for 500 mL samples of this leachate. However, since any one sorbent type is unlikely to extract all compounds of interest, the leachate was serially extracted through C_{18} and NH_2 (buffered as an anion exchange column) cartridges. The large reservoir cartridges were taped together so that the samples would pass through the C_{18} column to the NH_2 column.

For each leachate sample run, an equal volume of distilled water was treated identically. Some samples were spiked with 100 μg ATZ, 225 μg BZL and 300 μg BSF1, while others were unspiked. One sample had 10% (50 mL) methanol added while all five of the subsequent samples had only 1% (5 mL) methanol added. To avoid possible interferences from the citrate, the NH_2 columns were eluted with either the acidic or basic methanol solutions. Samples were analyzed by both LC/DAD and LC/MS.

2. SPE of an Industrial Wastewater

a. SPE Conditions: To fulfill one of the objectives of this research, the techniques developed on the leachate were applied to the analysis of a raw industrial waste stream known to be more concentrated than the leachate. That is, its COD was about two orders of magnitude greater than that of the leachate. The COD was determined by Section 508 B, *Standard Methods for the Examination of Water and Wastewater* (1985). Sample volumes of 25 mL of industrial wastewater were filtered and extracted in one step. Gelman Nylon acrodisc 0.45 μm filters were attached to a 30 mL leur lock glass syringe. The acrodisc was fitted into an adapter which connected to the 2.8 mL Bond Elut SPE cartridges.

The C_{18} cartridges were conditioned with two column volumes of methanol followed by two column volumes of the pH 7 phosphate buffer solution for the pH 7 samples and with a pH 10

NaOH distilled water solution for the pH 10 samples. Samples were drawn through by vacuum at approximately 15 psi. All extractions with the industrial wastewater were performed beneath a hood. The columns were washed with the same buffer and water solutions used to condition the columns and allowed to dry for 10 minutes before elution with methanol. Samples were eluted with three 500 μL fractions of methanol collected in one vial, concentrated and refrigerated overnight prior to analysis. The pH 10 samples were eluted with 0.1 N NaOH in methanol. All samples were analyzed by LC/DAD. Only unspiked samples were analyzed by Thermospray LC/MS and GC/MS.

An initial experiment with two C_{18} cartridges in tandem revealed that one would be sufficient for 25 mL samples.

b. Recovery Experiments: An attempt was made to quantify the recovery of two principle components in the wastewater, Diphenylamine and 2,4-DNT. Standards of 99% purity of these two compounds were weighed together in a 10 mL volumetric flask and dissolved in methanol to yield respective concentrations of 5 and 10 mg/mL. Synthetic wastewaters were made at two concentrations by mixing 50 μL and 500 μL of the standard solution with water (5% methanol) in 50 mL volumetric flasks. However, a precipitate formed, possibly due to the interactions between the two compounds and the relative insolubility of DPA in water. The CRC *Handbook of Chemistry and Physics*, (1979) (HOCAP) lists DPA as insoluble in water. Verschueren's *Handbook of Environmental Data on Organic Chemicals*, (1983), gives an aqueous solubility of 300 mg/L. However, the effects of pH, salts and other constituents in the same solution on DPA solubility are unknown. To determine the actual concentration of the DPA in the solution, 250 μg (Solution A, estimated to be 5 mg/L) and 1050 μg (Solution B, estimated to be 21 mg/L) of DPA were added to separate 50 mL volumetric flasks with distilled water and 1% methanol, and stirred overnight.

Three methods were utilized to determine the actual concentration of DPA in these solutions. In method one LC/DAD analysis was done to try to determine the concentrations of these solutions using a standard curve based on peak areas at five different mass loadings.

Method two utilized Lambert-Beer's Law:

$$A = C\varepsilon L$$

where: A = UV absorbance at a specific wavelenght, C = concentration in moles/L,

ε = molar absorptivity coefficient in L/mole-cm and L = cell path length in cm.

The CRC *Handbook of Chemistry and Physics* (1979), gives a logε value of 4.29 for DPA in alcohol at 286 nm. This equals an ε value of 19498 L/mole-cm.

To check the HOCAP ε value, three known concentrations of DPA in methanol (52.5, 210 and 420 μg/mL) were analyzed with a Beckman Instruments Model DU-6 Spectrophotometer at 286 nm zeroed against methanol. Absorbances were measured three times and a methanol blank was measured after each sample to confirm accuracy. Lambert-Beer's Law was used to calculate ε for each of the three solutions.

UV absorbance of Solutions A and B were measured directly with the DU-6 Spectrophotometer at 286 nm zeroed against a 1% methanol Milli-Q water solution. Concentrations of these samples were then calculated using Lambert-Beer's Law. Each fifty milliliters of Solution A or Solution B were applied to C_{18} columns and then extracted with methanol. Total DPA mass recovered from the extracted samples was determined from DPA standard curves at 230 nm and 286 nm using peak areas integrated by the LC/DAD. Lambert-Beer's Law, using the LC/DAD ε values (explained below) at 230 and 286 nm for the absorbances observed, was also used to determine the DPA mass recovered; peak heights were used as a measure of absorbance.

Since the LC/DAD was used to separate DPA and DNT, it was desirable to obtain ε values for DPA and DNT under the specific conditions used during LC/DAD analysis. Absorbances ob-

tained from the analyses of three solutions of DPA in methanol (52.5, 210 and 420 mg/L) were used in Lambert-Beer's Law to calculate ε values at 230 and 286 nm. (The cell path length for the diode array detector was 0.6 cm.) Values for these three individual concentrations were averaged. This averaged value (one at 230 nm and one at 286 nm) was designated "LC/DAD ε" An LC/DAD ε value was also calculated for DNT at 230 nm based on replicate runs of standard solutions at three different concentrations in methanol (210, 420 and 630 mg/L).

To test the SPE recoveries of a mixture of DPA and DNT, 100 μL of the standard mixture containing 5 mg/mL DPA and 10 mg/mL DNT was diluted to 100 mL of water (1% methanol) and stirred overnight. This solution was analyzed by LC/DAD at 230 and 286 nm prior to extraction. It was then divided into two 50 mL portions each of which was extracted with one C_{18} SPE cartridge as before.

Standard addition was another method for measuring recoveries that was investigated. Three 50 mL samples of wastewater were extracted by C_{18} SPE as before: one sample was unspiked, one had 52.5 μg DPA added, and 105 μg DPA were added to the other. Extracts were analyzed by LC/DAD. Since the DNT levels were off scale on the standard curve, 1:10 dilutions of the final extracted samples were made by mixing 100 μL of each sample with 900 μL methanol. Duplicate LC/DAD analyses were done on each of these dilute samples also.

E. INSTRUMENTAL ANALYSIS

1. LC/DAD

All liquid chromatographic (LC) analyses conducted at Virginia Tech were on a Hewlett-Packard Model 1090M fitted with a diode array detector (DAD). Absorbance was monitored

at 230, 254 and 280 nm with bandwidths of 10, 10 and 35 nm respectively unless noted otherwise. An Alltech Econosphere C_{18} 5 μm 250 mm x 4.6 mm i.d. column was used for all analyses with a Rainin guard column. The Alltech C_{18} pellicular guard column packing was changed about once a month. Column temperature was maintained at 40° C during all analyses to avoid variances due to ambient temperature fluctuations.

All samples were run under the following conditions unless otherwise stated. At the start of each session, the injector was washed for a few minutes. Sample volume injected was 15 μL. Methanol and water were the primary mobile phases used. All solvents were continuously purged with Helium. Prior to the analysis of the first sample for the day, methanol was injected for a 25 minute "start" run at a methanol/water gradient that went from 1% to 100% methanol in 7 minutes, was isocratic for 5 minutes and then returned to 1% methanol in 10 minutes where it remained for 3 minutes. This served to both flush the column and equilibrate it. A gradient was used during equilibration of the column because rapid changes in mobile phase composition can be detrimental to the column and the analysis. Each sample had a 40 minute run time as the gradient went from 1 to 60% methanol in 14 minutes, 60 to 68% in 8 minutes, and 68 to 100% in 18 minutes before returning to 1% methanol in 10 minutes. To be ready for the next injection, a 5 minute equilibration period preceded each run. Flow rate was 1.2 mL/min. Peaks were integrated according to the following integration events:

Peak Width	0.100
Threshold	1
Area Reject	1
Shoulders	ON

Most samples were analyzed twice and average areas calculated. Several samples were rerun using a 0.1 M Ammonium Acetate solution instead of water as the aqueous mobile phase to test for interferences with the chromatography before Thermospray LC/MS was performed.

LC fractions were manually collected from three extracted wastewater samples to try to isolate peaks or groups of peaks for LC/MS analysis. Fraction #1 was collected from 1.8 to 3.5 minutes, #2 from 6.9 to 7.9 min, #3 from 14.0 to 18.5 min, #4 from 18.5 to 19 min, #5 from 19 to 23 min, and #6 from 29.5 to 31 and 32 to 34 minutes. Each fraction was analyzed on the Waters LC system at NIEHS at 1 mL/min under isocratic conditions most closely resembling the likely mobile phase composition of each fraction. These fractions were later concentrated under N_2 to 200 - 300 μL and analyzed on the HP1090 LC system at 1 mL/min with a methanol/water gradient that went from 60 to 70% methanol in 20 minutes and 70 to 100% in 10 minutes before returning to 60% methanol in 5 minutes.

2. Thermospray LC/MS

All on-line mass spectrometric analyses were conducted at the LC/MS lab at the National Institute for Environmental Health and Sciences, Research Triangle Park, NC. The same LC column and guard column as used at Virginia Tech were used on a Gilson LC with a Rheodyne 6-port injector and a Waters UV detector at 254 nm. The column was not heated. The interface was a Vestec 701S Thermospray (TSP) device. The mass spectrometer was a quadrupole VG Analytical VG 12-250 operated in both positive and negative chemical ionization modes. Mobile phase was methanol and either water or 0.1 M Ammonium Acetate. All solvents were degassed in a sonicator prior to use. Leachate and wastewater samples and standards were analyzed by TSP LC/MS. The flow rate was 1 mL/min. The mobile phase conditions were modified slightly to facilitate use of the Thermospray interface. A five minute isocratic run at 10% methanol was followed by a 30 minute gradient increase to 100% methanol. This level was maintained for 5 minutes.

3. *OTLC-MS*

The open-tubular LC/MS system used was developed by deWit *et al.* (1987). It allows direct liquid introduction of the total effluent from an open-tubular liquid chromatography column into a mass spectrometer. A 10 μm i.d. fused silica capillary open tubular column tapered to 1 μm was inserted into an MS probe of a Finnigan 3300 mass spectrometer. The system was operated as follows: the mobile phase flow (60 nL/min methanol) was stopped while 200 μL of sample was injected into the sample reservoir. The sample was injected onto the column for 1 second. Flow resumed when the column was reopened. All but the few nanoliters of sample injected onto the column can be recovered. (See schematic) Positive and negative CI modes were used with methane as the ionization source. The source temperature was 150° C; the tip temperature was operated up to 300° C. Five individual standards were run on this system. Separation of a mixture of standards and analysis of a leachate sample was attempted with an OV-17 fused silica capillary column on this system.

4. *Off-line MS*

LC eluent fractions were manually collected for off-line EI MS analysis. Fractions from a mixture of four standards (309 mg/L BSF1, 9530 mg/L BZL, 10,000 mg/L BZD and 7530 mg/L DBP) were collected every minute in 2 mL vials with plastic caps. UV absorbance of each fraction was measured at 230 nm on the DU-6 UV Spectrophotometer. Spectra were obtained to confirm the identity of the standards. LC retention times were compared with the fraction collection times which allowed determination of the residence time for peaks to flow from the detector to the collection exit port.

The fractions that had UV absorbance were dried under N_2 and reconstituted in methanol before EI MS analysis. Analysis was done on a VG - 7070E - HF via direct probe inlet, heated if

necessary from 30 - 300° C. The electron energy was 70 eV; the accelerating voltage was 4 kV; and the source temperature was 200° C.

With an estimate of the eluant retention time determined for the analytical standards (above), fractions thought to contain peaks from a lyophilized leachate sample were collected from three LC/DAD analyses and combined. This was done in an effort to get as much sample as possible for EI-MS.

5. GC/MS

A Hewlett Packard HP-5890 Series II Gas Chromatograph with 5870 Mass Selective Detector was used for all GC/MS analysis. The column was a DB5 fused capillary column 30 meters long of 1.0 μm film thickness (J & W Scientific, Folsom, CA). The column oven temperature was programmed to rise from 55 to 320° C in 20 minutes. The transfer line was set at 280° C, injector at 250° C, and the detector at 130° C. Data were analyzed on the HP 9000 data system.

IV. RESULTS and DISCUSSION

A. Overview

The methods described in the previous chapter are, in fact, the results of much effort towards achievement of one of the objectives of this work: methods development. More data were collected from SPE than other extraction techniques because that procedure developed into the method that warranted the most attention.

Likewise, the LC/DAD conditions cited were the product of numerous trials where column temperature was the only variable kept constant. Standards of BZL, BSF1, BSF2, BHT, BZD, DBP and DOP were used to identify LC conditions suitable for separation of compounds likely to be found in leachate. Numerous mobile phase compositions and flow rates were used. The conditions finally adopted utilized a methanol/water gradient that went from 1 to 100 % methanol in 30 minutes. Since unknowns were being analyzed, this offered a system to separate a broad range of components according to polarity. The most polar or hydrophilic components eluted first. Results of the work with the standards mentioned above indicated good separation at a methanol concentration of 60 - 68%. Therefore, the gradient rate of in-

crease of methanol was slowed to 1% per minute between those values. These conditions were held constant through the remainder of this work for the sake of consistency.

All LC/MS work was performed in Research Triangle Park, NC by National Institute of Environmental Health and Sciences (NIEHS) staff expert in Thermospray and Open Tubular LC/MS. The LC elution conditions used on the LC/MS system were similar to those used on the HP 1090 system. The author relied on the expertise of NIEHS for acquisition of all of the Thermospray and OTLC/MS data. Data were interpreted at VPI & SU. Use of the OTLC/MS system was a result of the temporary incapacitation of the VG 12-250 mass spectrometer due to the failure of an RF generator. This is noted because instrumental problems are a very real result of this type of analysis.

B. Liquid-Liquid Extraction

The LLE procedure with ethyl acetate proved to be burdensome, time consuming and produced nebulous results. Due to its relatively high solubility in water, good phase separation did not occur until the second serial extraction. However, after concentration, the final sample separated into two phases. This made analysis much more difficult and questionable. Also, if organics were indeed extracted into the ethyl acetate and a sizeable portion of the solvent remained in the aqueous phase, then a large amount of analytes could be lost from analysis. This necessitated that disposal of the residual water fraction conform to that required of ethyl acetate.

Both phases of the concentrated extract of the spiked water sample were analyzed by LC/DAD. All three analytical standards were found, but the large number of interference peaks also present further limited the utility of this extraction technique. As a result of these difficulties, it was decided to abandon this procedure and explore other extraction techniques.

C. Lyophilization

Cresol was virtually unrecovered from all samples, even though it was spiked at levels close to 2600 mg/L. Recoveries of the other standards ranged from 6 to 17% for the distilled water sample (unsonicated), to 17 to 96% for the leachate sample which was sonicated during extraction with methanol. (See Table 2).

A chromatogram of a freeze dried leachate sample is shown in Figure 1. All of the LC/DAD data were acquired at 230 nanometers (nm) unless otherwise noted. Sensitivity was greatest at this wavelength. For example, the 1000 mL leachate sample had 12 LC peaks at 280 nm, but 47 peaks at 230 nm. The chromatographic results show a cluster of peaks eluting from 2.3 to 5.8 minutes including two very large peaks eluting at 2.3 and 2.7 minutes. Hewlett Packard software associated with the HP 1090M LC/DAD system was used to determine the purity of individual peaks. A purity match greater than or equal to 990 indicates very high purity (99%) while a purity match less than 990 indicates impure peaks. The first peak of the cluster gave an impure purity match (850) while the other indicated very high purity (match of 995). Another 18 peaks were fairly well separated between 15 and 31 minutes. Of the peaks where data were available, 8 peaks were identified as pure while 6 were impure. Aside from the standards, only 4 peaks had UV absorbance maxima at wavelengths greater than 210 nm.

One-minute LC eluent fractions were collected at 1, 3, 4, 16 18, 20, 21, 25, 26, 28 and 31 minutes. The same fraction was manually collected three times in the same vial as a crude way to collect and concentrate the separated peaks for off-line EI/MS analysis. The time for peaks to flow from the detector to the exit was determined to be from ½ to ¾ minutes by collecting fractions of a mix of standards, measuring their absorbances on the a scanning UV spectrophotometer and comparing the fractions with absorbance to the peak retention times on the LC.

Table 2. Percent Recovery of Spiked Standards from Lyophilized Samples

Standard	Distilled Water 500 mL	Distilled Water 1000 mL	Leachate 500 mL	Leachate 1000 mL
Aminopyrene	8%	17%	24%	17%
Atrazine	8%	6%	96%	54%
Cresol	0%	< 1%	0%	0%
Total # LC Peaks @ 230 nm	7	20	36	47

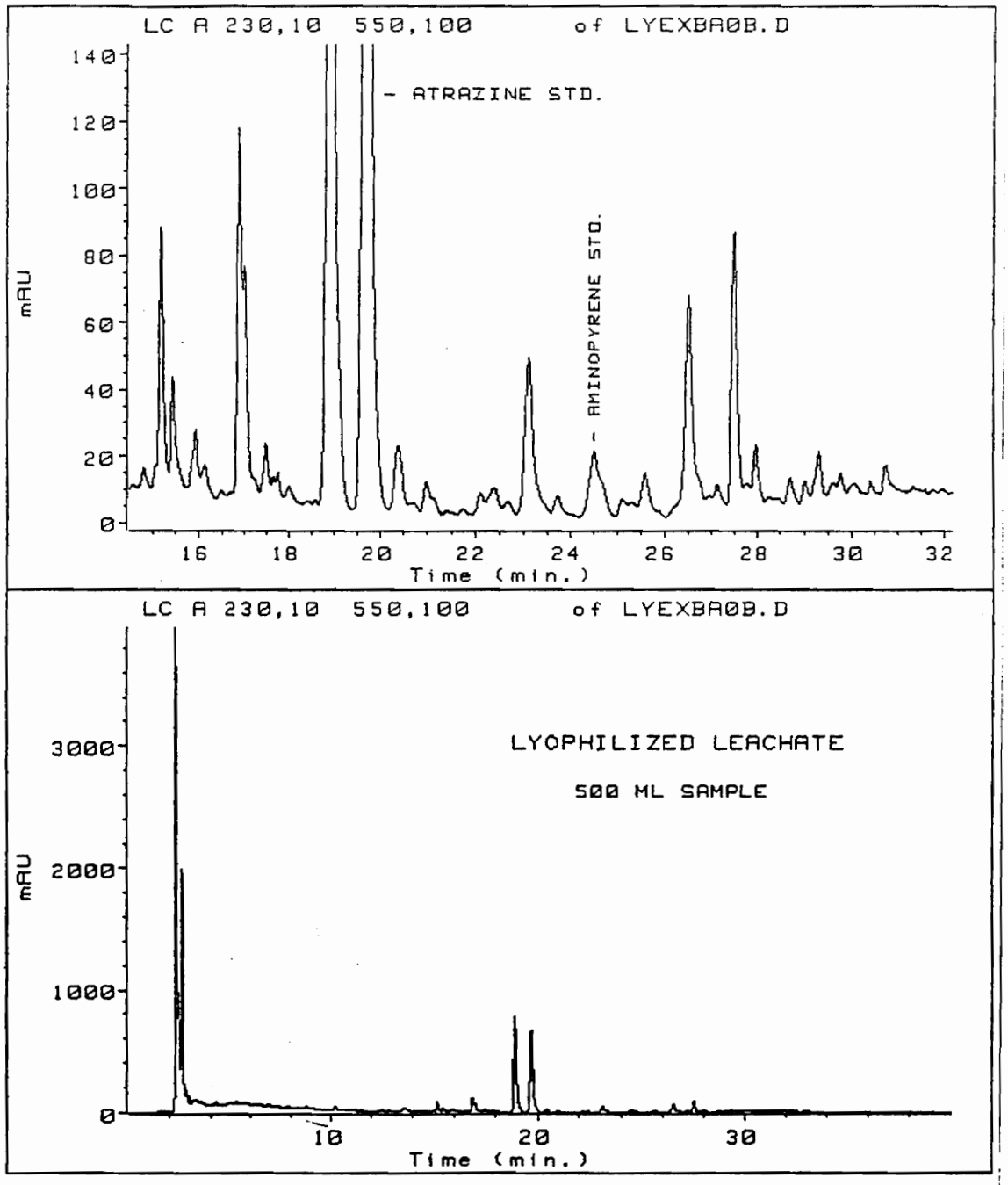

Figure 1. LC Chromatogram of Spiked Lyophilized Leachate: Top chromatogram magnified 25 times; bottom chromatogram is full scale.

Off-Line EI/MS results: Attempts were made to match the mass spectral results with results from an EI library. Confidence of each match can be correlated with the purity (P) and mixture (m) scales that range from 0 to 1000, where values over 500 denote confidence greater than 50%. The results of off-line EI/MS analysis of LC fractions of lyophilized leachate are summarized in Table 3. Atrazine standard was detected in peak 14 of fraction 21 at about the 1 μg level and confirmed by library matching (P 774, m 982). Silanes were also detected. Spectra of fractions collected at 16, 18, 20, 21 and 28 minutes had fragments separated by 30 mass units. This could indicate a polymer of CH_2O, cyclic ethers, methoxy aromatics or nitro aromatics. The spectrum of fraction 4 indicates a phthalate. The spectrum of peak 11 from fraction 16 gave a library fit (P 400, m 595) of 1-methoxy-4- 2-(4-nitrophenyl) ethyl benzene, molecular weight (MW) 255. The spectrum from fraction 18 was nearly identical to that of fraction 28, as was that of 20 to 21. Number 18 was library matched (P 359, m 472) to Phenyltris (trimethylsiloxyl) silane (MW 372). It is not known whether this compound originated from the leachate or is part of the silica solid support for the LC column packing material. However, direct probe EI/MS analysis of C_{18} packing material did not yield a similar spectrum. A library fit (P 333, m 372) of N-(3,4,5,6-tetraethyl-1-phenyl-2 (1H) benzeneamine (MW 358) was given for peak 16 from fraction 20. Fraction 25 contains the spectrum typical of a silicone. Fraction 28 yielded a library fit (P 304, m 718) of 1,2-dibenzofurandicarboxylic acid, 1,9B-dihydro-4 (MW 314). Spectra from fractions 4 and 31 suggest DBP. Since one would not expect phthalate isomers to elute so far apart, the phthalate in fraction 4 is likely the result of contamination during the manual collection, storage or transportation to the MS lab phases of the procedure. EI/MS analysis of HPLC fractions collected from injecting a mixture of 5 μg BSF1, 143 μg BZL, 150 μg BZD and 113 μg DBP onto the LC column, yielded probable confirmation of only the BSF1 and the phthalate. The MS may not be as sensitive for detection of the benzamide and benzothiazole or the results may indicate limitations of the off-line collection system.

Table 3. LC and Off-line EI/MS Data of Lyophilized Leachate

LC DATA				EI/MS DATA	
Fraction #, min.	Likely LC Peaks, min.	Purity Match	λ_{max}, nm	TIC Peak	Spectra: Most Intense Fragments, m/z
1	Blank			7 12	104, 90, 75, 62 167, 149
3	1.7 2.3	- 850	slope slope	13	144, 142, 104, 73
4	2.7 2.9 3.1	995 990 983	slope slope slope	5 11	279, 167, 149 174, 149, 104, 73
16	15.3 15.5	986 985	270 slope	4 11 12	255, 195, 165, 151 287, 255, 225, 195, 151 287, 255, 195, 151
18	16.9 17.1 17.5	996 999 998	212,266 214 slope	11	331, 299, 267, 237, 207, 177, 163
20	18.9	1000	214	4 & 16	375, 343, 313, 267, 237, 207, 177, 163
21	19.7 20.4	978 987	220,262 slope	14 4,20	215, 200, 173 343, 313, 267, 237, 207, 163
25	24.6	NA	240,280,356	3 10	429, 335, 281, 221, 149, 73 221, 149, 104, 73
26	24.6 25.6	NA NA	240,280,356 slope	4,8 9	167, 149 279, 167, 149
28	26.5 27.5	1000 1000	slope slope	13	299, 267, 237, 207, 163
31	blank	-	-	7	279, 167, 149

slope = no absorbance maxima observed between 210 and 400 nm

D. Solid Phase Extraction - Leachate

1. Elution Volume

The first experiment was to determine an appropriate solvent elution volume. This was done by looking at the number of LC peaks detected at 230 nm from each elution fraction for the 500 mL samples of water and leachate spiked with the same amounts of internal standards. The first three fractions were eluted with methanol (MeOH) while the last three were with methylene chloride ($MeCl_2$). The number of peaks detected per elution fraction for each sample were as follows:

Elution Fraction	Spiked Control	Spiked Leachate
1 MeOH	6	20
2 MeOH	2	5
3 MeOH	1	2
4 $MeCl_2$	0	0
5 $MeCl_2$	0	0
6 $MeCl_2$	0	0

These results confirm that three 500 μL volumes of methanol were adequate for eluting recoverable organics from the sorbent.

Recoveries of the standards were estimated by comparing the integrated areas of each compound from the samples with the areas for each compound from a known mixture of the standards. Areas used were averages of duplicate LC runs. The calculation is as follows:

$$\text{Mass recovered} = \frac{C}{A_{st}} \times A_s \times V_f$$

where:

C = concentration of the standard in the mixture, μg/mL

A_{st} = integrated area of the compound from the standard mix

A_s = integrated area of the compound from the sample

V_f = final extracted sample volume.

$$\% \text{ Recovery} = \frac{\text{Mass recovered}}{\text{Mass added}} \times 100$$

Recoveries given in Table 4 were corrected for purity by multiplying the percent recovery by the purity match achieved, e.g. a match of 1000 has a multiplication factor of 1.000 and 990 of 0.990.

2. *Breakthrough*

In the breakthrough experiment, 500 mL control and leachate samples were spiked with 120 μg of APY and ATZ and 5170 μg Cresol. The LC detected 7 peaks in the first C_{18} cartridge, 3 in the second; 3 in the first NH_2 cartridge and 3 in the second for the control sample. For the leachate samples, 13, 8, 6 and 3 peaks were detected, respectively. Citrate accounted for 2 peaks in the aminopropyl extracts. Extraction of the leachate onto two cartridges in tandem was deemed sufficient for the organics extractions performed.

These results led the investigator to believe that adding 10% methanol to the samples was excessive. Analytes may have been unretained on the cartridges due to a stronger affinity for the methanol. Both Cresol (2 peaks) and ATZ broke through to the second cartridge.

Table 4. Percent Recovery of Standards from C_{18} SPE Cartridges Eluted with 500 μL Fractions of Methanol

Standard	Distilled Water				Leachate			
	Fraction #				Fraction #			
	1	2	3	Total	1	2	3	Total
Cresol	5	0	0	5	4	0	0	4
Atrazine	66	9	0	75	55	21	1	77
Aminopyrene	17	23	2	42	4	9	2	15

In the second breakthrough experiment, time zero for the Cresol solution flowing through the column was assumed to be at the point when absorbance was first detected. This occurred at 3.2 minutes. Breakthrough was said to occur at the inflection point of the cresol chromatogram. The results indicated a cresol capacity of the C_{18} packing as approximately 414 μg per 44 mg of packing, or 1% and 530 μg cresol per 36 mg NH_2 packing or 1.5%. Non uniform packing of the column, channeling and wall effects could affect the breakthrough pattern, however.

3. *C_{18}/NH_2 Serial SPE of Leachate*

HPLC Results: The C_{18}/NH_2 tandem extraction of the leachate resulted in the detection of 15 to 32 separated peaks by the LC from the C_{18} extracts and from 0 to 3 peaks from the NH_2 extracts. Average recoveries of ATZ, BSF1 and BZL were determined for the C_{18} cartridges from two extractions as noted in Table 5. Many of the compounds detected by LC did not have a maximum UV absorbance at a specific wavelength. Rather, their spectra sloped downward from 210 to 400 nm. These are indicated by "slope" in the accompanying tables. While no positive identification was made, several peaks exhibited UV spectra similar to those identified for phthalates, that is, late eluting peaks absorbing near 222 and 274 nm. Retention times and absorbance maxima (λ_{max}) for a few samples are listed in Table 6.

TSP LC/MS Results: The results of a sample from one spiked leachate C_{18} extraction analyzed by LC/DAD and TSP LC/MS are given in Figure 2, Table 7 and Table 8. LC/DAD data are given in Table 7 for comparative purposes. In positive chemical ionization (PCI) mode, five unknown spectra indicative of compounds in the sample, were detected in addition to the detection of all three internal standards.

Table 5. Percent Recovery of Standards from C_{18} SPE Cartridges

Standard	Distilled Water	Leachate
Atrazine	58	68
Benzenesulfonamide	48	59
Benzothiazole	52	61

Table 6. LC/DAD Retention Times and UV Absorbance Maxima for Extracted Leachate Samples

Sample 1		Sample 2				Sample 3			
C_{18}		C_{18}		NH_2		C_{18}		NH_2	
t_R min	λ_{max}nm	t_R min	λ_{max}nm	t_R min	λ_{max}nm	t_R min	λ_{max}nm	t_R min	λ_{max}nm
1.4	222	1.4	222	1.5	222	1.7	slope	1.5	220
2.6	220	2.6	slope	2.6	slope	1.8	224	2.6	240, 272
2.8	220	2.8	220			3.2	slope		
2.9	222, 260	2.9	slope			3.4	224, 260		
4.9	slope	6.4	slope			6.1	222		
5.3	slope	8.3	slope			6.3	slope		
5.4	slope	8.7	slope			6.5	226		
8.0	slope	8.9	slope			13 peaks from 6.6 to 14.4 min	slope		
12.7	232	13.5	232						
		15.1	220						
		15.3	222						
15.6	slope	15.5	222						
15.9	(274)	15.9	222						
		16.6	222						
		16.8	220			14.6	232		
20.1	220, 254	16.9	220			15.9	slope		
27.7	270	17.1	220			16.3	slope		
30.9	222, 274	17.7	220, 254			17.1	slope		
31.1	222, 274	18.0	220, 254			17.6	220		
31.4	222, 274	18.4	222, 254			17.7	220		
31.6	222, 274	18.6	220, 254			18.5	222, 260		
31.8	222, 274	20.1	218, 252			19.5	218		
31.9	222, 274					20.0	218		
32.0	222, 274	34.0	230, 274			24.1	274	33.7	230, 274

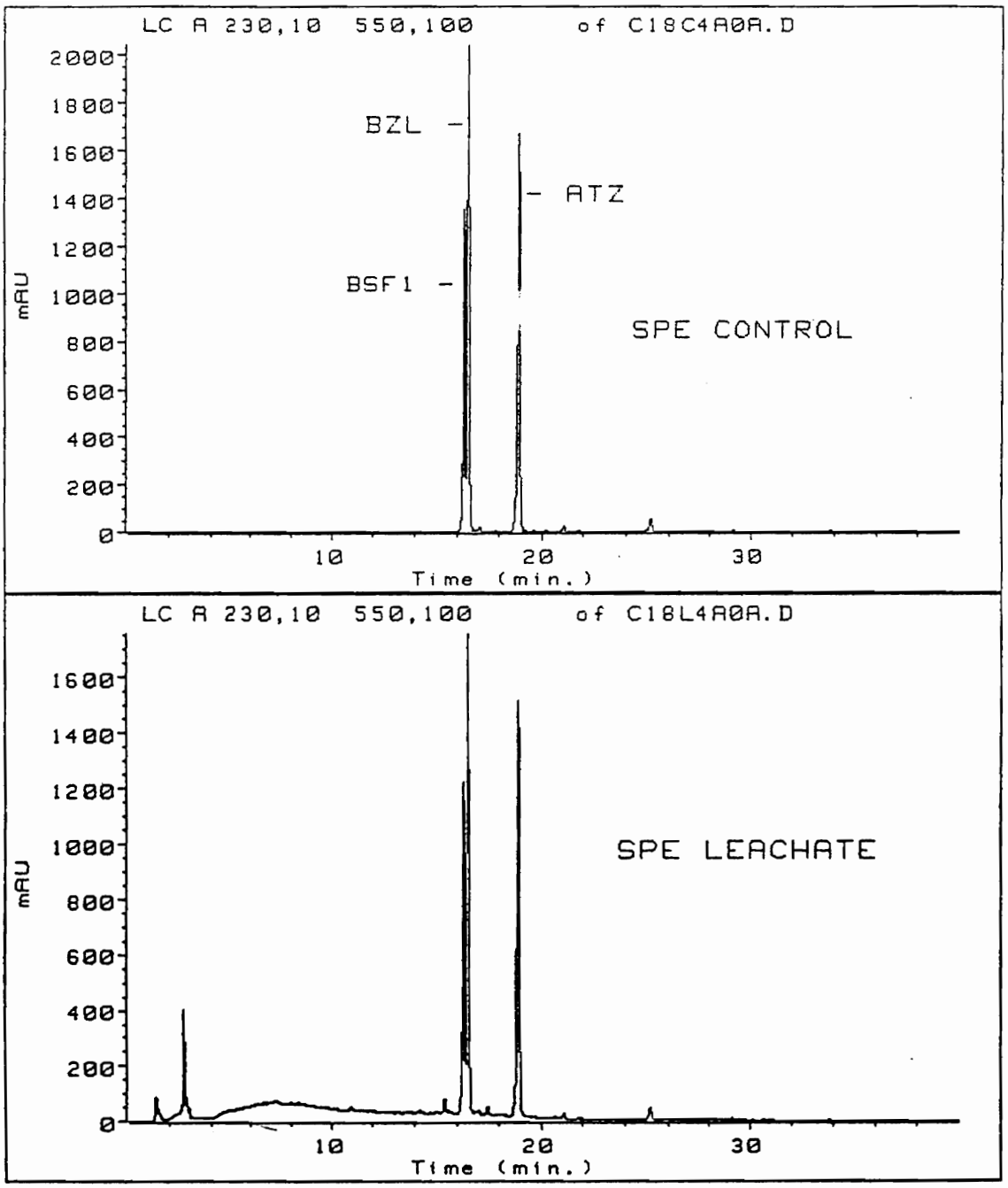

Figure 2. LC/DAD Chromatograms of Spiked Leachate and Control Samples from C_{18} SPE Extracts

Table 7. LC/DAD and Thermospray LC/MS Data of Solid Phase Extracted Leachate

LC/DAD DATA		TSP+ LC/MS		TSP- LC/MS	
t_R,min	λ_{max} nm	t_R	Spectrum,m/z	t_R	Spectrum, m/z
1.4	slope	2.2	201	2.0	127
2.7	224				
3.0	slope				
6.6	-	13.5	224		
6.7	-	16.0	169,128		
10.9	232	16.1	183,169,128		
15.2	244,278	16.7	286,270,251, 217,200,186		
16.3	226[1]	17.4	217[1]	17.5	311,215,198, 155
16.5	214,250, 284[2]	18.1 18.2	136[2] 168,154	18.3	286,256,226, 196,166
17.5	218,278			20.2	287,225,169
18.8	222,262[3]	23.0	216,218[3]	22.5	249
		28.2	258,229,170, 125	23.1	278,248
				26.0	219,195,169
				28.4	317,257,233, 197,169
				30.0	300,233,181, 169,137
				38.4	319,287,227, 169

[1]BSF1

[2]BZL

[3]ATZ

Table 8. Results of Thermospray LC/MS Data of Solid Phase Extracted Leachate

Sample	Scan #	m/z	Probable Molecular Ion	Likely Compound
TSP^+ Standard Mix	503	217	$[M + NH_4]^+$	BSF1
	526	136	$[M + 1]^+$	BZL
	657	216, 218	$[M + 1]^+$	ATZ
	864	218	$[M + 1]^+$	APY
TSP^+ Leachate	504	217,	$[M + NH_4]^+$	BSF1
		200	$[M + 1]^+$	BSF1
	516	136,	$[M + 1]^+$	BZL
		168	$[M + 1 + MeOH]^+$	BZL
	658	216, 218	$[M + 1]^+$	ATZ
TSP^- Leachate	508	311,		
		215,	$[M - 1 + NH_3]^-$	BSF1
		198,	$[M - 1]^-$	BSF1
		155		
	521	286, 256, 226, 196, 166		Not BZL
	660	278, 248		Not ATZ
	815	317, 257, 233, 197, 169		
	1100	319, 287, 227, 169		

Solvent ions are not included in the spectra listed. In PCI, the ion at m/z 110 was from the protonated solvent cluster of ammonium acetate and methanol. In negative chemical ionization (NCI) mode, the solvent clusters appeared at m/z 119 and 151.

TSP identification of the standards was made by matching LC/MS retention times and probable molecular ions from the analysis of a mixture of the standards to those from the sample (Table 8). Retention times in the LC/MS were later than for the LC/DAD and typically varied between trials due to slight changes in flows and back pressures from the LC and Thermospray interface. All three internal standards were detected in the spiked leachate sample with PCI, but not with NCI (Table 8). The ammonium adduct ions resulted from the ammonium acetate in the mobile phase. Atrazine is represented by two protonated molecular ions (at m/z 216 and 218) due to the chlorine isomers. No NCI data were available on the mixture of standards alone. In NCI of the leachate, BSF1 was the likely compound at scan #508 because the retention time matches that of the standard mix. One must also assume that the peak at m/z 311 is insignificant. The NCI spectra of the unknowns occurring at the retention times similar to those of the other two standards (scans # 521 and 660) do not match the spectra expected for those standards. Indeed, they are marked by a fragmentation pattern of a serial loss of 30 mass units.

Of the five probable mass ions observed in leachate in PCI mode, as noted in Table 7, three, with m/z's of 170, 224 and 286, indicate compounds with an odd number of nitrogen atoms. The molecular ion in the spectrum at 28.3 minutes is thought to be at m/z 170 because the peaks at m/z 258 and 229 have low signal to noise ratios. The other ions detected had m/z's of 169 and 183. The ion at m/z 201 appears to be a background ion.

In NCI mode, ions were detected at m/z's of 219, 249, 278, 286, 287, 300, 311, 317 and 319. The 278 ion appears to be little more than noise. The 286 ion at 18.3 minutes exhibited a fragmentation pattern denoting multiple losses of 30 mass units while the spectra at 28.4 and 38.4 minutes showed losses of 60 mass units. The most abundant non-solvent ion at 30 minutes

was at m/z 169. Anion exchange extracts were not analyzed by LC/MS. However, it is possible that they contained compounds that are ionizable that don't have UV chromaphores.

GC/MS Results: GC/MS analysis (with library matches in parentheses) confirmed the presence of the two benzenesulfonamides (BSF1 and BSF2) and two phthalates (DBP and DOP) found previosly (Freedman, 1989). Tentative identification was made of 3-tertbutylphenol (TBP), a large alkane (hexatriacontane), and two acids of alkanes (9-octadecanoic acid and tetradecanoic acid). TBP may be a degradation product of the preservative Butylated hydroxyanisol (BHA).

OTLC-MS Results: The five standards tested were individually detected by OTLC-MS in both PCI and NCI modes. Table 9 shows the likely mass ions formed for each standard in both modes along with their intensities. The responses for Cresol, APY, and BZL were more intense in PCI than NCI while the opposite was true for ATZ and BSF1. All of the positive ions were $(M + 1)^+$; all of the negative ions were $(M - 1)^-$. The three standards ATZ, BZL and BSF1 in a SPE control sample, were identified by PCI. A SPE leachate sample clogged the column when tip temperatures were greater than 200 °C. An OV-17-V 10 μm i.d. column separated ATZ, BZL, BSF1 and Cresol at the picogram level in less than 30 seconds. (Schematic and TIC in Appendix)

E. Solid Phase Extraction - Industrial Wastewater

1. General Results

An industrial wastewater was analyzed by the SPE and LC/MS procedures utilized on the leachate. The wastewater was reported by the industry to contain high levels of 2,4-DNT (300

Table 9. OTLC - LC/MS Results for Five Standards from Analysis of Leachate

	PCI			NCI		
Standard	m/e	Likely Ion	Response	m/e	Likely Ion	Response
ATZ	216	$[M + 1]^+$	328192	214	$[M - 1]^-$	363520
Cresol	109	$[M + 1]^+$	279040	107	$[M - 1]^-$	34112
APY	218	$[M + 1]^+$	58176	216	$[M - 1]^-$	12864
BZL	136	$[M + 1]^+$	266752	134	$[M - 1]^-$	10720
BSF1	200	$[M + 1]^+$	118912	198	$[M - 1]^-$	351744

ppm) and DPA (5 ppm) in a largely aqueous solution. (Industry staff determined solvent composition to be less than one percent of ether and ethanol.) The pH remained stable at about 7. One of the reasons this waste was accepted for analysis was that it was about two orders of magnitude more concentrated than the leachate. The COD of the wastewater was stated to be 8625 mg/L by the industry; when measured in house, the COD was 7450 mg/L. The leachate had a COD of 75 - 100 mg/L.

The waste was supersaturated with a compound in crystalline form which was identified as DNT using LC/DAD analysis of a filtered residue washed with an aliquot of methanol. The analysis of DPA in solution also received special attention.

2. *Diphenylamine Analysis Results*

Since diphenylamine was a major component of this wastewater, much attention was given to characterizing it and related compounds.

HPLC Results: The results from the analysis of the DPA standard were especially interesting. Although claimed by Aldrich to represent over 99% purity, the LC/DAD analysis of the diphenylamine standard yielded six distinct components as shown in Figure 3. The major peak was labeled DPA and the others were arbitrarily assigned letters a - e. All peaks except b and e showed purity matches of 999 or 1000. The purity matches for peaks b and e were 986 and 981, respectively. The amounts of these constituents relative to DPA are illustrated in Table 10 for direct LC/DAD analysis of the standard, LC/DAD analysis of the standard following solid phase extraction and for LC/DAD analysis of extracted wastewater samples. Note that with but one exception, the ratios are greater when peak area is compared than when absorbance (peak height) is compared. Components b and e were not sufficiently recovered from the extracted standards to be integrated. Component c was the only one recovered from the actual wastewater and at a relative abundance significantly greater than found in the DPA

standard. The area and peak height ratios for the a, c, d and e impurities relative to DPA were substantially different before and after SPE extraction of the standard solution.

Thermospray LC/MS Results: In PCI the solvent serves as the ionization reagent. It is important to recognize the solvent ions likely to form that can interfere with sample ions as well as enhance sample ions. With the wastewater samples, better results were achieved without ammonium acetate as an ionization agent in both PCI and NCI modes. Therefore, methanol/water was used as the mobile phase. CI reagent gas, with the discharge electrode, produced the ionization. Yinon and Hwang, (1983) identified the following mass ions formed by methanol and water in PCI (m/z in parentheses): CH_3^+ (15), H_3O^+ (19), $CH_3OH_2^+$ (33), $(2CH_3OH + H)^+$ (65), $(2CH_3OH + CH_3)^+$ (79), $(3CH_3OH + H)^+$ (97), $(3CH_3OH + H_3O)^+$ (115), and $(4CH_3OH + H)^+$ (129) .

TSP PCI LC/MS of the DPA standard yielded a total ion current (TIC) with two major peaks at scan #s 906 and 530 and several less abundant peaks at scan #s 53, 422, 587, 715 and 784. The spectra of each of these scans except #906 contained only the ion of m/z 125. The spectra of scans 906 and 910 had an m/z of 170 which could be the $(M+1)^+$ ion of DPA alone or DPA coeluting with another compound of MW 169. The 125 ion, which possibly represents a protonated solvent cluster consisting of acetic acid and two methanol molecules, dominates nearly every spectrum at very high intensities. The 157 ion, which appears as a background ion, would logically be a solvent cluster with an additional methanol molecule attached. Other spectra of note are of scans: #580 with an ion at m/z 185; #806 with an ion as m/z 186; #1025 with an ion at m/z 399; and #1160 with an ion at m/z 391. Background ions were subtracted from these scans to verify the presence of these ions identified. Positive identification was not made of any of these four scans, though the latter spectrum is representative of dioctyl phthalate.

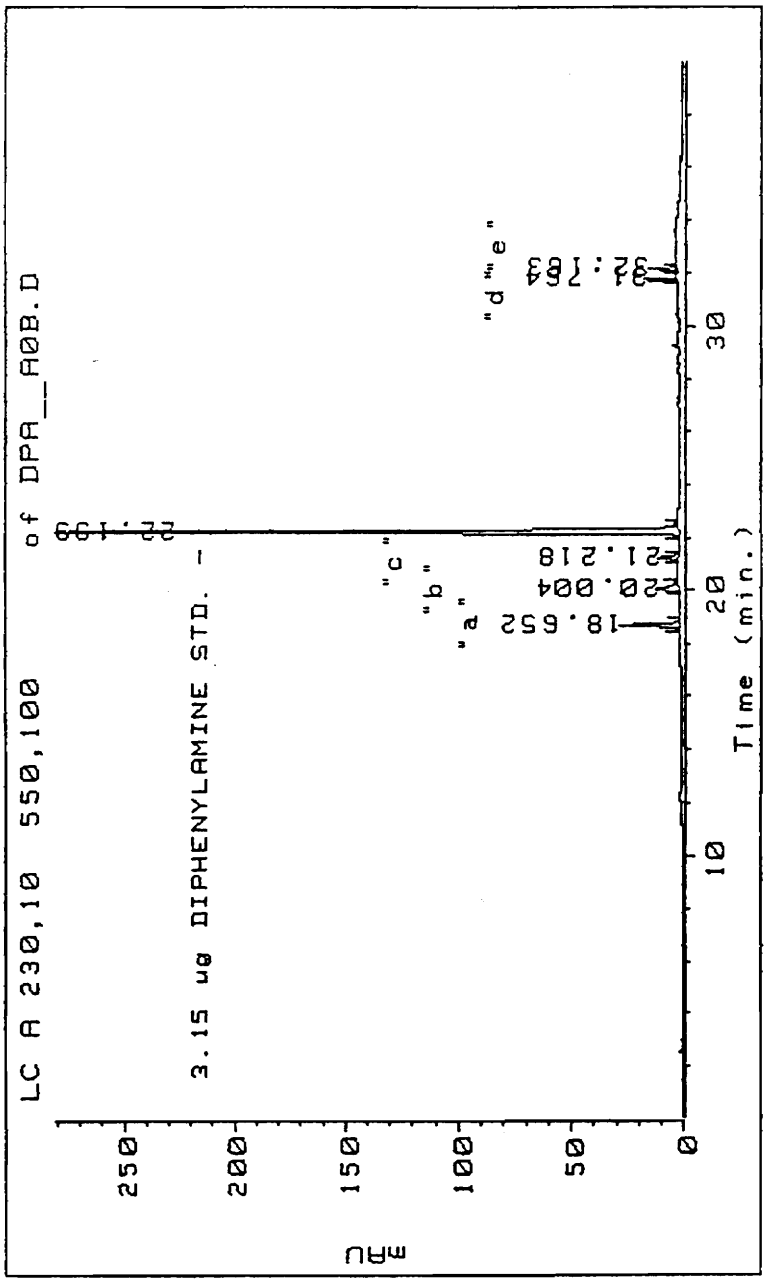

Figure 3. LC/DAD Chromatogram of Diphenylamine Standard

Table 10. Area and Peak Height Ratios Measured at 230 nm for DPA Impurities Relative to DPA for the Pure Standard, an SPE Extracted Standard and Extracted Wastewater Samples

	DPA Standard		Extracted Standard		Extracted Samples	
Compo-nents	Peak Height	Area	Peak Height	Area	Peak Height	Area
a:DPA	1:10	1:12	1:14	1:18		
b:DPA	1:28	1:18	1:28			
c:DPA	1:21	1:25	1:12	1:13	1:3	1:5
d:DPA	1:15	1:38	1:28			
e:DPA	1:23	1:37	1:30	1:80		

GC/MS Results: Electron ionization (EI) GC/MS of DPA revealed no other constituents aside from the DPA, even though as much as 10 ng of the sample were injected onto the column. This reinforces the notion that many compounds detected by LC are not amenable to GC analysis.

3. Wastewater Analysis

The pH 10 extracts were more magenta colored than the pH 7 extracts indicating the occurrence of some type of pH mediated reactions such as the formation of Schiff bases. However, the results from LC, LC/MS and GC/MS analyses were similar for samples at both pH's. In the spiked samples, ATZ appeared from LC/DAD analysis to coelute with an unknown analyte. No aminopyrene was recovered from any of the spiked samples. Only unspiked samples were analyzed by TSP LC/MS. LC analysis of controls of three unspiked samples showed small levels of DNT contamination. This was probably due to an inadequately rinsed pH probe contaminated with DNT from a previous sample. Subsequent control samples showed no further DNT residues.

LC/DAD Results: Information pertinent to LC analysis of chemical standards relating to this wastewater are provided in Table 11. This information includes the observed LC/DAD retention times and UV absorbance maxima (λ_{max}) plus the λ_{max} values presented in the 1979 CRC *Handbook of Chemistry and Physics* (HOCAP) where available. Some of the observed values are slightly different than reported in HOCAP. This could be due to interpolation by the DAD system. DPA was found to be a constituent of the NNDPA standard in LC/DAD analysis also, but none of the other previously noted DPA constituents were apparent in this standard. The information on 2-Nitrosodiphenylamine (2NDPA) was deduced from the chromatographic results of a mixture of standards received by the investigator (from the industry) known to con-

tain 2,4-DNT, DPA, NNDPA, 2NDPA and DBP. This mixture is referred to as "wastewater standards" in Table 13 showing PCI and NCI LC/MS results.

The typical C_{18}-extracted waste sample revealed 16 distinguishable peaks on the LC under the conditions described in the previous chapter. The 2,4-DNT peak went off scale at 4000 mAU. Figure 4 shows a typical chromatogram of the wastewater magnified to show the smaller peaks dwarfed by DNT at full scale. The LC/DAD retention times and UV absorbance maxima (λ_{max}) for five extractions of the wastewater are presented in Table 12.

LC/MS Results: Data from the Thermospray LC/MS analysis of the mixture of five known wastewater standards and of the extracted wastewater samples, are summarized in Table 13 and Table 14, respectively. Spectra, with the most abundant ions underscored, of each scan listed are given for both positive (TSP^+) and negative (TSP^-) ionization modes. Likely molecular ions are suggested for spectra where identification of compounds is attempted. This was done for 4 of the 10 positive ion spectra and 5 of the 11 negative ion spectra selected for the standards mixture, and for 3 of the 10 positive ion spectra and 7 of the 10 negative ion spectra selected for the wastewater sample.

Very poor response was noted for the LC/DAD analysis of the LC effluent fractions collected, concentrated and reinjected. Although a number of peaks were detected, apparently the amounts collected were too small to be identified for all but the 2,4-DNT. The DNT appeared in fractions 3-5 indicating tailing of the large DNT peak and mixing in the LC effluent line.

GC/MS Results: Retention times and molecular weights of eleven lab standards determined by GC/MS analysis are found in Table 15. The NNDPA standard revealed a molecular ion for DPA only. This was likely due to the thermal degradation of the nitrosamine to the corresponding amine in the GC. Retention times from the analysis of extracted wastewater samples are also given in Table 15 along with the probable identification suggested by the Wiley library search in the data system used.

Table 11. LC/DAD Retention Times and UV Absorbance Maxima (λ_{max}) of Chemical Standards for the Industrial Wastewater

Chemical Standard	t_R, min.	LC/DAD λ_{max}, nm	HOCAP[1] λ_{max}, nm
ABP	19.8	272	278
Acetone	15.8	240	
DBP	29.5	224, 274	225, 275
2,4-DNT	18.1	246	252 (5% al)
2,6-DNT	17.7	236	241 (5% al)
DPA	22.2	282	208, 286
DPA "a"	18.7	266	
DPA "b"	20.0	276	
DPA "c"	21.2	230, 254, 290	
DPA "d"	31.8	306	
DPA "e"	32.2	256, 292	
DOP	34.0	224, 274	
4N2AM	15.1	224, 246, 290, 360	231, 253, 288, 373
NBZ	17.0	262	260
2NDPA	27.2	258	220, 259
NNDPA	21.9	(224), 290	290
TNT	15.6	228	225

[1] HOCAP = CRC Handbook of Chemistry and Physics (1979), where available

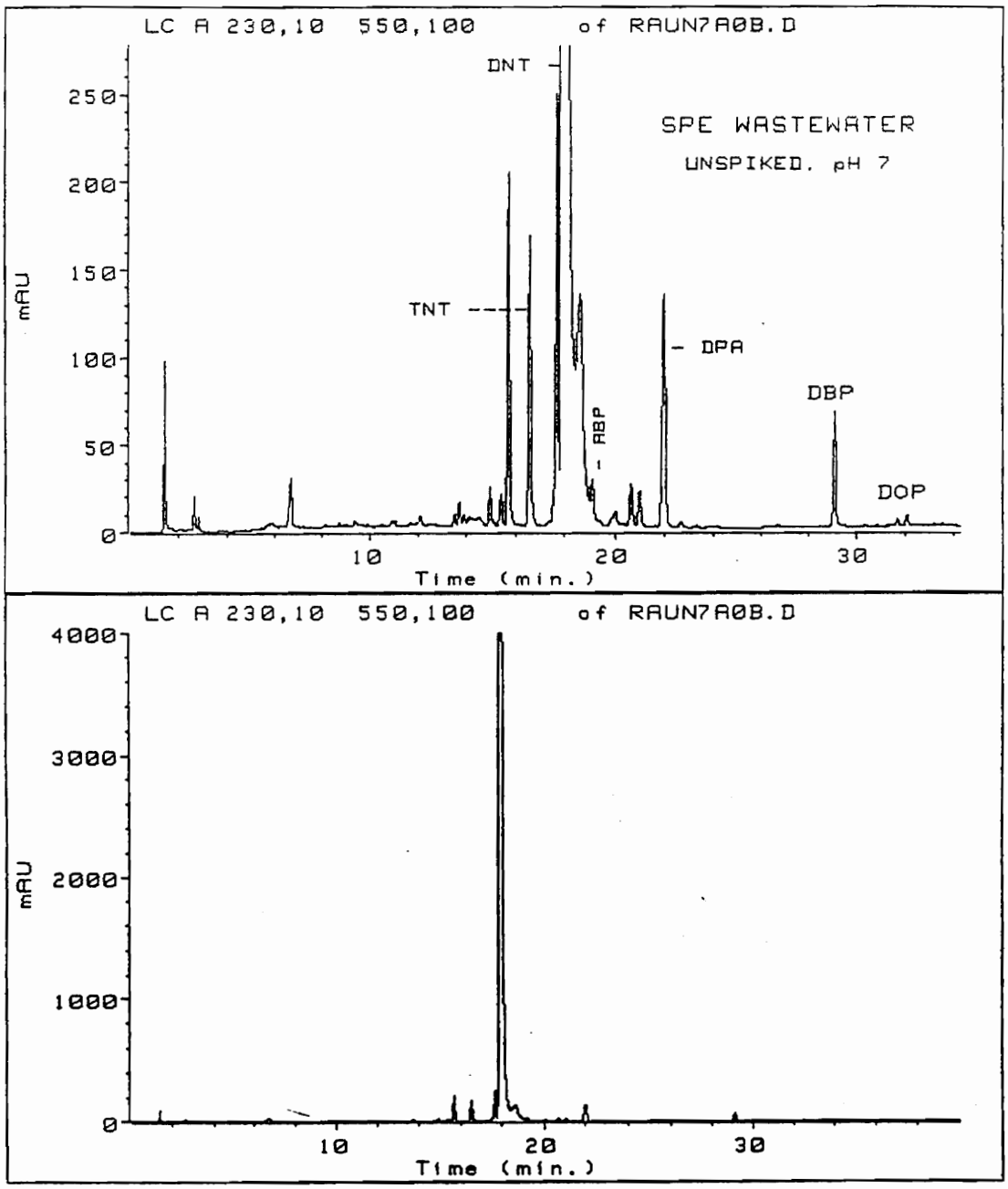

Figure 4. LC Chromatogram of Unspiked Solid Phase Extracted Wastewater

Table 12. LC/DAD Retention Times and UV Absorbance Maxima for Solid Phase Extracted Industrial Wastewater Samples

Sample 1, pH 7		Sample 2, pH 10		Sample 3, pH 10		Sample 4, pH 7		Sample 5, pH 7 (spiked)		Likely Compound Identification
t_R min.	λ_{max} nm	t_R min.	λ_{max} nm	t_R min.	λ_{max} nm	t_R min.	λ_{max} nm	t_R min.	λ_{max} nm	
		1.4	slope	1.4	slope					
2.6	bumpy slope	2.6	bumpy slope	2.6	248	2.6	248, 272	2.7	-	
		6.9	280	6.1	bumpy slope			3.0	230, 274	
						7.5	-			
13.8	244	13.8	244	13.7	244	13.6	242	13.8	240	
				14.5	236, 274					
15.1	228	15.1	230	15.0	236, 274 & 232, 274	14.9	228	15.0	230	
15.5	236	15.5	226 & 236	15.4	224,244; 236	15.3	236; 228, 290	15.4	240	Acetone
15.9	236	15.9	236	15.7	236	15.6	236	15.7	236	
16.7	228	16.7	228			16.4	228	16.5	228; 234	TNT
								16.6	264	NBZ
17.7	258	17.7	258 & 216, 264	17.7	254; 216,264	17.4	236; 260	17.4	222, 262	
17.8	216, 264	17.9	216, 264			17.6	216, 264	17.5	260	
								17.7	216, 264	
18.2	246	18.2	244	18.0	246	17.9	244	18.0	246	2,4-DNT
18.8	248-250	18.8	248-250	18.6	248; 252	18.5	246, 248	18.6	246	
								19.3	230, 262	ATZ
19.4	278	19.4	278	19.2	274-276					ABP
		21.0	246	20.7	244					
21.3	232, 254, 290	21.3	232, 254, 290	21.1	232, 254, 290	20.9	232, 254, 290	21.1	232, 254, 290	DPA "c"
						21.5	226, 290			NNDPA
22.3	282	22.3	282	22.0	282	21.8	282	22.1	282	DPA
29.3	224, 274	29.3	224, 274	29.1	272	29.0	224, 274	29.2	224, 274	DBP
		32.2	256, 290							DPA "e"
33.8	226, 276									DOP

Table 13. Results of Thermospray LC/MS Analysis of Wastewater Standards

Sample	Scan #	Spectrum m/z	Probable Molecular Ion	Probable Compound
TSP^+	97	418, 265, 218		?
	899	186, 153		?
	902	321, 186		?
	981	170	$[M + 1]^+$	DPA[1]
	995	199	$[M + 1]^+$	NNDPA[2]
	1034	215	$[M + 1]^+$	2NDPA[1]
		185	$[M + 1 - 30]^+$	
	1053	279, 278, 205, 149	$[M + 1]^+$	DBP[1]
	1059	399, 279, 205, 149		?
	1174	391, 343, 279, 149	$[M + 1]^+$	DOP[1]
TSP^-	779	227, 205	$[M]^-$	TNT[1]
	837	182, 165, 153	$[M]^-$	2,4-DNT[1]
	935	239, 224, 205, 183, 169		?
	1000	214, 197, 162	$[M]^-$	2NDPA[1]
	1029	278, 238, 205, 182, 161, 148	$[M]^-$	DBP[c]

[1] confirmed identification

2 tentative identification

Table 14. Results of Thermospray LC/MS Analysis of Extracted Wastewater Sample, pH 7

Ionization Mode	Scan #	Spectrum m/z	Probable Molecular Ion	Probable Compound
TSP^+	78	181, 162, 136, 123		?
	93	311, 279, 223, 162		?
	713	151, 135, 119, 109		?
	797	153, 138, 123	$[M + 1]^+$	4N2AM[1]
	811	185, 153, 137, 123		?
	922	170	$[M + 1]^+$	DPA[2]
	944	217, 199, 170, 129	$[M + 1 + H_2O]^+$, $[M + 1]^+$	NNDPA[1]
	1024	279, 205, 149	$[M + 1]^+$	DBP[2]
	1032	399, 279, 205, 149, 119		?
	1165	391, 343, 311, 279, 261, 149	$[M + 1]^+$	DOP[2]
TSP^-	82	408, 364, 317, 222		?
	636	198, 152		?
	663	196, 136		?
	693	199, 183, 168		?
	696	199, 183, 168, 152, 138	$[M + MeOH - 1]^-$, $[M]^-$	DNB[1*]
	715	227, 210, 198	$[M]^-$	TNT[2]
	741	214, 182, 165, 153, 135	$[M + MeOH]^-$, $[M]^-$	2,6-DNT[1]
	754	214, 197, 182, 165, 153	$[M + MeOH]^-$, $[M]^-$	2,4-DNT[2]
	824	224, 214, 182, 168, 161		?
	997	278,	$[M]^-$	DBP[1]

[1] tentative identification

[2] confirmed identification

* DNB = Dinitrobenzene

Table 15. **Results of EI GC/MS Analysis of Standards and Wastewater Samples**

Chemical Standards Analyzed	t_R min.	Wastewater Analysis Suggested by EI Spectral Library	t_R min.	Molecular Weight
NBZ	3.6			123
		1-methyl-4-NBZ	4.7	137
		dinitrobenzene	7.4	168
2,6-DNT	7.5			182
2,4-DNT	8.5	2,4-DNT	8.7	182
		3,4-DNT	9.3	182
4N2AM	9.5	4N2AM	9.4	152
NNDPA	see DPA[1]			198
DPA	9.8	DPA	9.7	169
		Nitrobenzene-amine	10.1	138
TNT	10.8	TNT	10.7	227
ABP	11.6			169
2NDPA	15.0			214
DBP	15.1	DBP	14.4	278
DOP	46.0			390

[1] No NNDPA ions were detected; only DPA

4. Discussion

Identification of Wastewater Components: These data suggest several confident identifications and other more tentative identifications of the constituents of this particular wastewater. As expected, 2,4-DNT and DPA were identified by all three analytical methods employed. DPA "c" was consistently detected by LC/DAD in all samples (Table 12). The UV spectrum of DPA "c" is similar to that found for 4N2AM, but the retention times do not match. Its retention time and UV absorbance are very similar to those of NNDPA except for the UV maximum at 254 nm. Retention times can vary 0.5 or so minutes over time on the same column due to build up on the guard column and column packing gradually sloughing off. NNDPA appeared to show up on the LC/DAD chromatogram of sample #4. DPA "e" ostensibly appears in sample #2. The retention time and UV spectrum of this peak match those of DPA "e" in the analysis of the DPA standard. It's identity, however, remains unknown. DPA "b" is very similar to ABP in both retention time and UV spectrum. It's appearance in samples #1 - 3 warrants a tentative identification. However, confirmation of ABP was not made by either LC/MS or GC/MS.

Since ABP and DPA are aromatic molecules with the same molecular weight (169 amu), it was thought spectra with the same molecular ion would occur at two different times if both compounds were present in the same sample. However, only one peak was noted at m/z 170 in PCI mode (scan #922) of the wastewater sample which would account for the DPA only, based on retention time data. Negative ion thermospray yielded more than one hit at m/z 169 and 168, but NCI is generally more sensitive to nitroaromatics than amines. The ABP standard was detected by GC/MS, but ABP was not found in GC/MS analysis of the sample.

Positive identification of DPA and DOP and tentative identification of 2-NDPA, was made in PCI analysis of the standards mix (Table 13). The spectrum of scan 1020 of the wastewater sample most closely resembles that of DBP. Spectra of PCI scans 1059 and 1174 of the wastewater standards are similar to those of 1032 and 1165 of the wastewater sample respectively. The

former may be a DPA dimer while the spectra of the latter scans confirm the presence of DOP. PCI scan 995 of the wastewater standards may be NNDPA if one assumes m/z 199 is the $[M + 1]^+$ ion.

The GC/MS results suggest a possibility for the identity of scan 797 of the PCI Thermospray analysis of the wastewater. If m/z 153 is the $[M + 1]^+$ ion, then it could be 4N2AM as indicated in Table 14. It is difficult to discern if scan 797 is not related to scan 811 which appears to have a molecular ion at m/z 185 also.

The negative ion Thermospray results indicate the presence of DNB, TNT, 2,6-DNT, 2,4-DNT and DBP as noted in Table 14.

The results from GC/MS analysis (Table 15) suggest the presence of a methyl-nitrobenzene, a dinitrobenzene, two DNT isomers, TNT, 4N2AM, DPA, DBP and a compound of mass 166 at 10.1 minutes. It is interesting to note that the 3,4-DNT isomer was matched by the EI GC/MS library, not the more common 2,6-DNT isomer. The spectrum at 10.1 minutes had a molecular ion at m/z 166. A nitro acetanilide or an aminonitrobenzaldehyde are feasible explanations for this compound.

Diphenylamine is photoactive and high molecular weight dimers and trimers could form if DPA was exposed to light before it went into solution. One of its industrial uses is as a stabilizer, which absorbs nitric oxide gases emitted during the decomposition of cellulose nitrate. Therefore, mono, di and tri nitro DPA isomers are nitration products likely to form.

The presence of DBP was confirmed by all three methods. The LC peak at around 29.3 minutes matches very well with the standard (Table 11 and Table 12). The same is true for GC/MS. NCI thermospray revealed an intense ion current at scan #997 whose spectrum correlates well to that of DBP. A match for DOP was found in only sample #1 on the LC, not on the GC/MS and only in the PCI TSP analysis of the standards.

Peaks #6 and 7 in all the LC samples occur at about 15.4 and 15.8 minutes respectively and have a UV absorbance maximum at 236 nm. This is very close to the LC results for the analysis of pure acetone which eluted at 15.8 minutes and had a UV absorbance maximum at 240 nm (Table 11). Acetone is used extensively in the plant where the wastewater originated, although it was not applied directly to this particular waste stream. It appears in sample #5 because it was the solvent for one of the standards used to spike the sample.

LC peak #8 suggested the presence of TNT in this wastewater (Table 12). This was confirmed by analysis of a TNT standard. A number of additional factors support the presence of TNT. First, the observed λ_{max} of 228 nm is very close to the published value of 225 nm (HOCAP). Second, since it has one more nitro group than DNT, one would expect it to elute before DNT in a reversed phase system, and this peak does. Third, it may be reasonable to expect to find TNT with DNT in industrial applications.

The presence of TNT was also confirmed by GC/MS analysis of the standard. The TNT standard was not analyzed by Thermospray, but LC/MS data suggest the presence of this compound. Negative ion TSP revealed an intense ion of m/z 227 at scan #715 (Table 14) which could depict the M^- ion of TNT. The m/z 197 would represent the loss of -NO (Voyksner and Yinon, 1986).

Parker *et al.* (1982) found NCI to be more sensitive than PCI in the analysis of explosives by DLI. Voyksner and Yinon (1986) found TNT not amenable to TSP ionization in the positive ion mode. But Yinon and Hwang (1983) observed good spectral results with PCI analysis of TNT on a DLI LC/MS system. They observed the following spectral ions for TNT using methanol-water as the mobile phase: MH^+ ion at m/z 228, the adduct ion $(M + CH_3OH + H)^+$ at m/z 260, and the molecular ion M^+ at m/z 227. Fragment ions included $(M - OH)^+$ at m/z 210 and the $(MH - 30)^+$ ion at m/z 198. The last ion results from the loss of NO from the MH^+ ion or reduction of a nitro group to the corresponding amine. Serial reduction of the remaining nitro groups yields mass ions of m/z 168 and 138. The reduction procedes through a hydroxylamino

intermediate. Oxidation and coupling of hydroxylaminodinitrotoluenes can result in the formation of azoxy compounds which have a MW of 407 (Yinon and Hwang, 1985).

Similarly, the positive chemical ionization mass spectrum of 2,4-DNT with a methanol-water reagent has been identified as MH^+ at m/z 183, $(M - OH)^+$ at m/z 165 and $(MH - 30)^+$ at m/z 153 (Yinon and Hwang, 1983). The mass ions at m/z 135 and 137 may likely be $(M - OH - 30)^+$ and $(MH - NO_2)^+$, respectively.

The Thermospray LC/MS analysis of wastewater and standards containing 2,4-DNT did not achieve the successful ionization of 2,4-DNT in the PCI mode reported above, although spectra of scans #899 and 811 from Table 13 and Table 14 respectively, come the closest. NCI proved to be more sensitive in the analysis of the wastewater, especially for the nitroaromatics.

Nitroglycerin (NG), like TNT, also has a molecular weight of 227 and is readily analyzable by NCI TSP LC/MS. Even though DNT, TNT and NG may coelute or elute closely together, negative ion TSP LC/MS offers the specificity required to resolve these compounds by mass (Voyksner and Yinon, 1986). Spectral ions of NG include $(M + ONO_2)^-$ at m/z 289, $(M + CH_3COO)^-$ at m/z 286, $(M + CH_3COO - COOH)^-$ at m/z 241, M^- at m/z 227 and $(M - H)^-$ at m/z 226 and $(M + CH_3COO - 2COOH)^-$ at m/z 196. The data from the analysis of the wastewater does not support the presence of NG. The EI mass spectrum for NG does not match any spectra found for the waste sample. Curiously though, NCI spectra of the landfill leachate at 18.3 and 38.4 minutes contain NG - like mass ions (Table 7).

5. Recovery Experiments

In making a synthetic wastewater consisting of DPA and DNT, it proved difficult to get mg/L amounts of the two components into an aqueous solution together. Addition of 500 μL of a 5 mg/mL DPA, 10 mg/mL DNT mixture (in methanol) to 50 mL of water caused formation of a

orange droplet. When 50 μL were added, the question of the actual amount of DPA in solution still existed because visual inspection for signs of insolubility was not reliable. The aqueous solubility of DPA appears to be affected by the presence of DNT. The aqueous solubility of DNT is reported to be 300 mg/L (Howard, 1990).

Recoveries are determined by comparing the mass applied to the SPE cartridge with the mass recovered. Is the mass applied based only on the concentration of the compound in the solution, or does it include the insoluble fraction also? How is the actual concentration determined? The question of aqueous DPA solubility, both by itself and in solution with DNT, prompted several approaches to the investigation of SPE recoveries.

The two DPA solutions in Table 16 and the DPA/DNT mixture in Table 17 were prepared to certain target concentrations. The actual concentrations were measured by spectrophotometric analysis using the LC/DAD. Each solution was analyzed prior to solid phase extraction. Analyte concentrations were measured by applying absorbance (peak height) to Lambert-Beer's Law and also by comparing integrated peak areas to standard curves. For the latter, concentrations could be determined by fitting the areas to the standard curves for the two compounds (Figure 5 and Figure 6). This method proved unsuccessful because the responses fell below the linear range of the standard curves (denoted as "off scale" in Table 16 and Table 17). However, peak areas were useful in measuring masses recovered from the SPE extracted samples.

The actual aqueous concentrations of DPA solutions A and B were calculated using Lambert-Beer's Law based on UV absorbances. To do this, the molar absorptivity coefficients (ε) were determined for known concentrations of DPA in methanol; ε values were determined for these methanol solutions by both LC/DAD and the DU-6 Spectrophotometer. An ε value of 21152 (SD = 374) was calculated using Lambert-Beer's Law for three different DPA solutions in methanol at 286 nm on the DU-6 Spectrophotometer. This results in an ε value within 8 percent of that reported in the *Handbook of Chemistry and Physics* (1979), (Chapter III). The

Table 16. Concentrations of DPA Solutions and Recoveries from C_{18} SPE Columns

Method	Solution A (5 mg/L DPA)*				Solution B (21 mg/L DPA)*			
			SPE Recovery				SPE Recovery	
	DPA conc.	Mass Applied to SPE	Mass		DPA conc.	Mass Applied to SPE	Mass	
	mg/L	µg	µg	%	mg/L	µg	µg	%
Beer's Law								
UV ε=21152	3.4	170			13.9	695		
LC-DAD ε = 393 @ 230 nm	ND	ND	155	62[1]	16.5	825	955	91[2]
LC-DAD ε = 1060 @ 286 nm	4.3	217	160	64[1]	17.0	850	714	68[2]
Standard Curve								
DPA @ 230 nm	ND	ND	155	62[1]	off scale		877	83[2]
DPA @ 286 mn	off scale		141	56[1]	off scale		883	84[2]
Average Recovery			153	61[1]			857	82[2]

* Theoretical Concentration, see text
ε = Molar Absorptivity Coefficient
ND = Not Detected

[1] Assumes 250 µG DPA applied to C18 SPE column

[2] Assumes 1050 µG DPA applied to C18 SPE column

Table 17. Concentrations of DPA and DNT in a Solution and Recoveries of Each from C_{18} SPE Columns

Method	Solution C (Mixture of 5 mg/L DPA & 10.3 mg/L DNT)*							
	DPA		DNT		Mass Recovered		%Recovery	
	Conc. mg/L	Mass Applied to SPE µg	Conc. mg/L	Mass Applied to SPE µg	DPA µg	DNT µg	DPA	DNT
Beer's Law								
LC-DAD ε = 393 @ 230 nm	5.7	570			390		78[1]	
LC-DAD ε = 1060 @ 286 nm	1.1	110			397		79[1]	
LC-DAD ε = 1602 @ 230 nm			11.4	1140		959		93[2]
Standard Curve								
DPA @ 230 nm	off scale				362		72[1]	
DPA @ 286 mn	off scale				342		68[1]	
DNT @ 230 nm			off scale			1007		98[2]
Average Recovery					373	983	74[1]	96[2]

* Theoretical Concentration, see text
ε = Molar Absorptivity Coefficient

[1] Assumes 500 µG DPA applied to C18 SPE column

[2] Assumes 1030 µG DNT applied to C18 SPE column

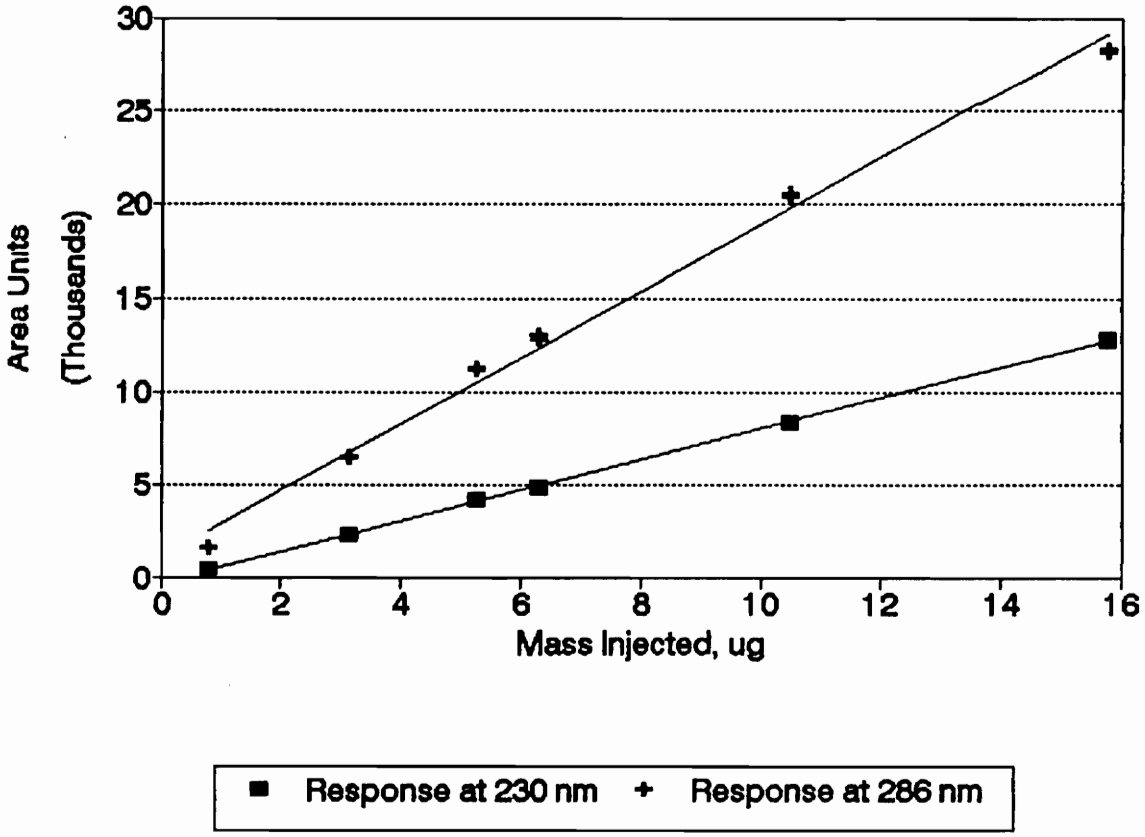

Figure 5. Standard Curve for Diphenylamine: Based on peak areas integrated at 230 and 286 nm

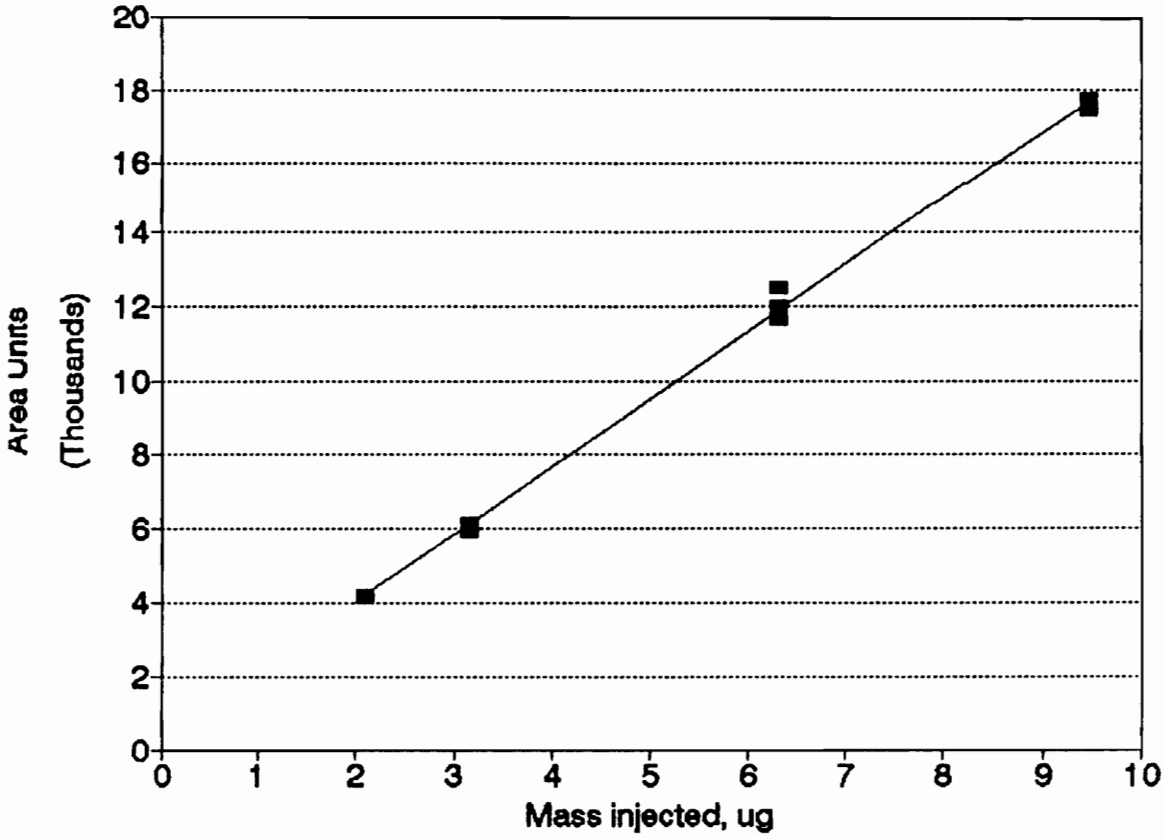

Figure 6. Standard Curve for 2,4-Dinitrotoluene: Based on peak areas integrated at 230 nm

LC/DAD molar absorptivity values calculated using these same 3 DPA solutions were 1060 L/mole-cm (SD = 23) at 286 nm and 393 (SD = 13) at 230 nm.

Using the LC/DAD ε values and DU-6 Spectrophotometer ε value, concentrations of solutions A and B were calculated. The DPA concentrations calculated from the DU-6 Spectrophotometer were significantly lower than those calculated from the LC/DAD which were less than the theoretical concentrations based upon the known amount of standards added to the solutions (Table 16).

Masses of the analytes recovered were calculated using Lambert-Beer's Law and the standard curves. Since percent recoveries depend on the initial masses applied and since the data for the concentrations vary, percent recoveries were based on the actual amount of standards added to the solutions. It is assumed that regardless of the actual solubilities, all of the mass was applied to the SPE columns. Thus, the percent recoveries given in Table 16 are minimum values for the three possible mass loadings. The average recoveries reported are the means of the recoveries determined by both Lambert-Beer's Law and by standard curves as explained above.

The data indicate an apparent effect of concentration on the recovery of DPA. Sample B, which had a greater DPA concentration than Sample A, showed better average recovery (82% vs. 61%).

The results of the analysis of the DPA/DNT mixture (Solution C) are shown in Table 17. The calculated concentration of DPA in this mixture was inconclusive due to the variability of responses at 230 and 286 nm. The observed absorbances were likely below the linear range of Lambert-Beer's Law. The LC/DAD ε value for 2,4-DNT was calculated to be 1602 L/mole-cm (SD = 22) at 230 nm. This was used in Lambert-Beer's Law to calculate the concentration of DNT in Sample C as shown:

$$\text{Concentration} = \frac{(0.060\ \text{AU} \times 182{,}000\ \text{mg/mole})}{(0.6\ \text{cm} \times 1602\ \text{L/mole-cm})} = 11.4\ \text{mg/L}$$

This was slightly greater than the theoretical value of 10.3 mg/L. The average DPA recovery from Sample C was 74% even though the amount applied to each SPE column was the same as that in Sample A. The average recovery of DNT from Sample C was 96%.

The recoveries of DPA by the standard addition method are based on averages of the integrated areas of the duplicates for each sample. A linear regression of the integrated areas of the DPA versus the concentration added is shown in Figure 7. The absolute value of the x-intercept is taken to be the DPA concentration of the unspiked wastewater sample. Based on this value of 2.23 mg/L, the DPA concentrations of samples 2 and 3 were 3.28 mg/L and 4.33 mg/L, respectively. The concentrations of the extracted samples were determined from the standard curves for the integrated peak areas at 230 nm of the DPA and DNT recovered. Extrapolation to a volume of 50 mL yielded DPA concentrations of the three sample extracts of 1.88, 2.14 and 2.98 mg/L respectively. Thus, the recovery of DPA was 89% from sample one, 65% from sample 2 and 69% from sample 3.

An interesting result of the experiment is that the DNT response increased with the addition of DPA even though the volume of wastewater was constant for each sample. The DNT concentration of Sample 2 (163 mg/L), was 9% greater, while that of Sample 3 (196 mg/L), was 30% greater than the concentration of DNT found to be in Sample 1 (149 mg/L). The reasons for this response are not known.

This response was not observed for three other compounds monitored by LC/DAD. The areas of the peaks identified previously as TNT, DPA "b" and DBP remained virtually constant in all six of the samples analyzed, i.e. both the full strength and 1 to 10 dilutions of the sample extracts.

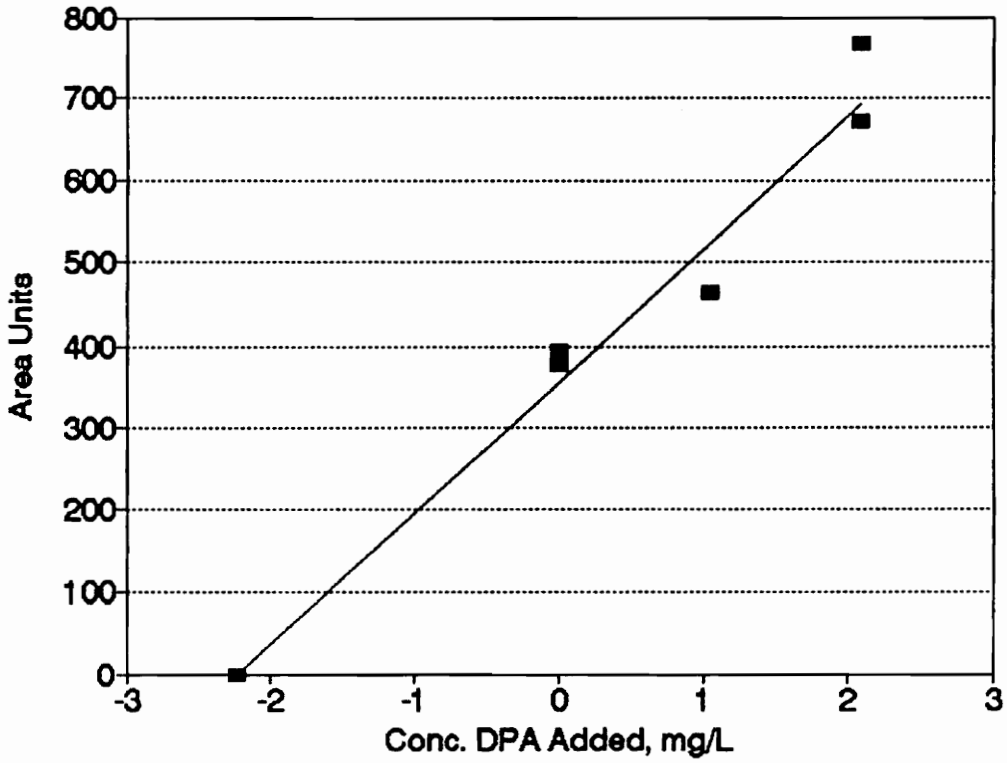

Figure 7. Linear Regression of Standard Addition of DPA to Wastewater

F. Comparison of Analytical Techniques

A secondary objective of this research was to compare the results acquired from the various analytical techniques utilized. A comparison of LC/MS to GC/MS was of particular importance. This section serves as a summary and subjective evaluation of the analytical methods applied to this research.

- The presence in the industrial wastewater of DBP, DPA, 2,4-DNT and 2,4,6-TNT were confirmed by LC/DAD, TSP LC/MS and GC/MS.

- More sample components were detected by LC/DAD and TSP LC/MS than by GC/MS.

- TSP LC/MS primarily provided molecular weight information and only limited structural data. Identification of unknowns was further complicated by the difficulty in distinguishing molecular ions from the wide array of adduct ions that might form depending on the ionization mode used and the ionization agent applied. Individual ions were more readily identified when analyzing standards than when analyzing unknowns. TSP is further limited by the difficulty in differentiating two or more compounds that coelute off an LC column.

- EI GC/MS analysis offers structural data. Identification of unknowns can be assisted by matching spectra with an EI spectral library. However, library matching should not be relied upon as the sole means of identification. The limitations of spectral library matching was evident with the analysis of TNT. The presence of TNT in the wastewater sample was first suggested by a library match of an unknown peak from GC/MS analysis of the industrial wastewater sample. Yet the library match from GC/MS analysis of a known TNT standard was 1,2-dichloropropane as illustrated in Figure 8. However, it is clear that the spectrum for TNT contained in the same library (Figure 9) matches very

favorably with the spectrum obtained for the standard analyzed. Confirmation of the presence of TNT in the wastewater sample was further enhanced by the matching of retention times (Table 15). Thus, spectral libraries should be used carefully and not exclusive of other methods of evaluation.

- A limitation of GC/MS analysis is the detection of compounds that may not have been in the original sample, but may be byproducts of the analytical conditions. For example, the methylnitrobenzene and dinitrobenzene detected in the wastewater samples may have been formed by thermal degradation of dinitrotoluene in the GC. Analysis of N-nitrososdiphenylamine resulted in the detection of only diphenylamine due to likely thermal degradation of the parent compound.

- Of the two nitrobenzenamines detected by GC/MS, the presence of only methylnitrobenzeneamine (4N2AM) was suggested by LC/MS. The presence of nitrobenzeneamines was likely the result of the ability of DPA to absorb nitric oxides in conjunction with splitting the amino-phenyl bond.

- Three detectors were used to analyze the same sample: LC/DAD, TSP LC/MS and GC/MS. Is one system better than the others? Each system is better at providing information unique to its design yet no one system is able to adeqautely characterize an unknown sample by itself. In this sense, the different systems complement each other. However, detailed analysis on three different systems is tedious and time consuming.

- In the analysis performed for this research, LC/DAD was able to detect more components than GC. Even though it does not definitively identify compounds (unless verified by standards), it does identify compounds by both retention times and UV spectra. The effectiveness of such an evaluation was shown in the analysis of TNT. The presence of TNT in the industrial wastewater was first suggested by observation of a peak in the LC/DAD chromatograms that eluted before DNT (TNT, which is more polar than DNT, would be

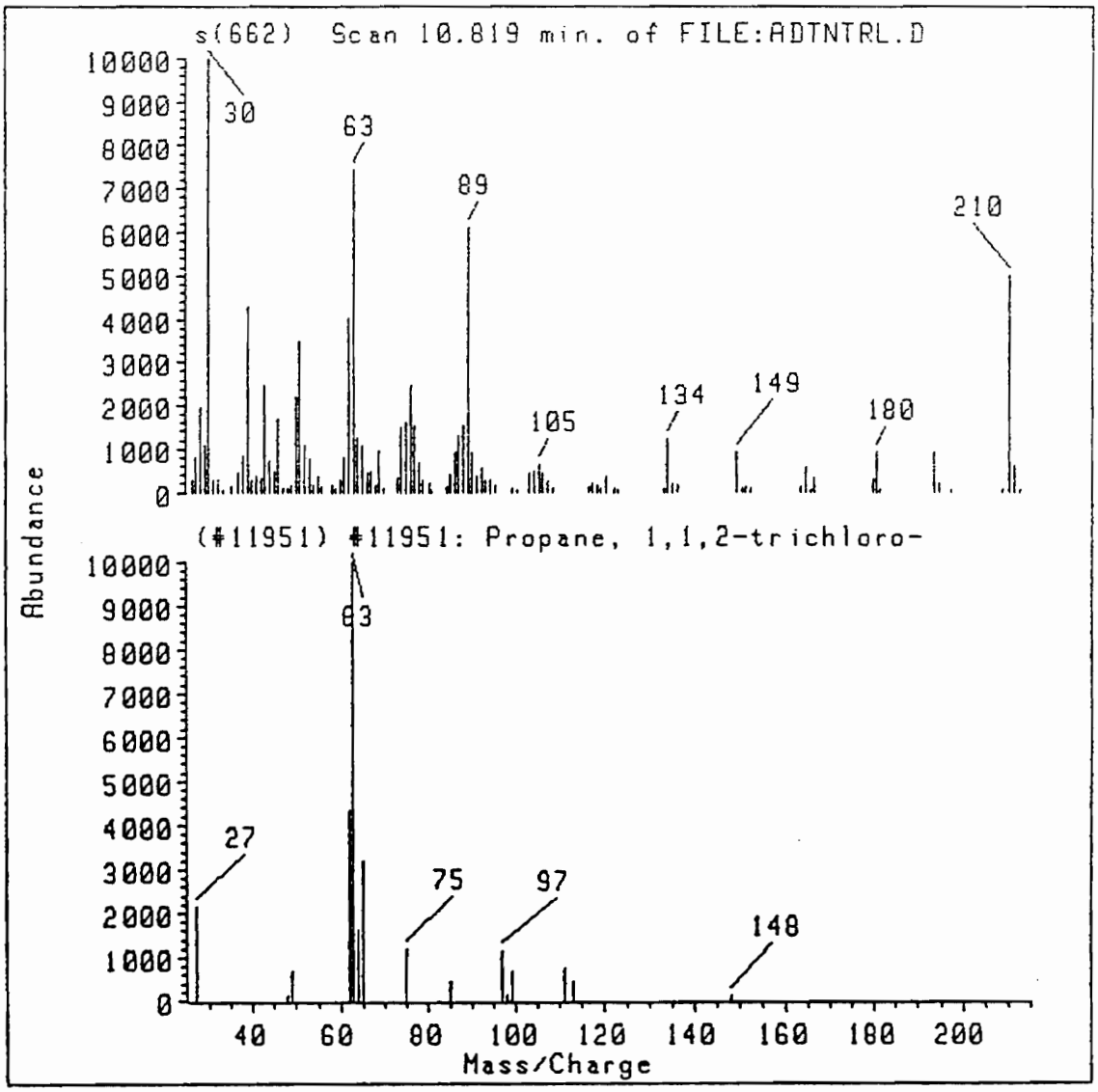

Figure 8. EI Wiley Library Match of Spectrum of TNT Standard

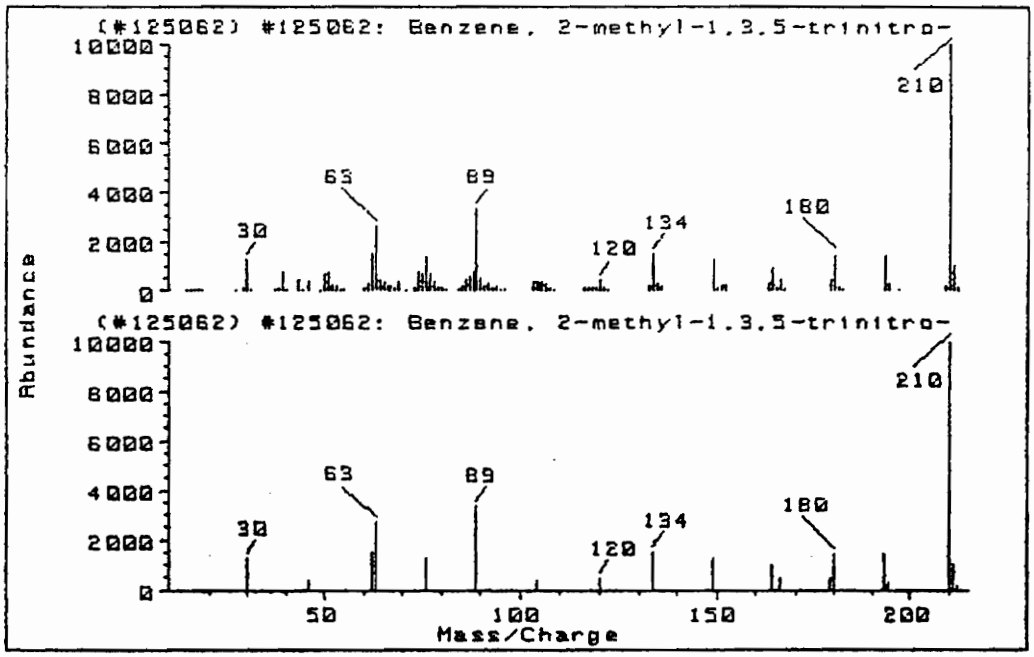

Figure 9. Wiley Library Entry for TNT (CAS # 118-96-7)

expected to elute first under the reversed phase conditions used) and that had a UV absorbance maximum similar to that listed for TNT in HOCAP. The identity of this compound was confirmed by LC/DAD analysis of a TNT standard. Figure 10 shows an exact match of the UV spectrum for the unknown peak in the wastewater with that of the TNT standard. The relative retention times also matched precisely. These data can be effectively used to compare influent to and effluent from a wastewater treatment system. For the purposes of monitoring the effectiveness of treatment, LC/DAD would be the preferred analytical system.

- If it is desired to identify components in an unknown sample, one would prefer to have the excellent separation, without thermal decomposition, provided by LC, the absorbance data generated by the DAD, and both EI and CI mass spectral data, in one system. Fortunately, current developments are occurring in this area. Particle beam LC/MS interfaces that provide both EI and CI mass spectral data have been developed. If they can be coupled to LC/DAD systems, then perhaps many of the current analytical limitations, including those encountered in this work, will be overcome.

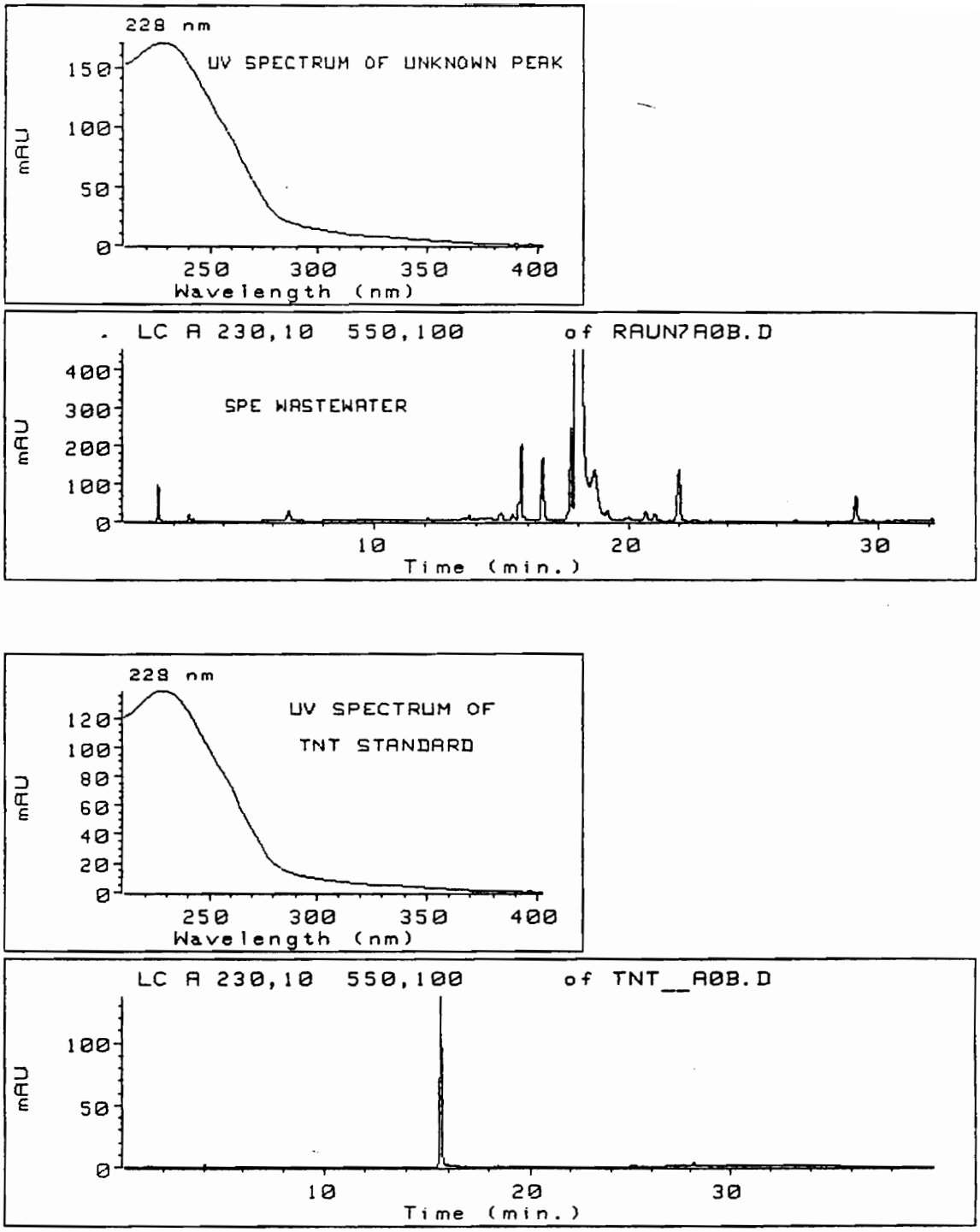

Figure 10. LC/DAD Analysis of TNT: Comparison of TNT Standard With Component of Wastewater Sample

V. CONCLUSIONS and RECOMMENDATIONS

The following conclusions and recommendations seem warranted from the results of the research described herein:

1. Liquid-liquid extraction with ethyl acetate is not a recommended procedure for extracting organics from aqueous samples. Emulsions and poor phase separation are problems that are difficult to overcome.

2. Lyophilization is a viable extraction procedure, but it's usefulness is limited by the methods necessary to control for interferences which are prevalent with complex samples.

   a. Lyophilization poorly recovers compounds that sublime under vacuum.

   b. A major problem with the procedure was due to interferences from salts in the freeze-dried residues. Lyophilization is a much more complicated procedure that requires much greater operation time than solid phase extraction procedures, especially for large sample volumes.

3. Solid phase extraction is a viable procedure for extracting organics from complex aqueous samples.

a. The C_{18} solid phase extraction sorbent was effective at retaining a variety of compounds from the two aqueous samples studied.

b. Additional refinement of the SPE techniques applied may prove useful in overcoming the variability in the recoveries of the compounds studied.

c. More work needs to be done to develop SPE techniques for the extraction of polar, nonvolatile compounds.

- The aminopropyl sorbent, used as a weak anion exchange column, was ineffectual in extracting organics from the leachate.

- It is recommended that use of a strong ion exchanger, such as quaternary amine, be explored for this purpose.

4. Excellent separation of compounds in complex samples was achieved by liquid chromatography with the conditions used.

5. Liquid chromatography with a diode array detector offers great versatility in the analysis of nonvolatile compounds and complements liquid chromatography/mass spectrometry (LC/MS) in broad spectrum, non-target analysis. This versatility would be enhanced if both detectors were used in series linked to the same LC. In this research each detector was on a separate LC system operating under slightly different conditions.

6. The methods developed for the analysis of leachate samples were successfully applied to the analysis of an industrial wastewater (within the limitations described belo

7. Thermospray (TSP) LC/MS is useful for identifying standards and providing molecular weight information, but has severe limitations in its ability to characterize unknown constituents.

- Positive ion TSP was more successful for the detection of amines while negative ion TSP was more successful for the detection of nitroaromatics in the industrial wastewater.

8. EI GC/MS in conjunction with an EI spectral library was useful in suggesting identities of only some of the components detected by LC/MS.

9. The presence of a major component in the wastewater sample may have caused decreased sensitivity in the detection of trace components in the sample.

10. If column clogging difficulties can be overcome, open tubular and capillary column LC/MS may be viable and sensitive methods of analysis.

11. Particle beam LC/MS, as reported in the literature, provides both EI and CI mass spectral data. Such a versatile system warrants consideration in the type of analyses performed in this research.

12. Recommendations: The application of these techniques to monitor the effectiveness of wastewater treatment deserves consideration. After all, it is the presence of organics in the treated effluent that warrants the most attention. The techniques utilized in the characterization studies reported here can be usefully applied to treated wastewater. Specific compounds, even those not identified by name, can still be identified by retention time, UV spectra and mass spectra. Treatment effectiveness can then be monitored by the presence or absence in the effluent of those compounds identified in the influent to the treatment process. These procedures could also be serve to identify degradation or transformation products that may result from treatment.

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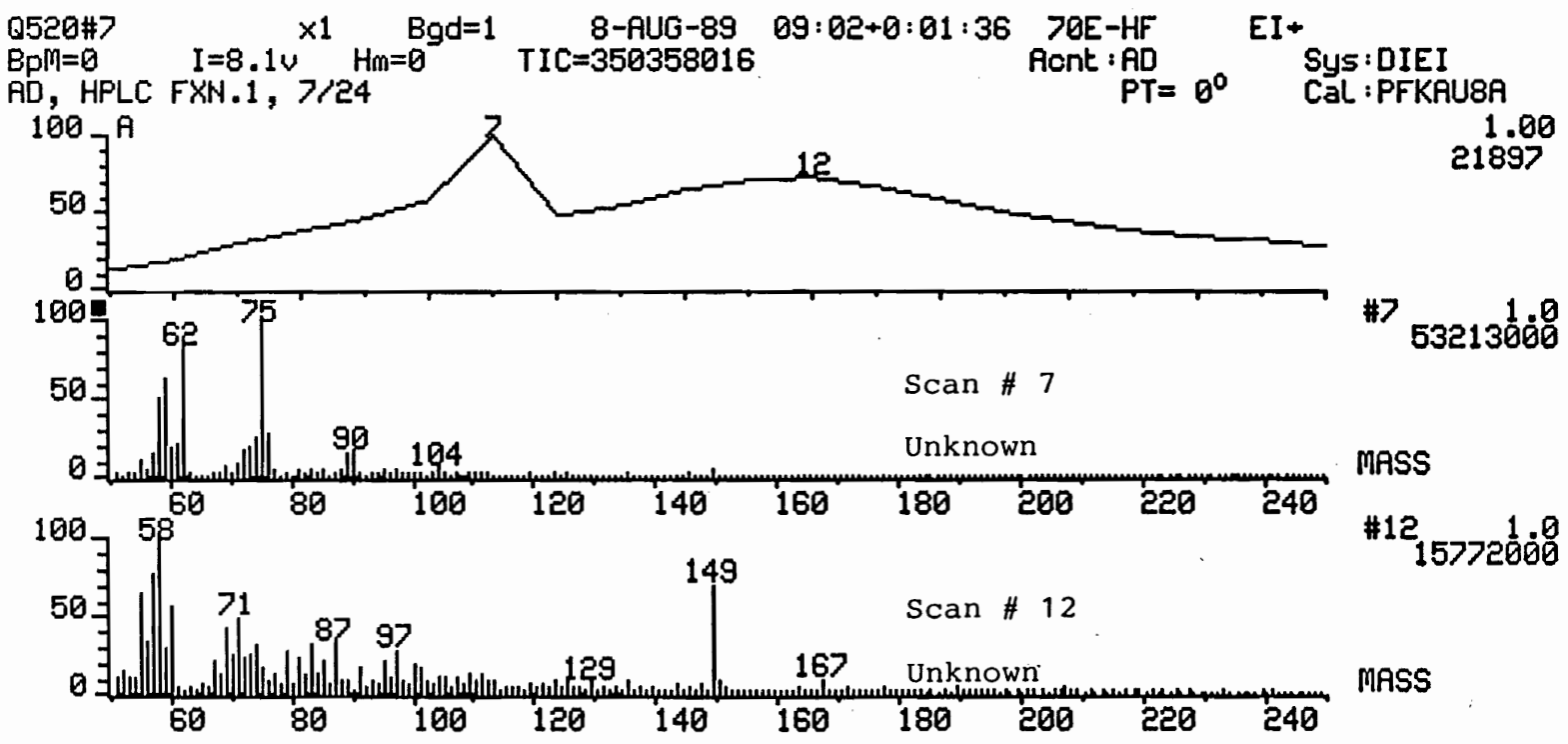
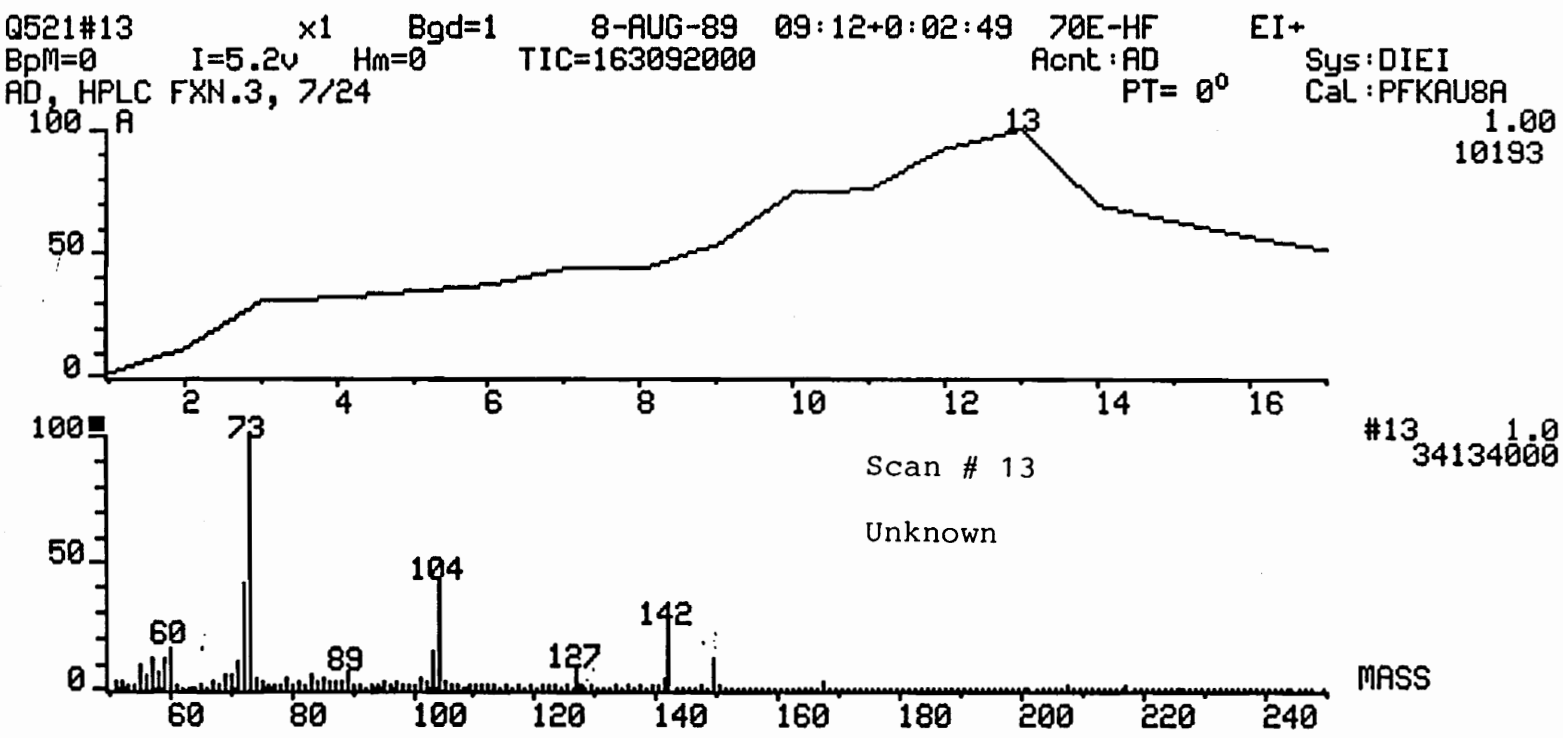
Appendix A. Offline EI Mass Spectra from Leachate Analysis (Table 3)



HPLC Fraction 1



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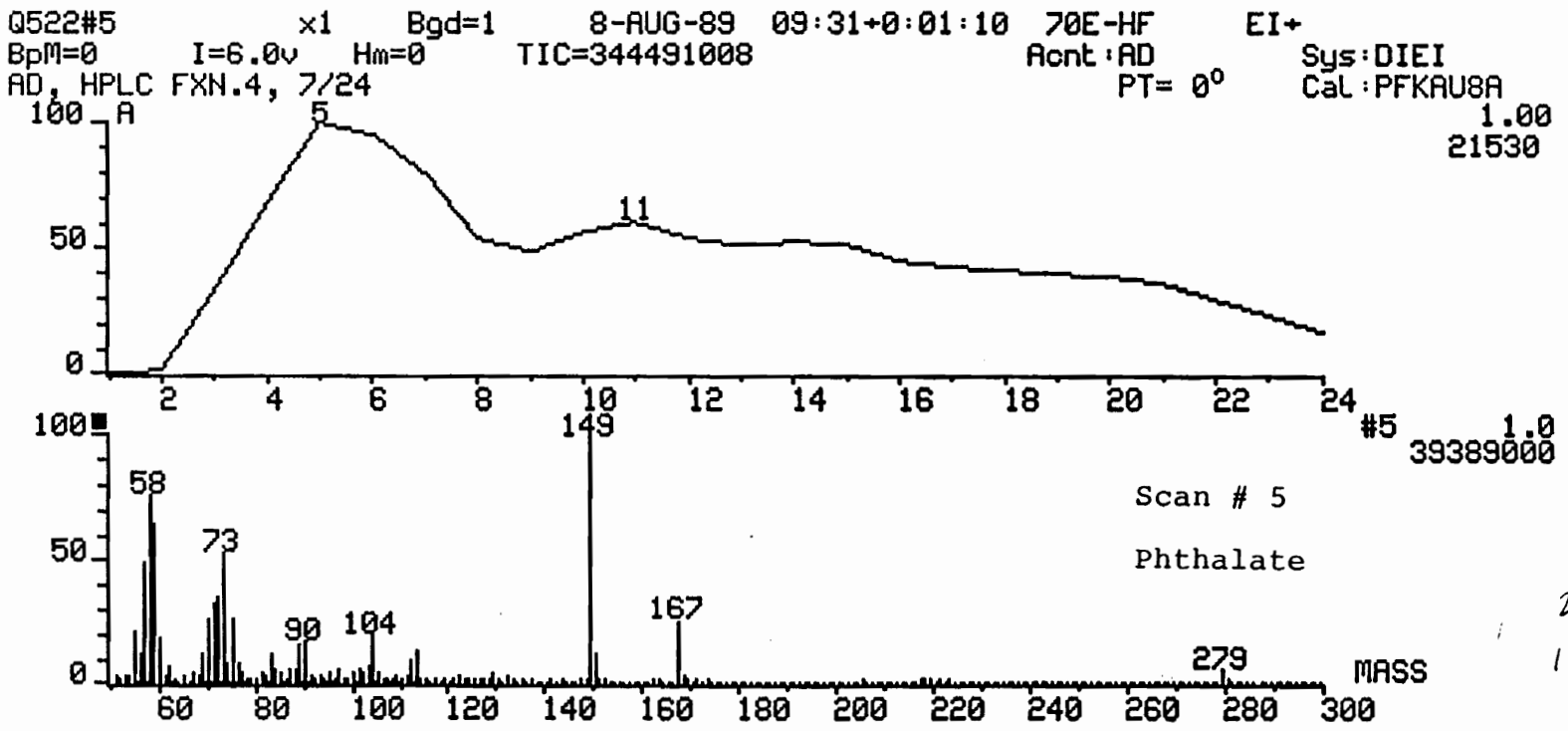

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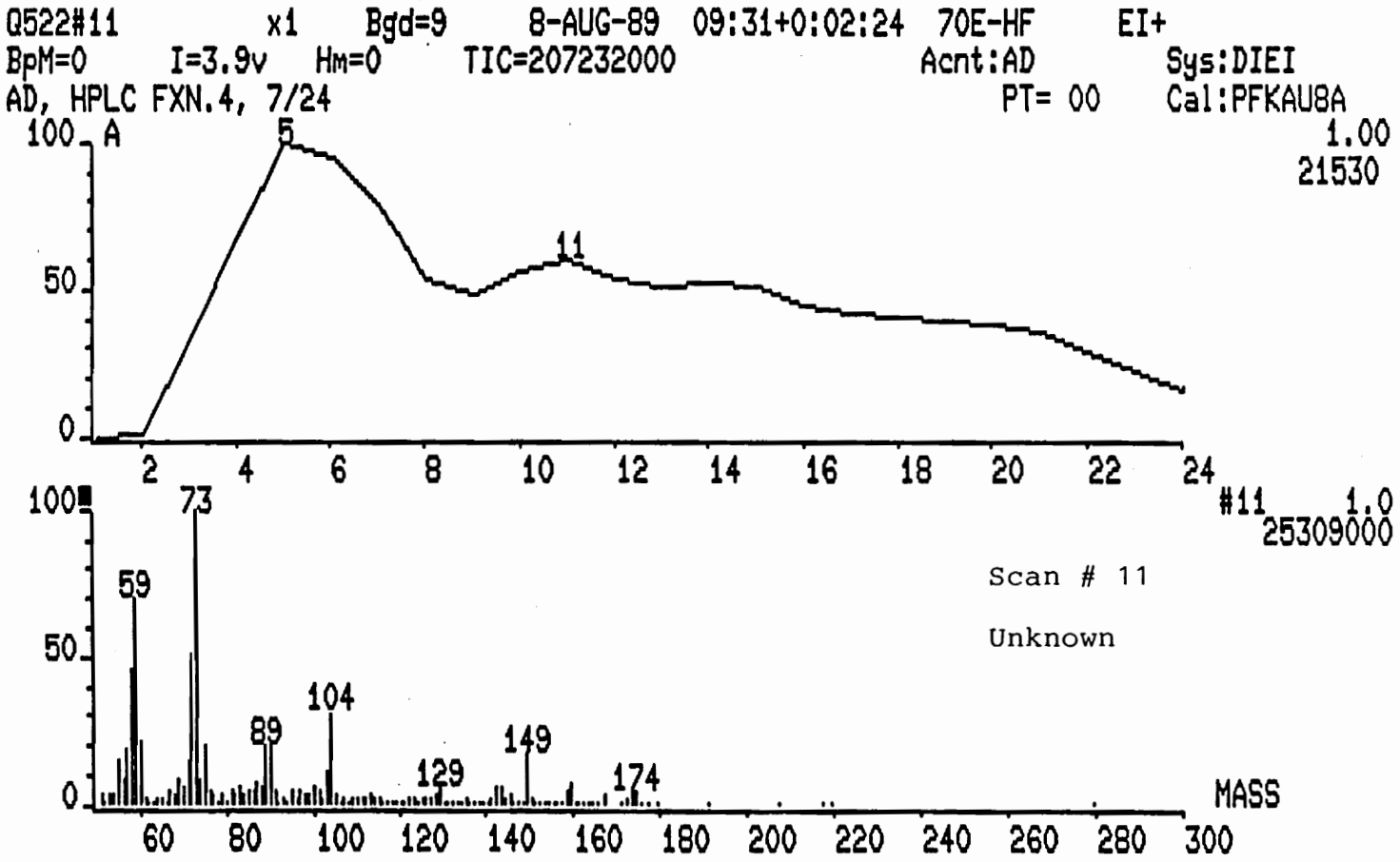

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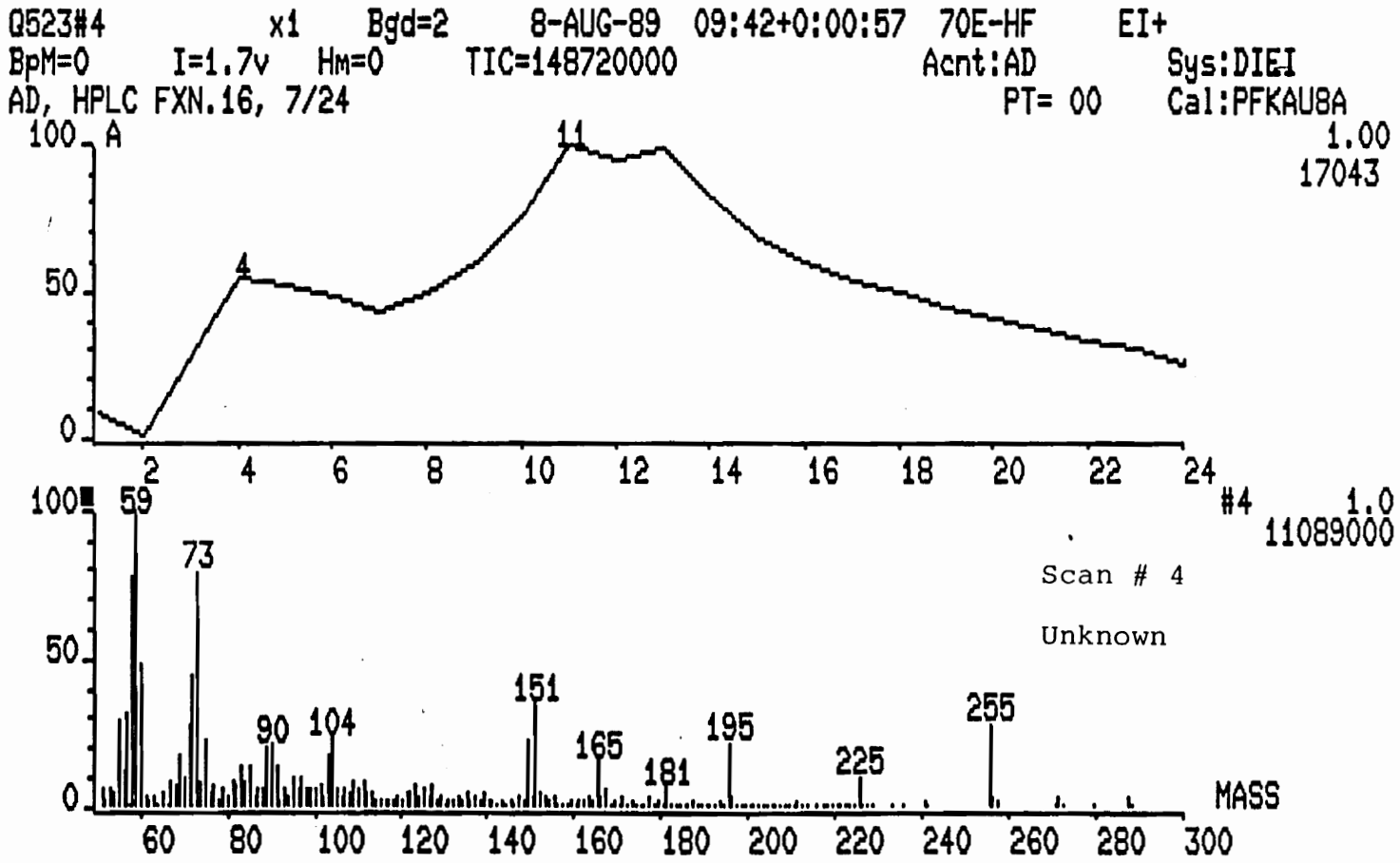

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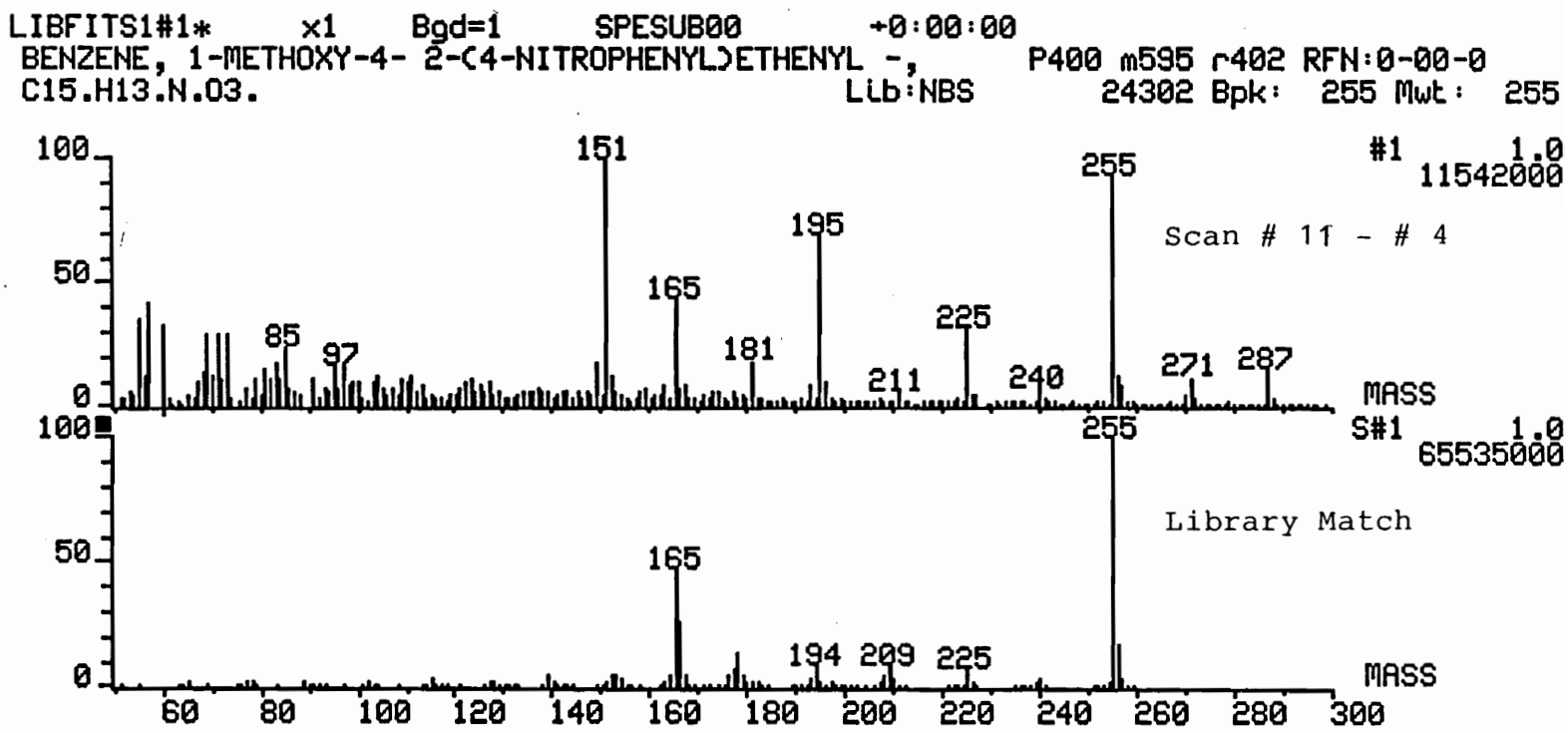
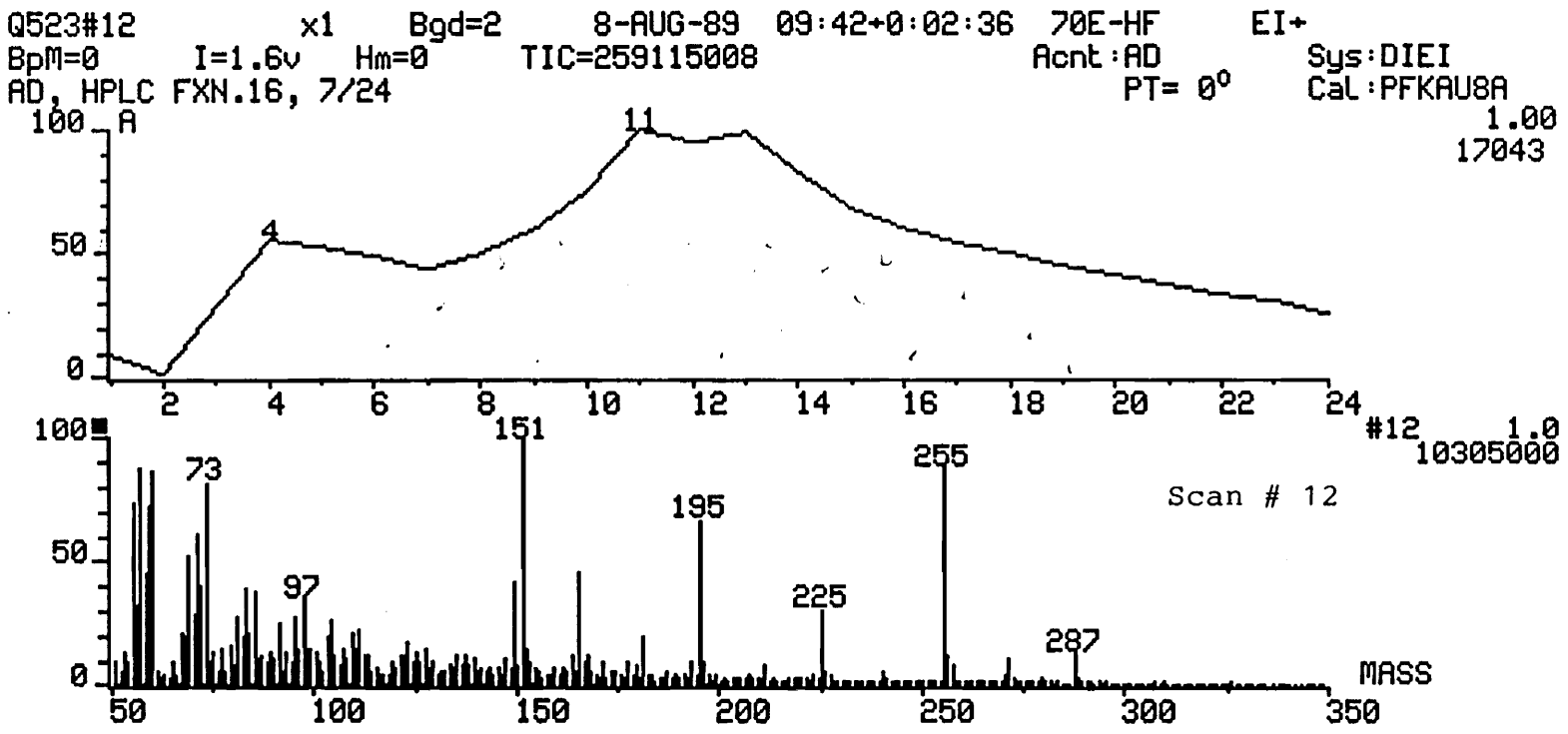
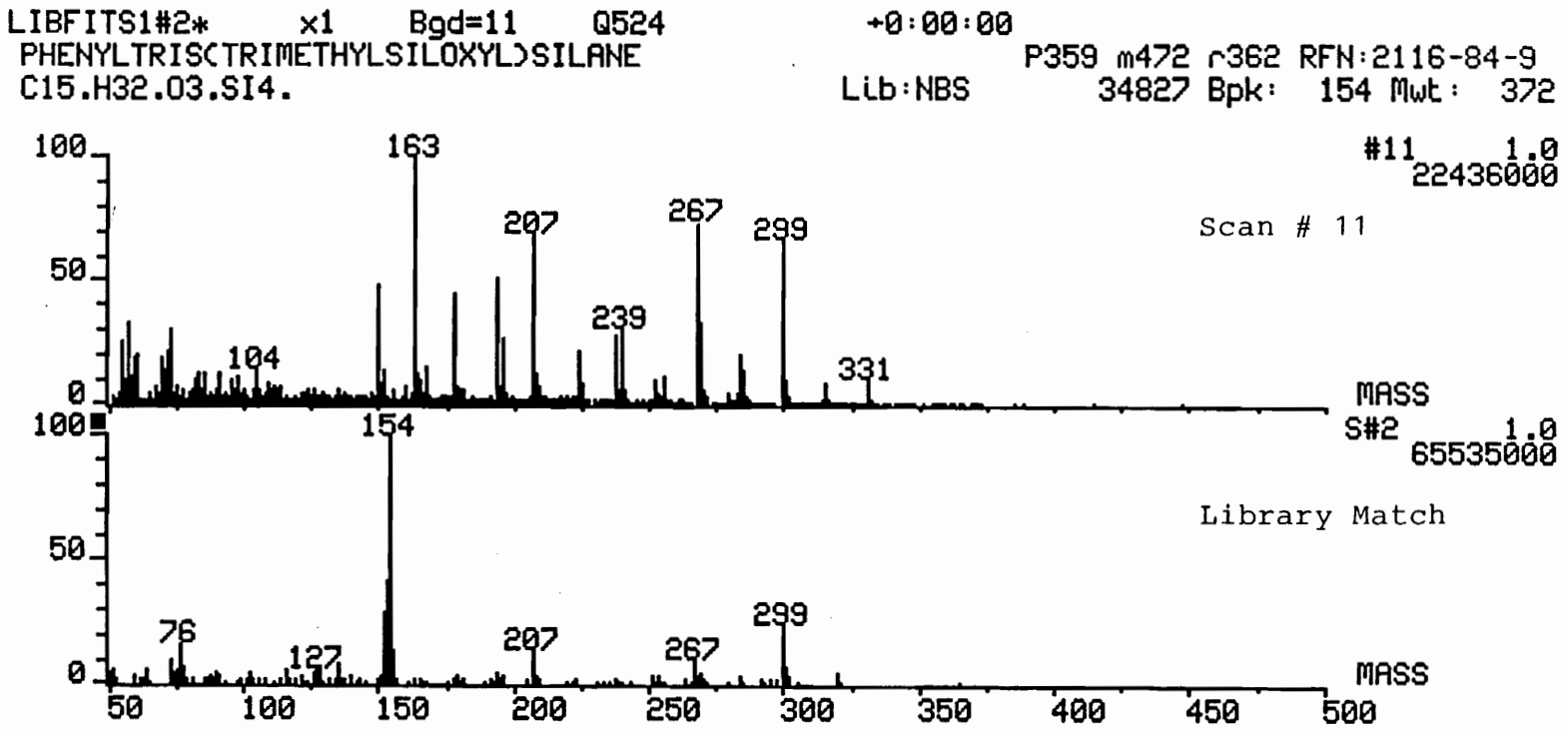


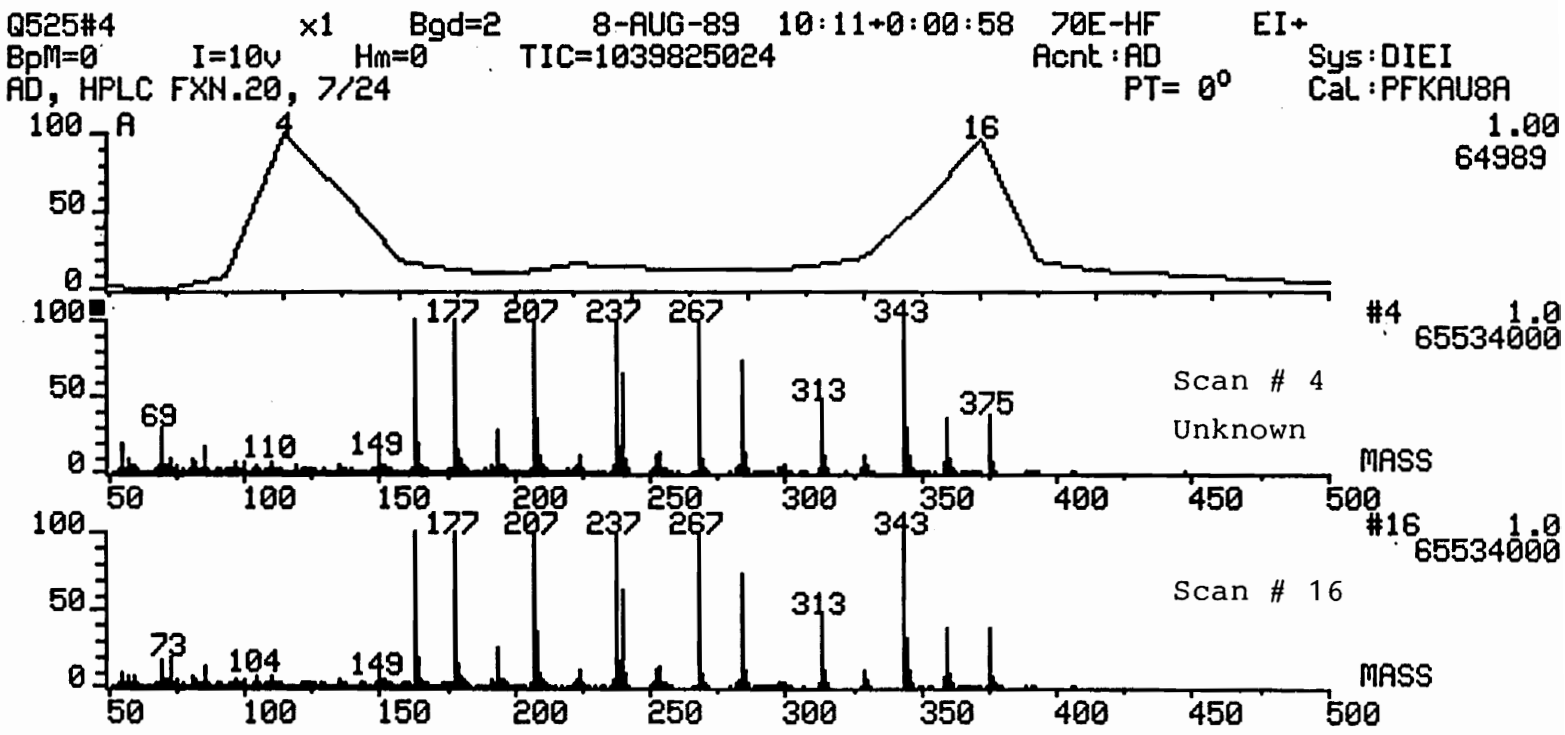
HPLC Fraction 16



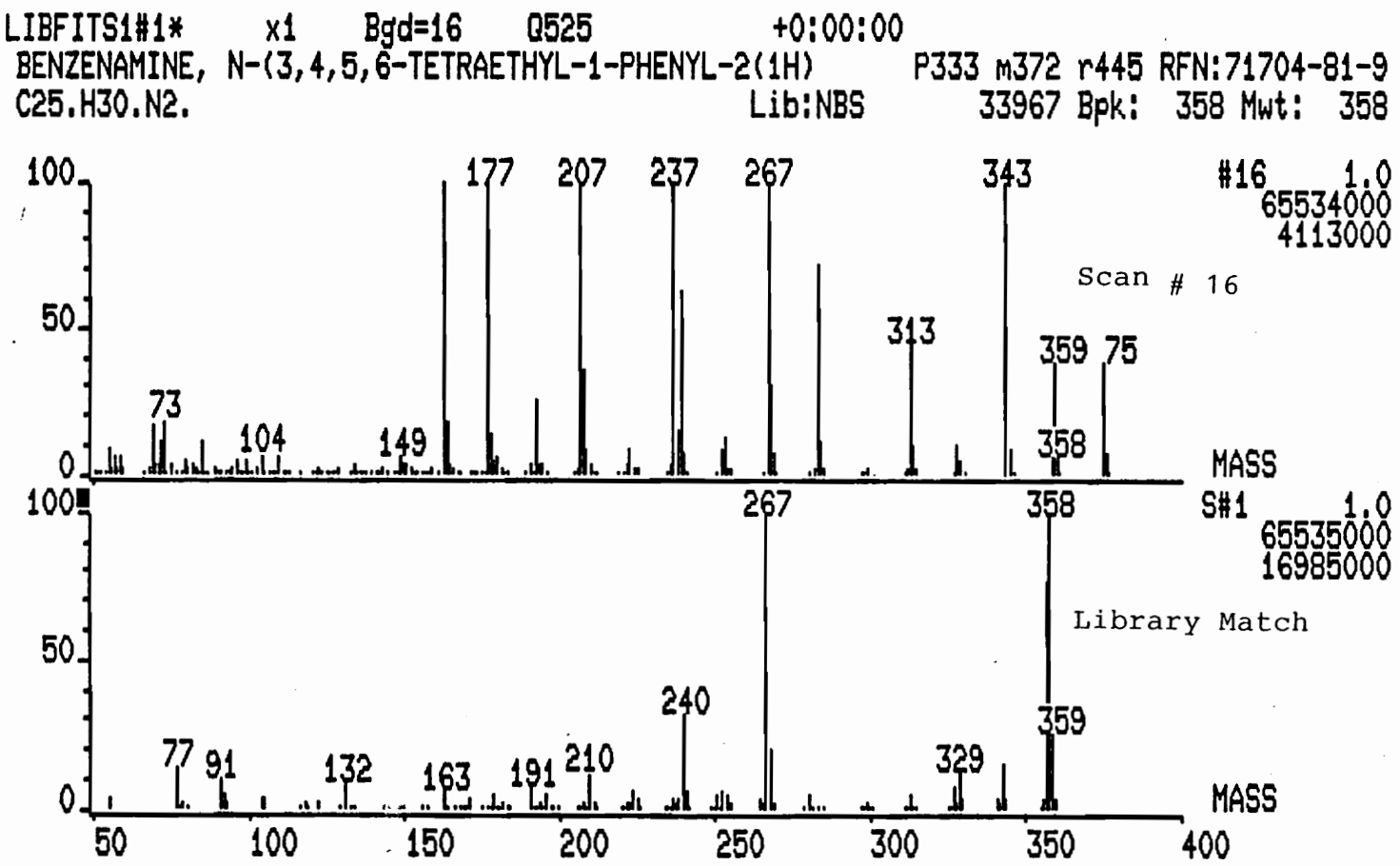
HPLC Fraction 16



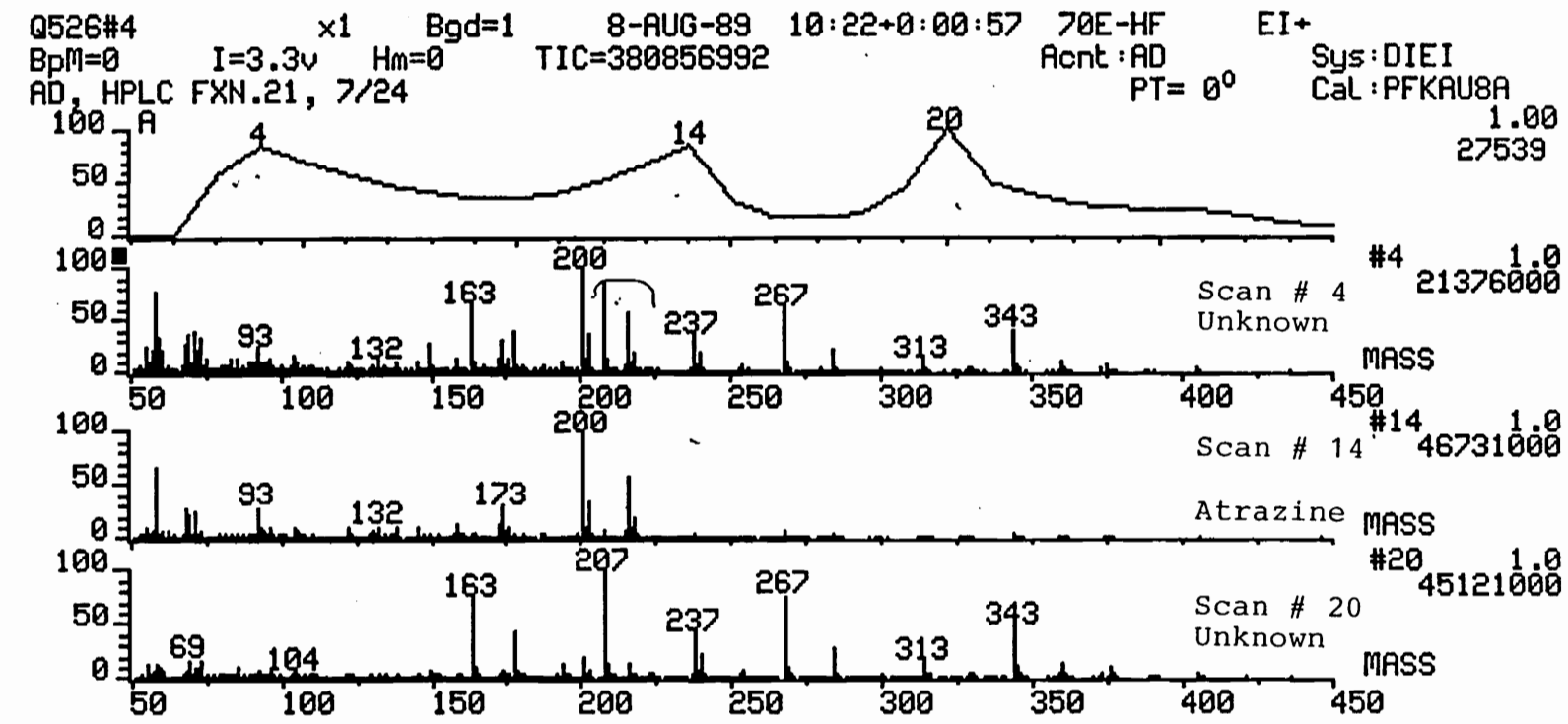
HPLC Fraction 18



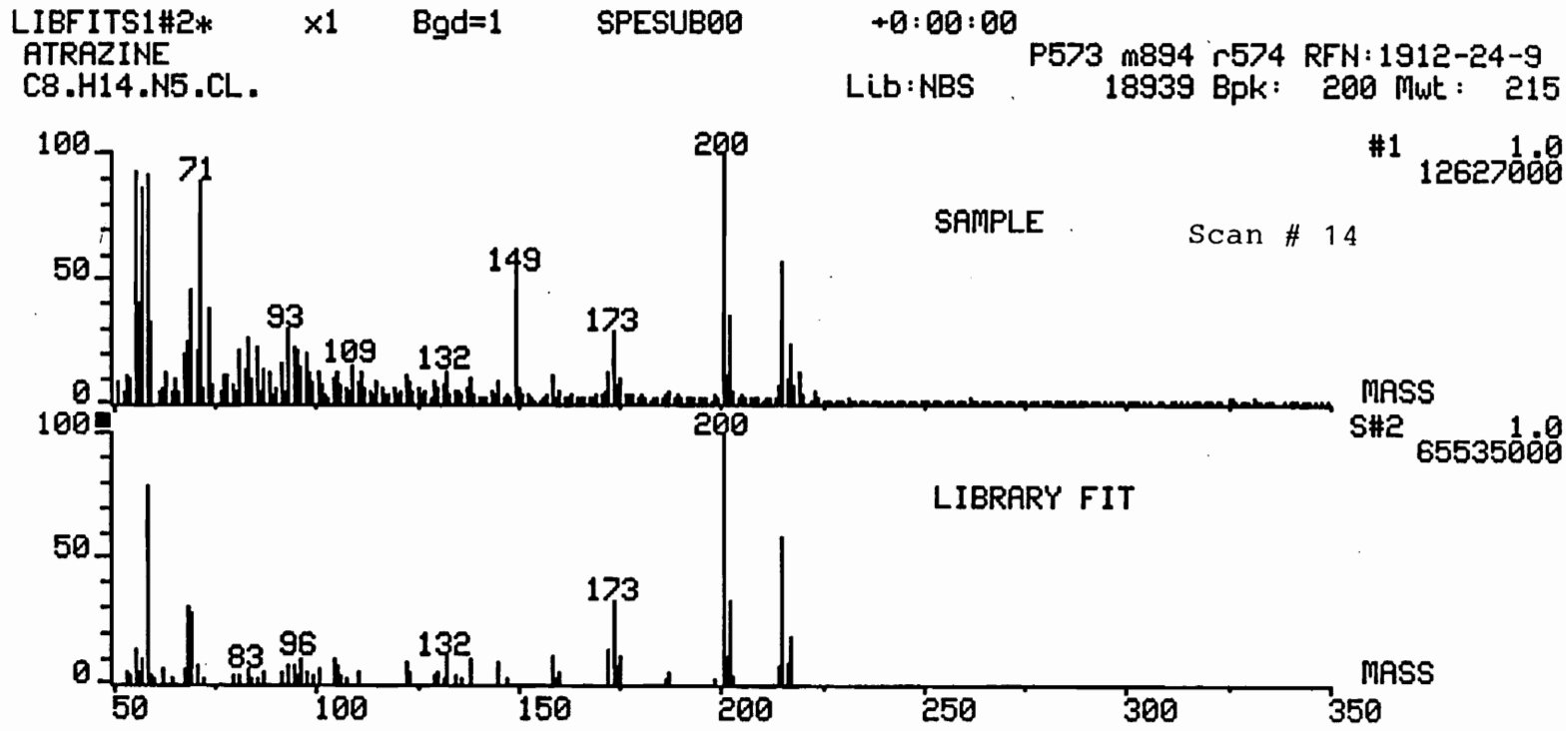
HPLC Fraction 20



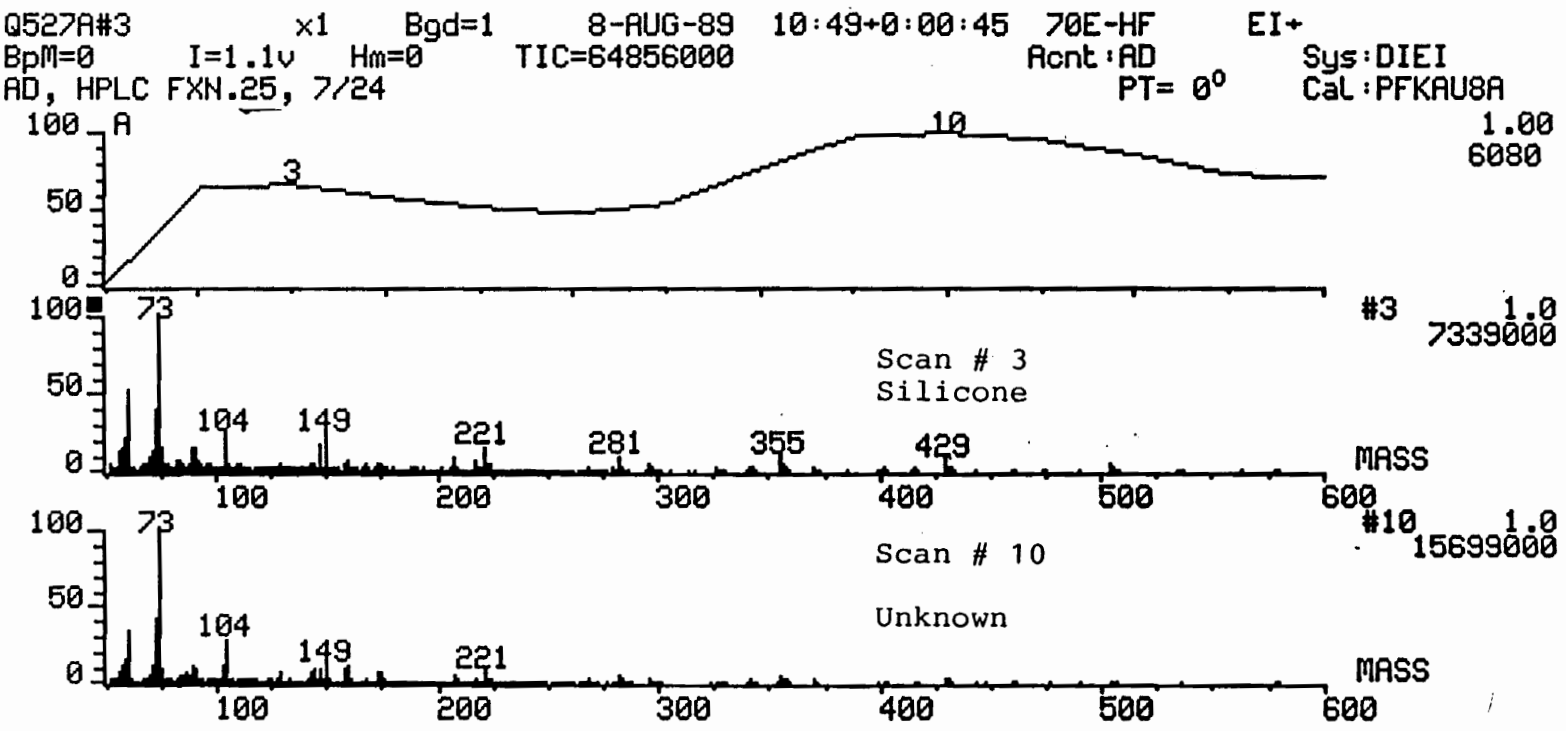
HPLC Fraction 20



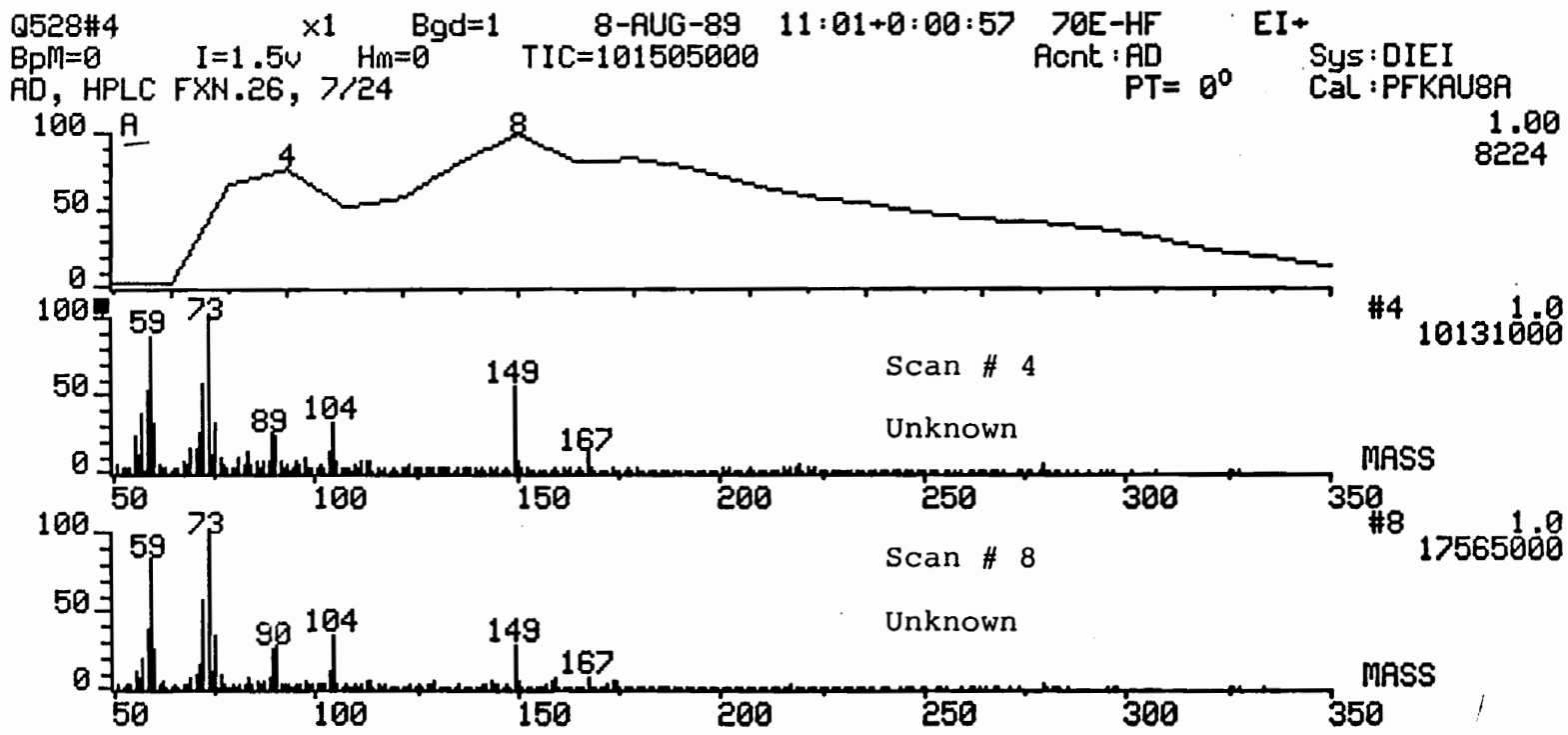
HPLC Fraction 21



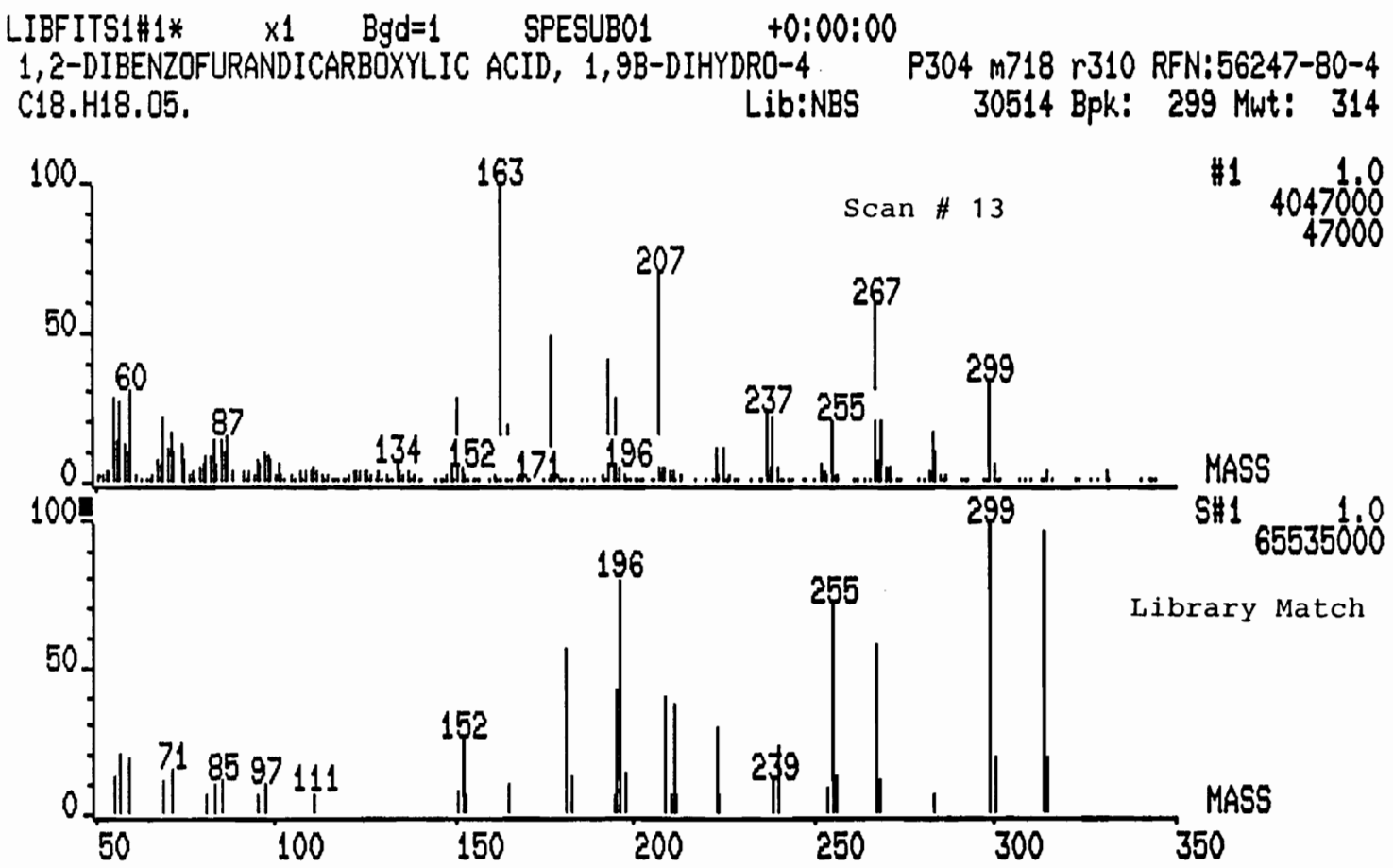
HPLC Fraction 21



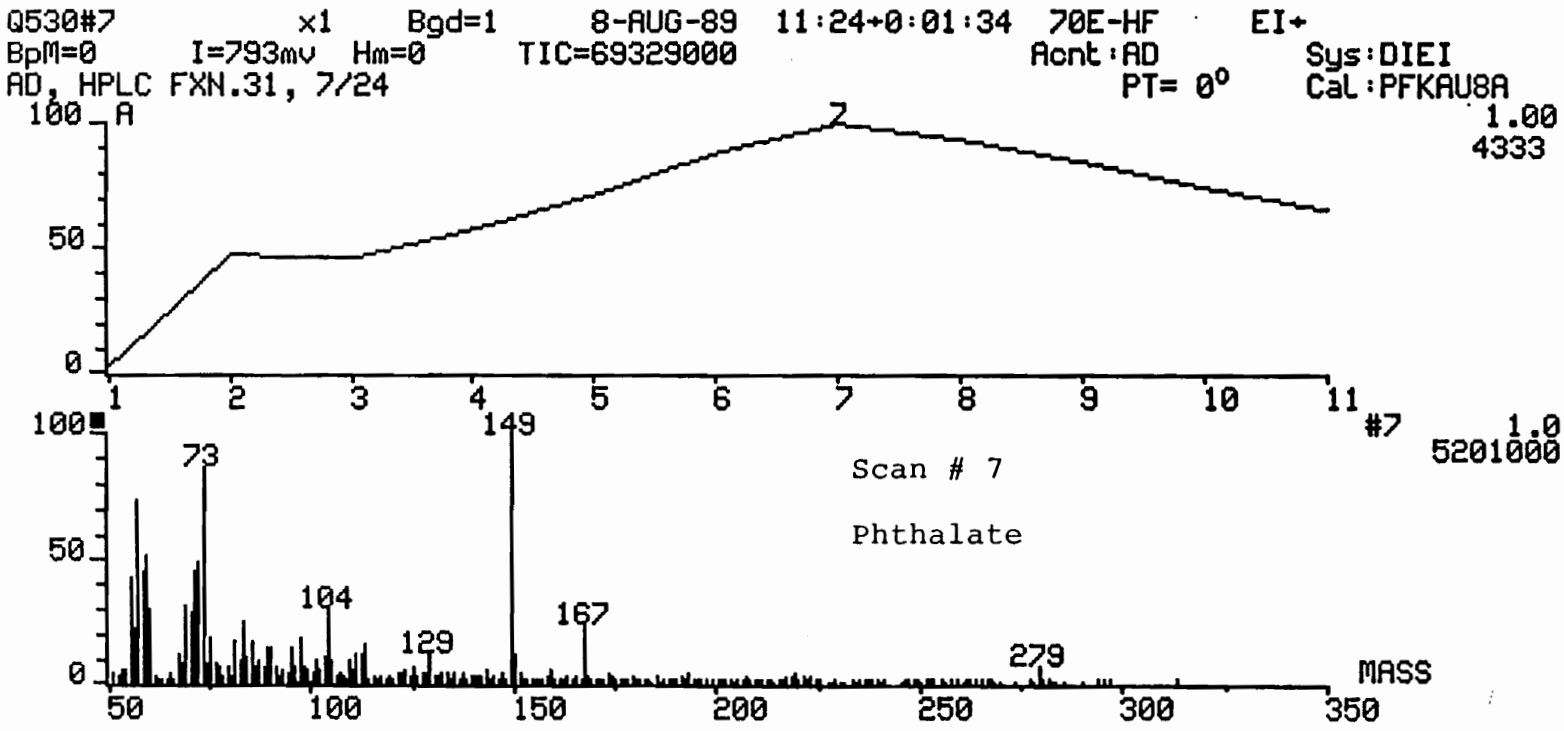
HPLC Fraction 25



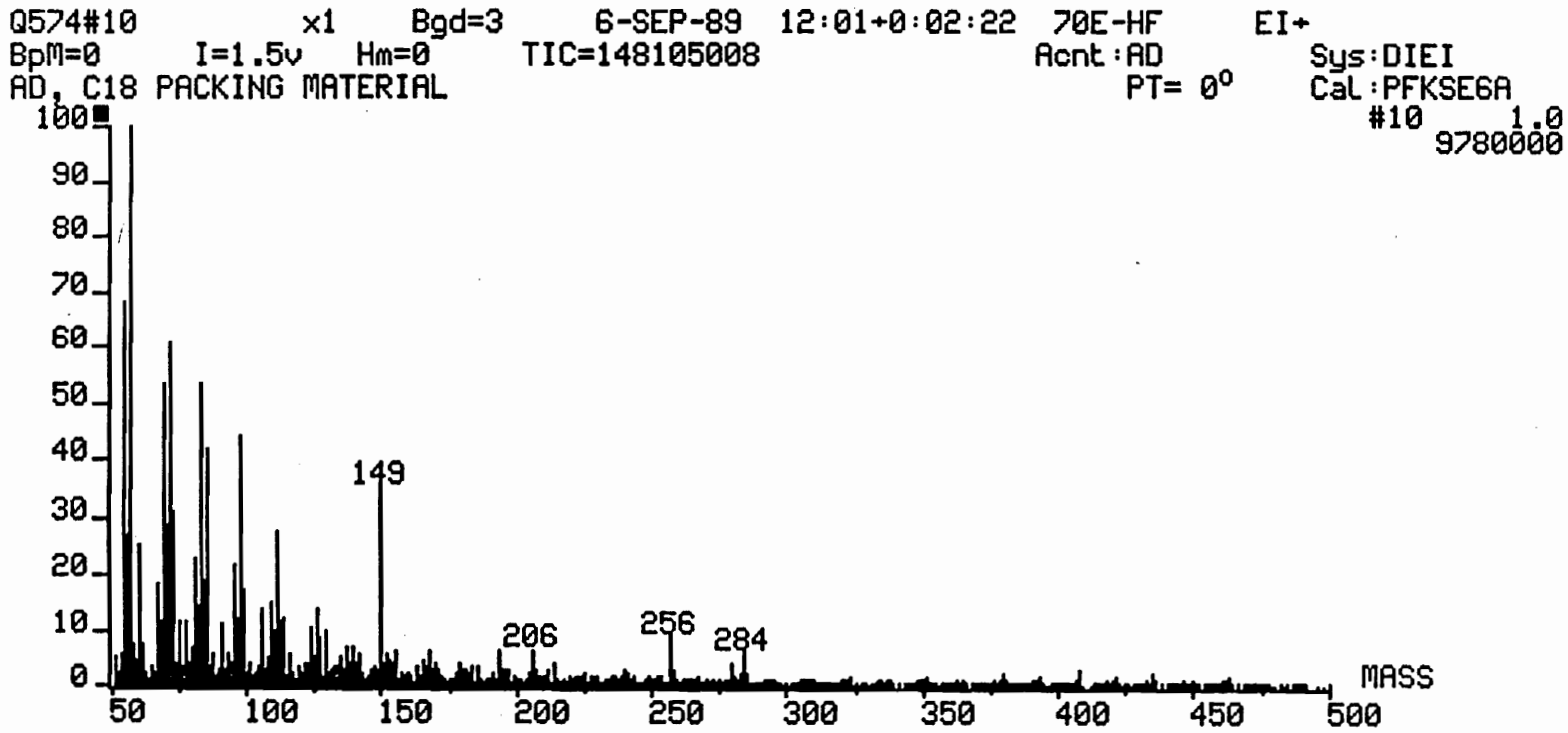
HPLC Fraction 26



HPLC Fraction 28

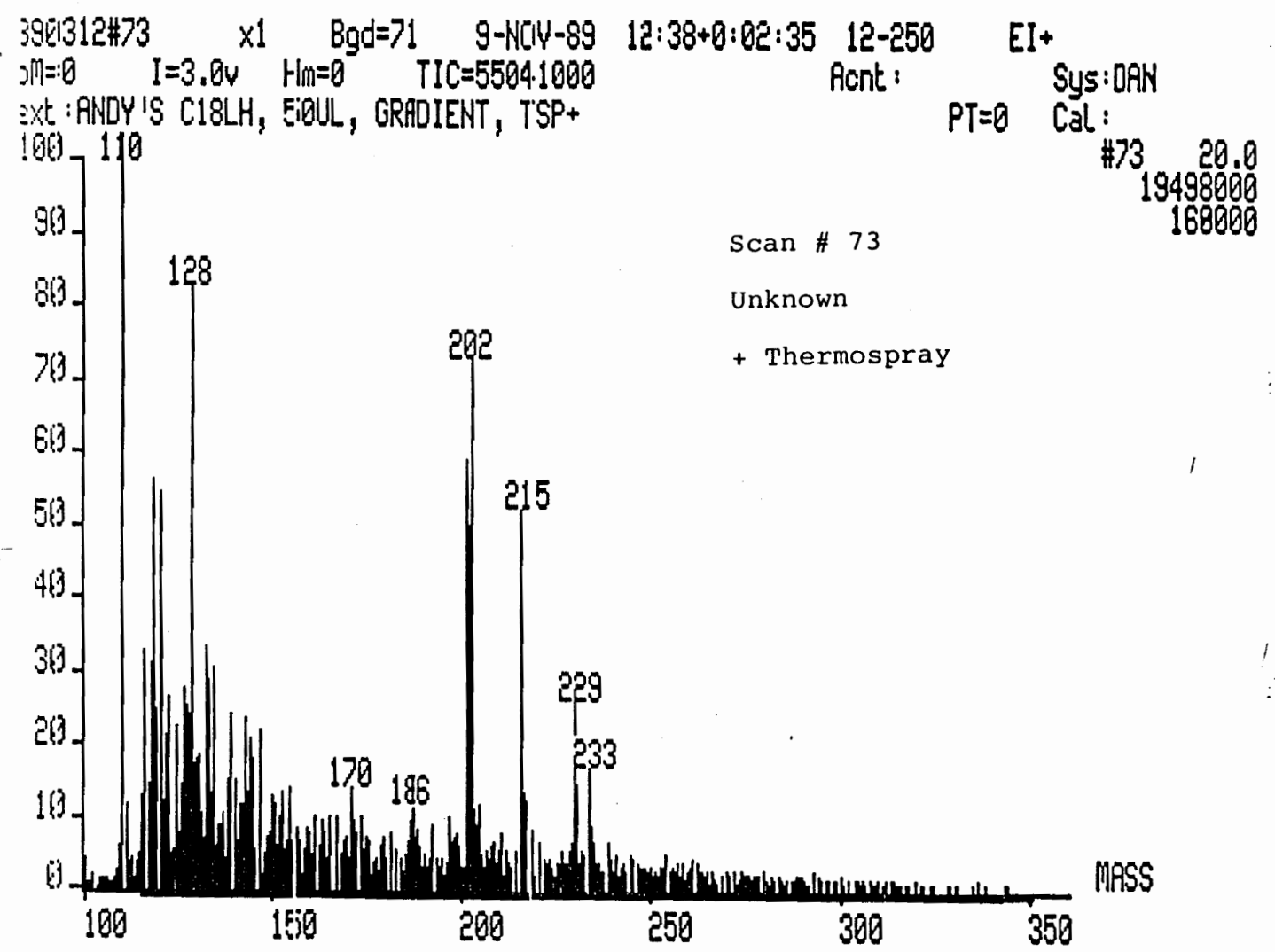


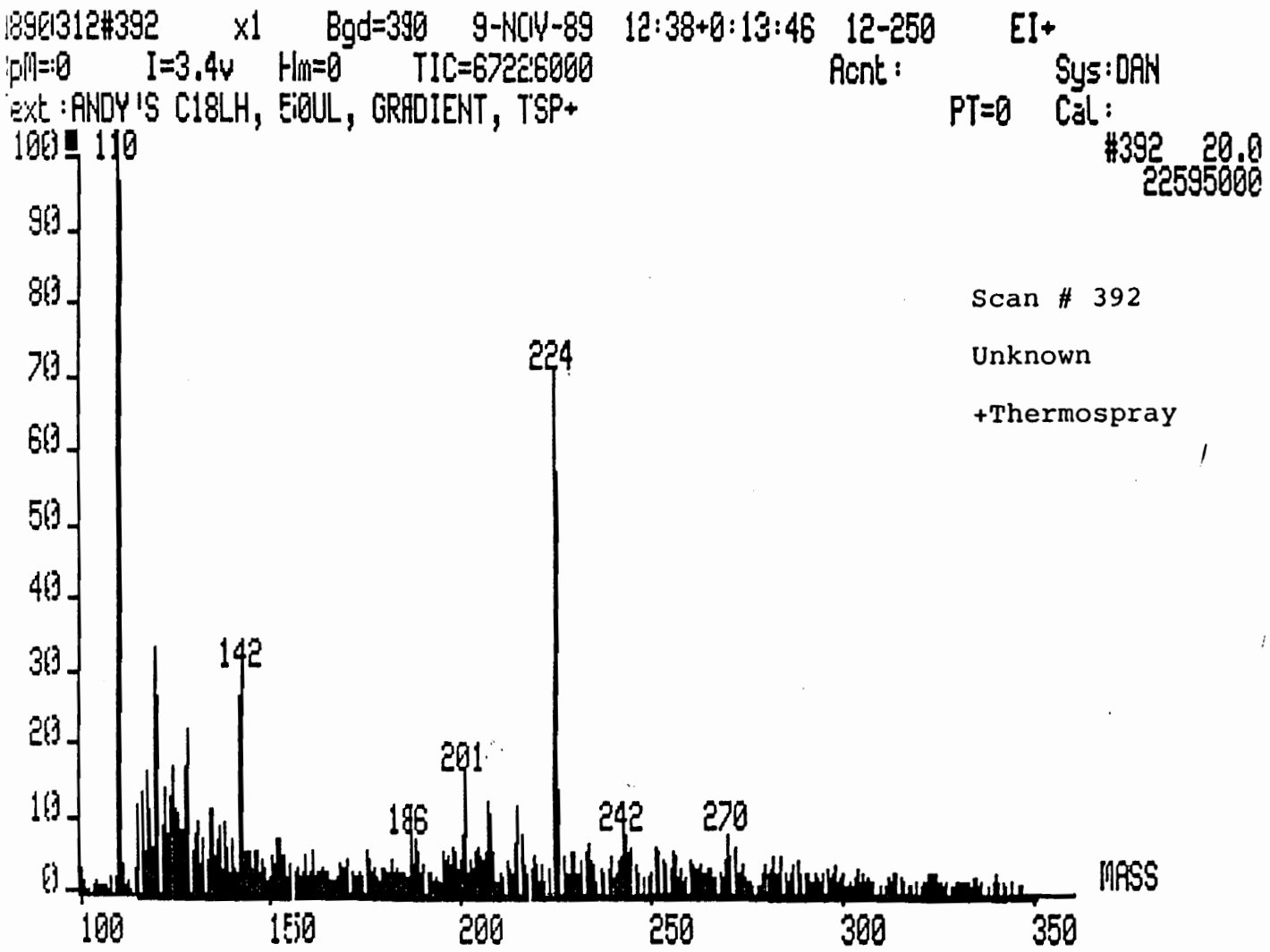
HPLC Fraction 31

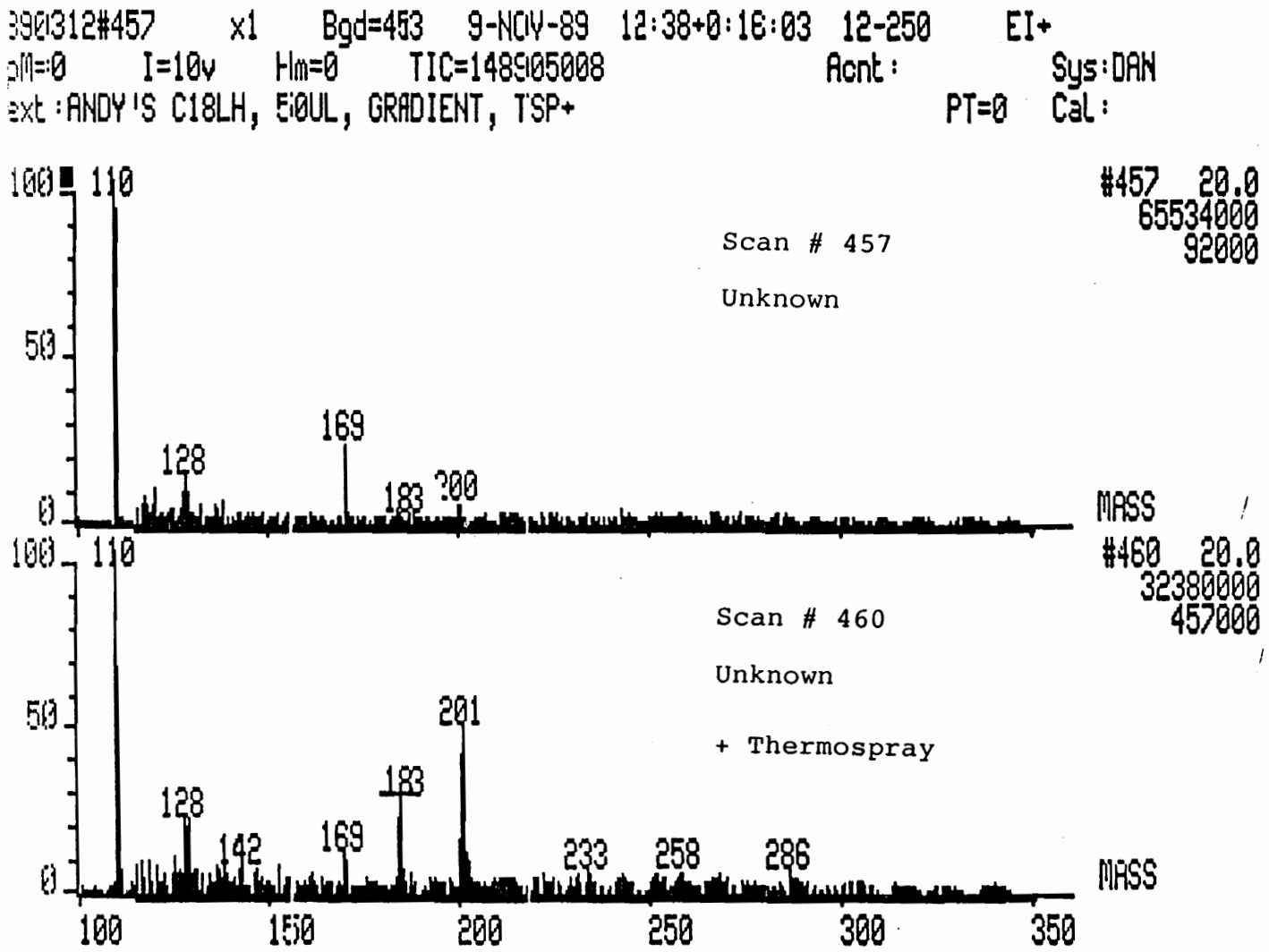


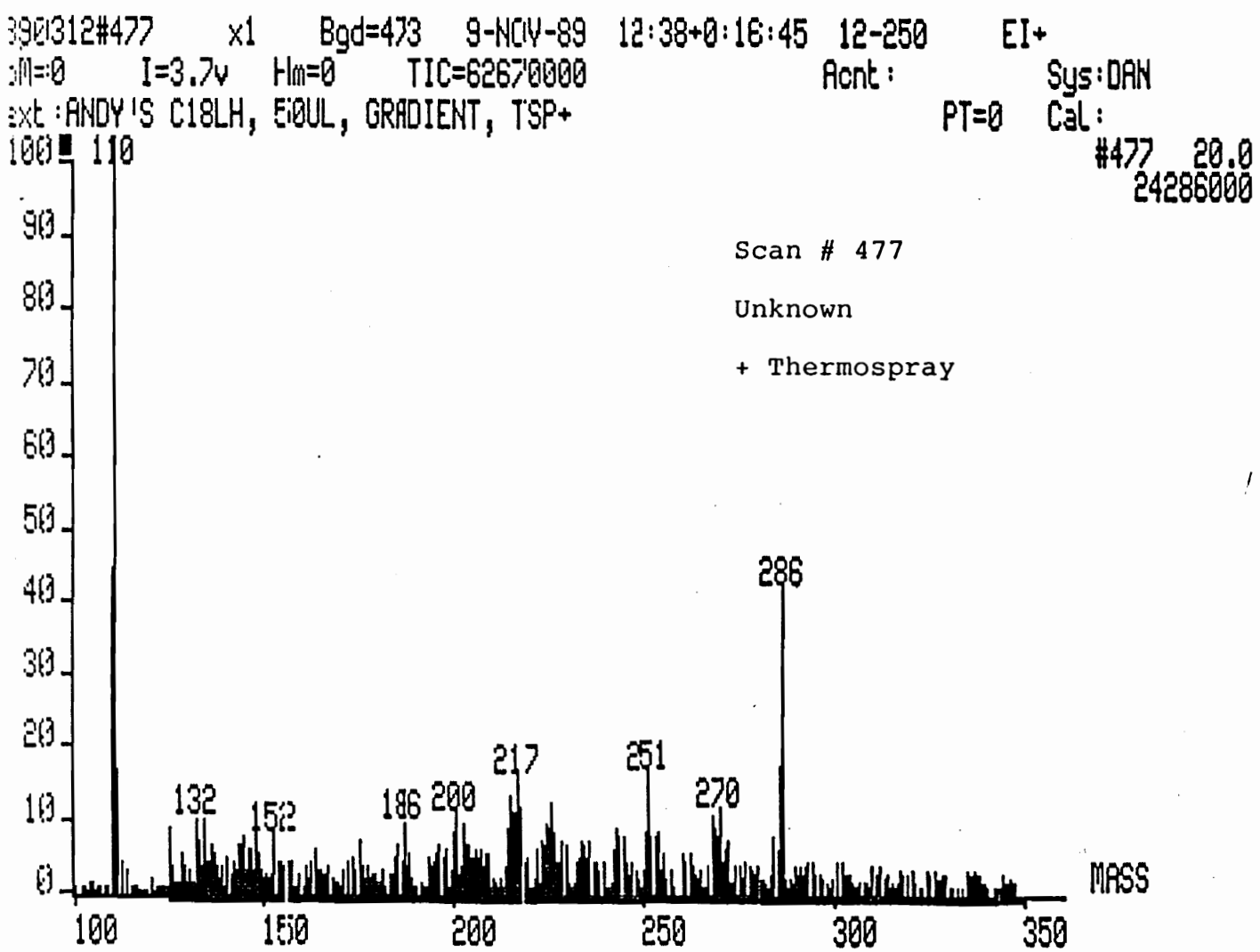
C18 Packing Material

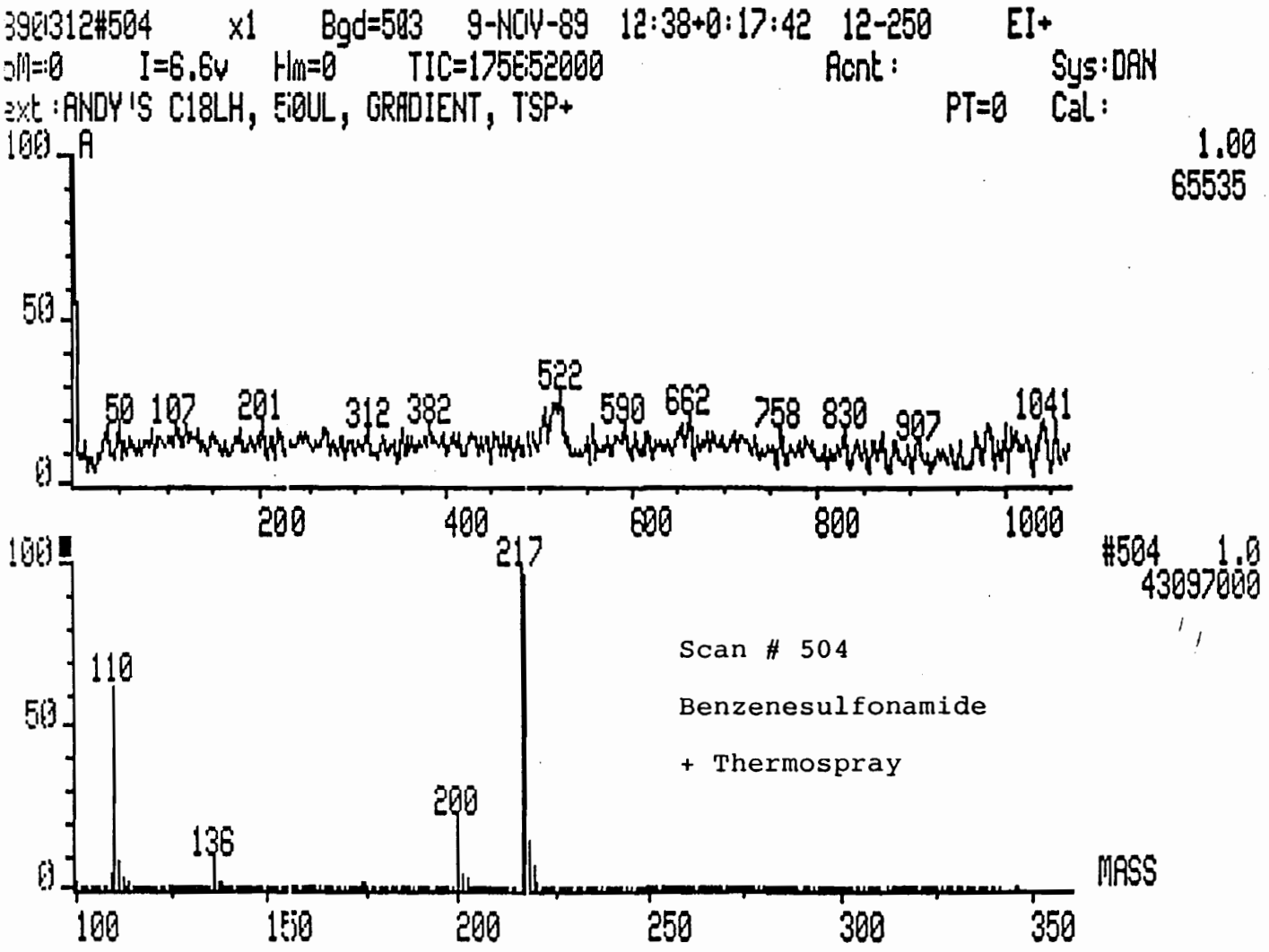
Appendix B. Thermospray LC/MS Spectra from Leachate Analysis (Table 7)

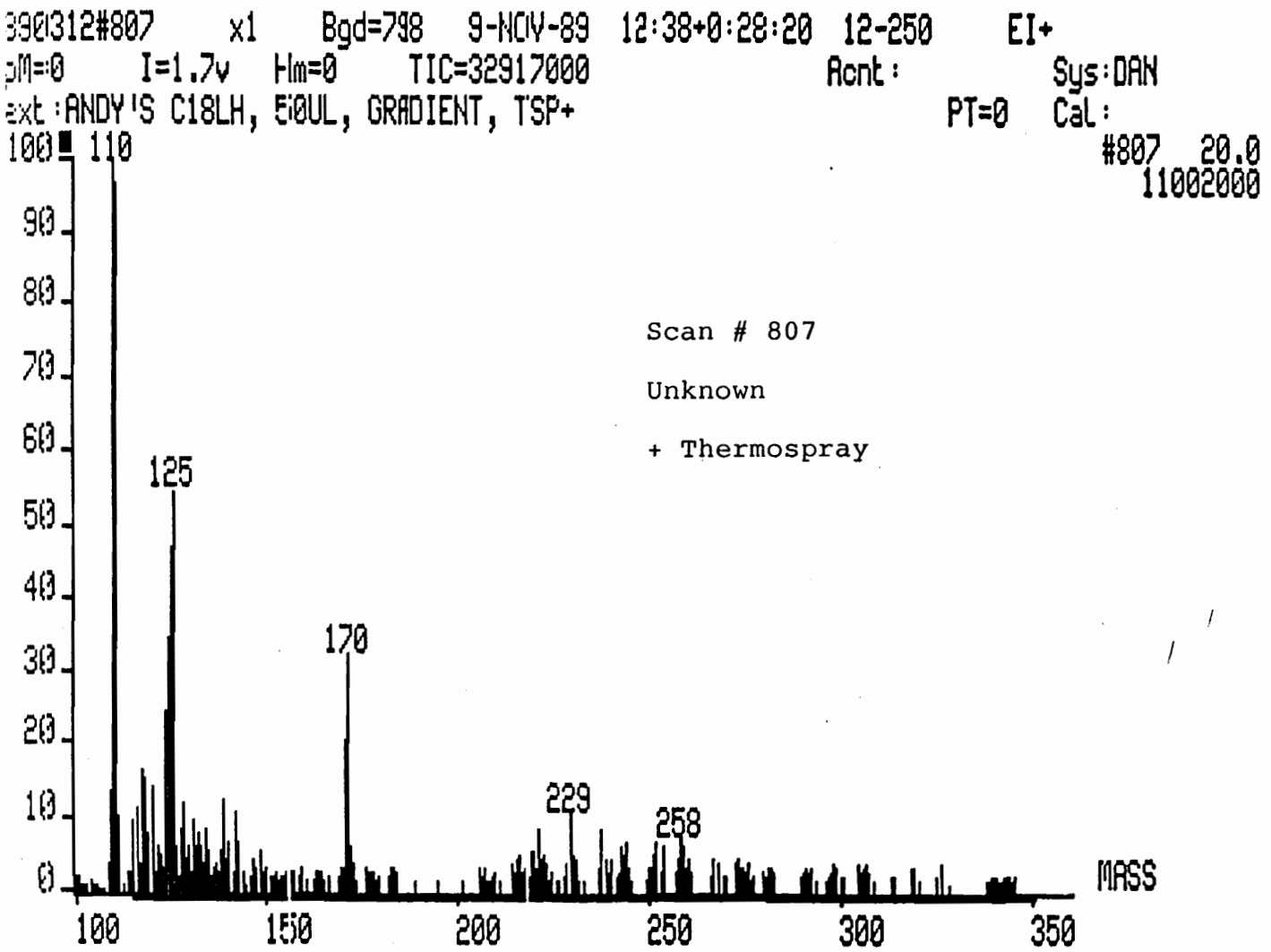

390312#73 x1 Bgd=71 9-NOV-89 12:38+0:02:35 12-250 EI+
oM=0 I=3.0v Hm=0 TIC=5504000 Acnt: Sys:DAN
ext:ANDY'S C18LH, 50UL, GRADIENT, TSP+ PT=0 Cal:
#73 20.0
19498000
160000
Scan # 73
Unknown
+ Thermospray
110
128
202
215
229
233
170
186
100
90
80
70
60
50
40
30
20
10
0
100
150
200
250
300
350
MASS

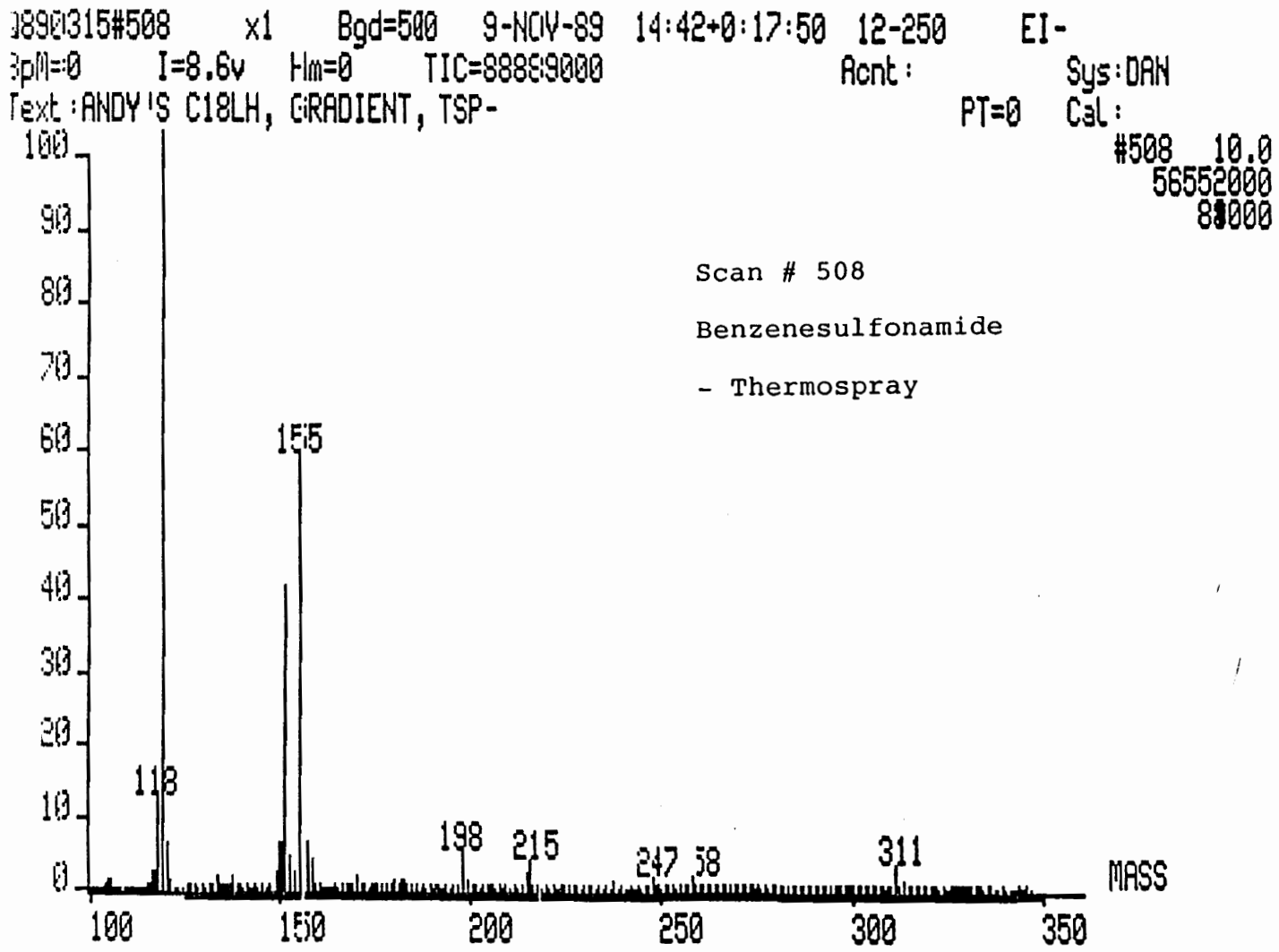

8901312#392 x1 Bgd=390 9-NOV-89 12:38+0:13:46 12-250 EI+
pM=0 I=3.4v Hm=0 TIC=6722:6000 Acnt: Sys:DAN
ext:ANDY'S C18LH, 50UL, GRADIENT, TSP+ PT=0 CaL:
#392 20.0
22595000
Scan # 392
Unknown
+Thermospray
110
224
142
201
186
242
270
MASS
100
90
80
70
60
50
40
30
20
10
0
100
150
200
250
300
350

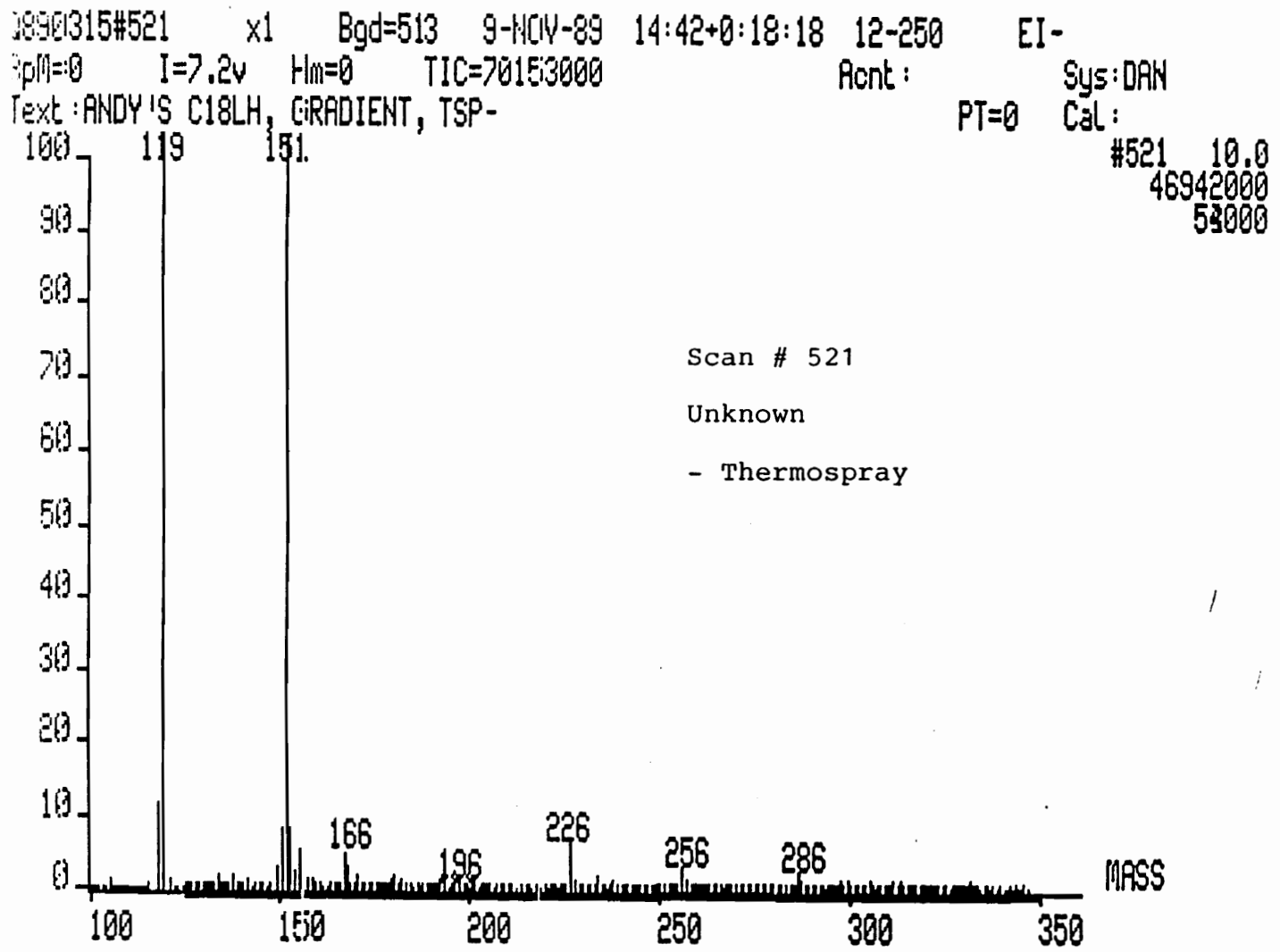

390312#457 x1 Bgd=453 9-NOV-89 12:38+0:16:03 12-250 EI+
pM=0 I=10v Hm=0 TIC=148905008 Acnt: Sys:DAN
ext:ANDY'S C18LH, 50UL, GRADIENT, TSP+ PT=0 Cal:
#457 20.0
65534000
92000
Scan # 457
Unknown
MASS
#460 20.0
32380000
457000
Scan # 460
Unknown
+ Thermospray
MASS
100
50
0
110
128
169
183
200
142
201
233
258
286
100
150
200
250
300
350

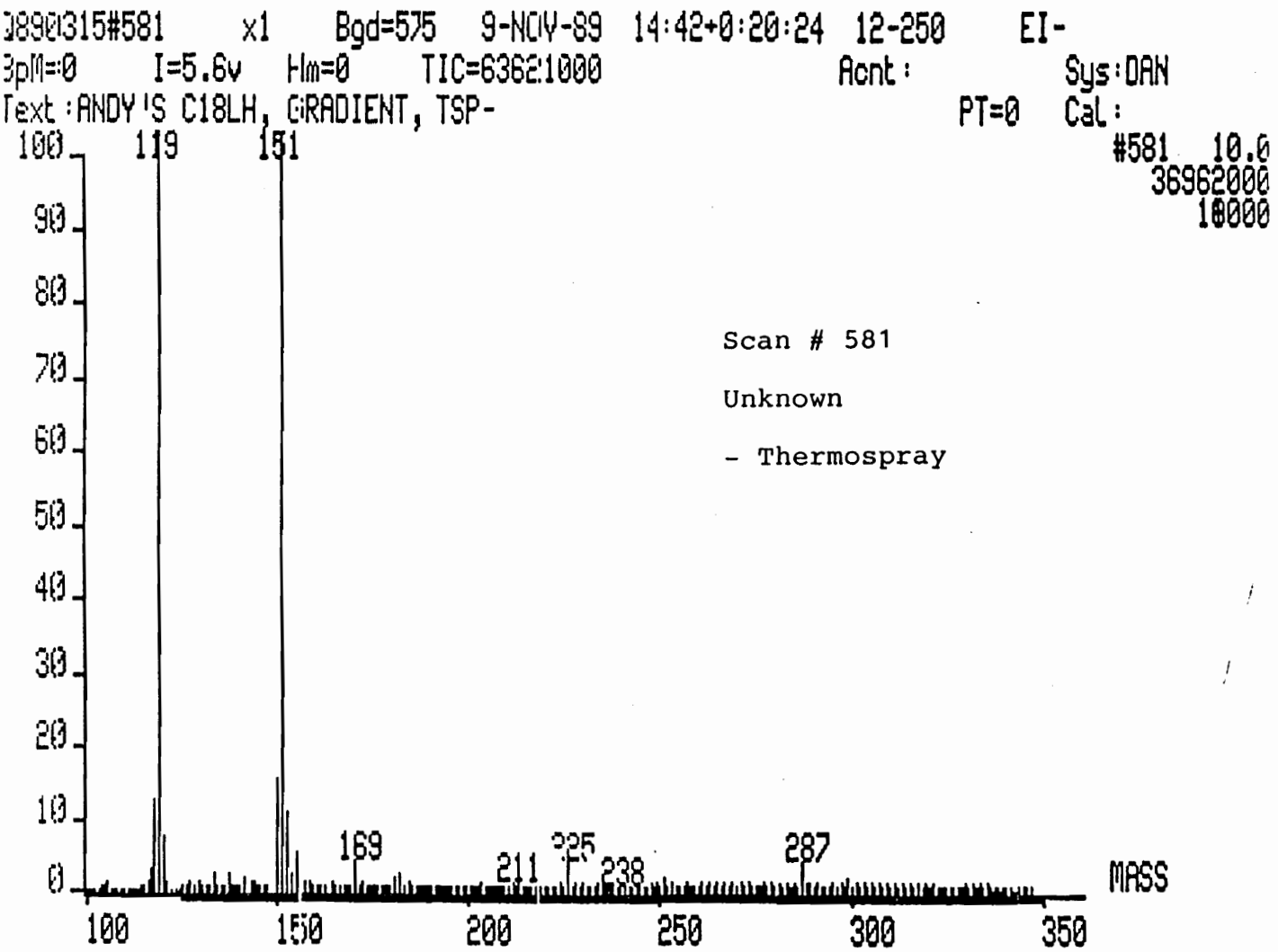

390312#477 x1 Bgd=473 9-NOV-89 12:38+0:16:45 12-250 EI+
:M=0 I=3.7v Hm=0 TIC=62670000 Acnt: Sys:DAN
ext:ANDY'S C18LH, 50UL, GRADIENT, TSP+ PT=0 Cal:
#477 20.0
24286000
Scan # 477
Unknown
+ Thermospray
110
286
217
251
132
152
186
200
270
MASS
100
150
200
250
300
350

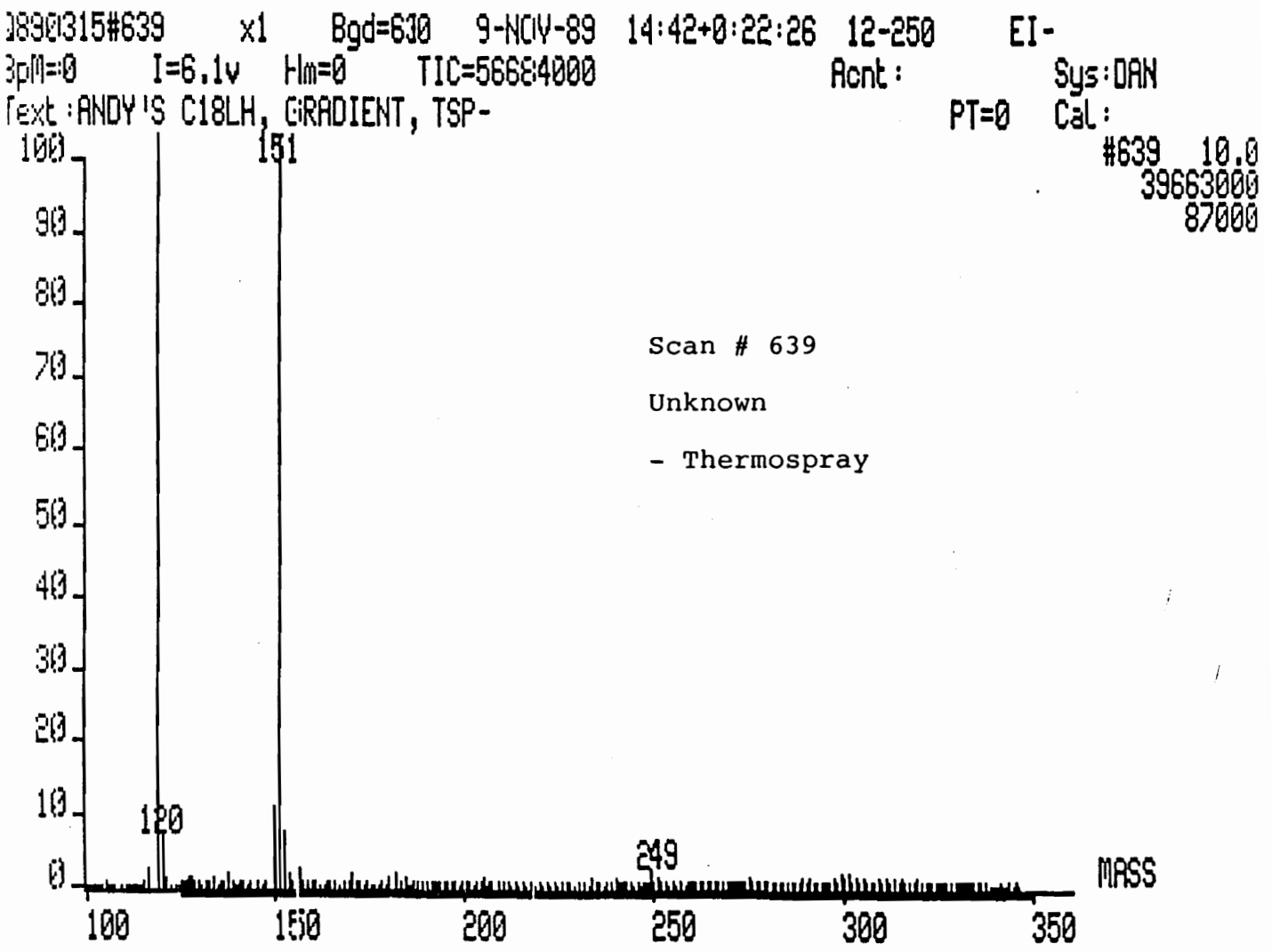

390312#504 x1 Bgd=503 9-NOV-89 12:38+0:17:42 12-250 EI+
BM=0 I=6.6v Hm=0 TIC=175652000 Acnt: Sys:DAN
ext:ANDY'S C18LH, 50UL, GRADIENT, TSP+ PT=0 Cal:
100 A
1.00
65535
50
0
50 107 201 312 382 522 590 662 758 830 907 1041
200 400 600 800 1000
100
#504 1.0
43097000
217
110
50
200
136
0
MASS
100 150 200 250 300 350
Scan # 504
Benzenesulfonamide
+ Thermospray

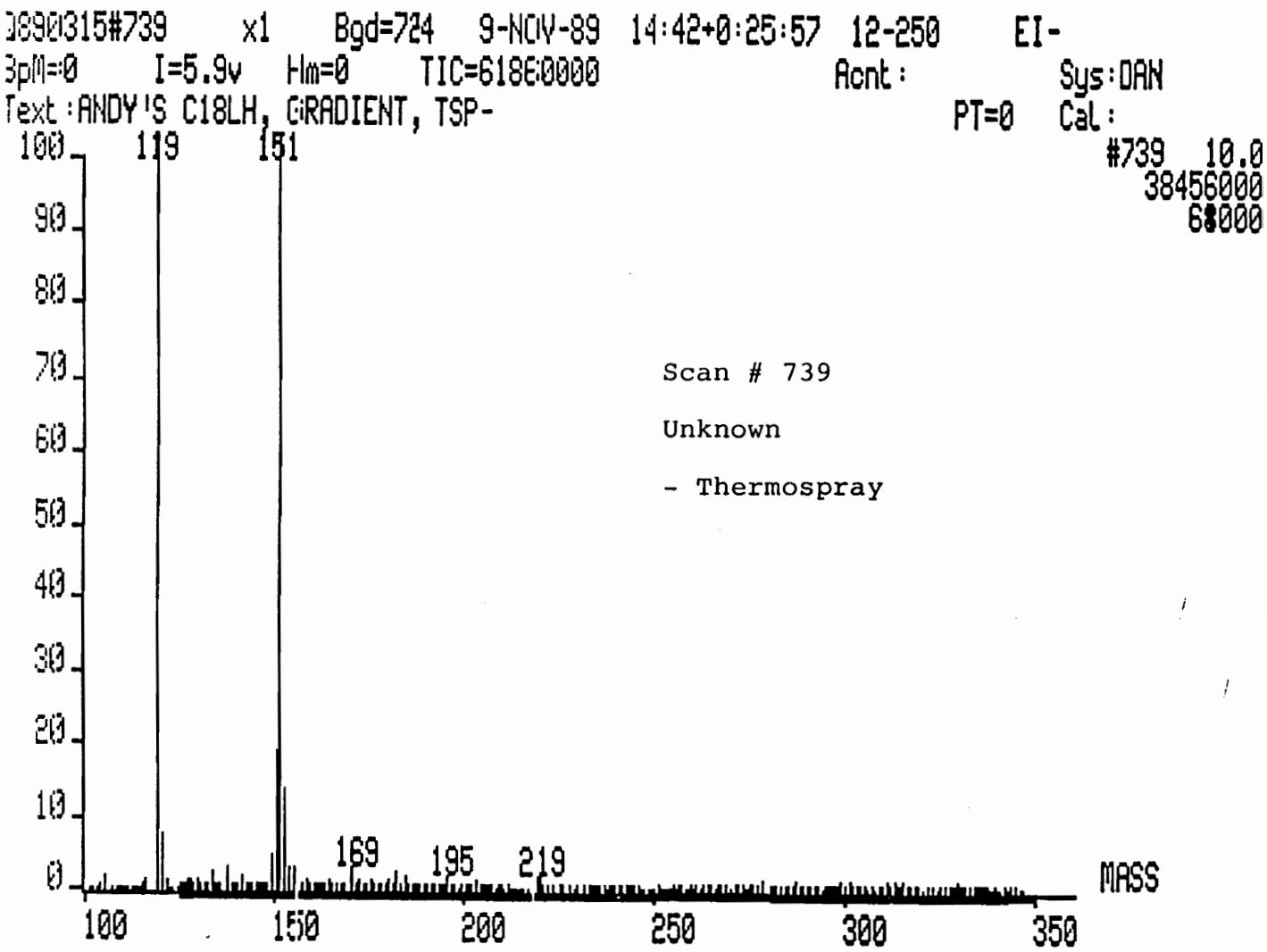

390312#807 x1 Bgd=798 9-NOV-89 12:38+0:28:20 12-250 EI+
pM=0 I=1.7v Hm=0 TIC=32917000 Acnt: Sys:DAN
ext:ANDY'S C18LH, 50UL, GRADIENT, TSP+ PT=0 Cal:
#807 20.0
11002000
Scan # 807
Unknown
+ Thermospray
110
125
170
229
258
MASS
100
150
200
250
300
350

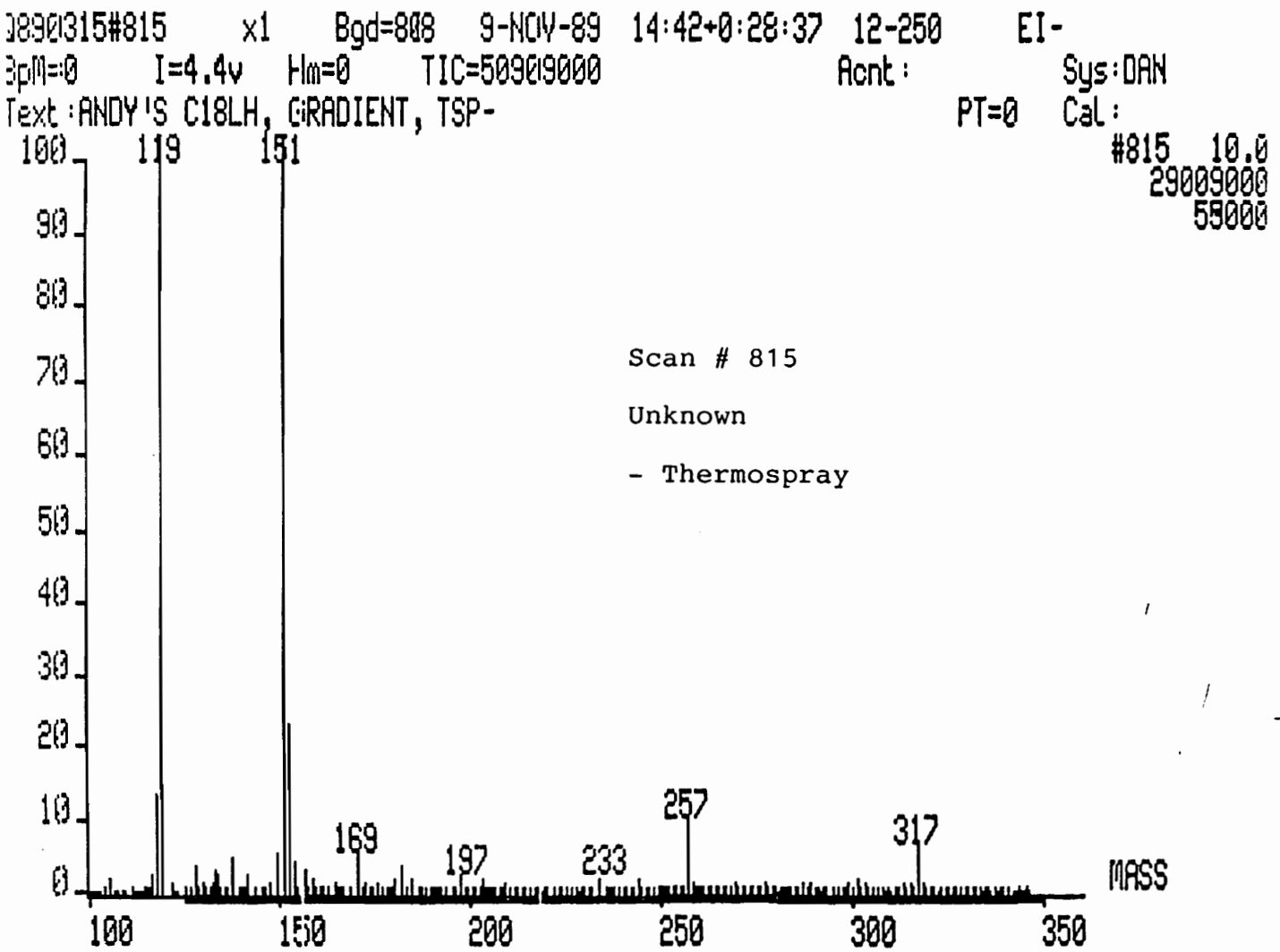

)890315#508 x1 Bgd=500 9-NOV-89 14:42+0:17:50 12-250 EI-
3pM=0 I=8.6v Hm=0 TIC=8886:9000 Acnt: Sys:DAN
Text:ANDY'S C18LH, GRADIENT, TSP- PT=0 Cal:
#508 10.0
56552000
8 000
Scan # 508
Benzenesulfonamide
- Thermospray
100
90
80
70
60
50
40
30
20
10
0
118
155
198
215
247 58
311
MASS
100
150
200
250
300
350

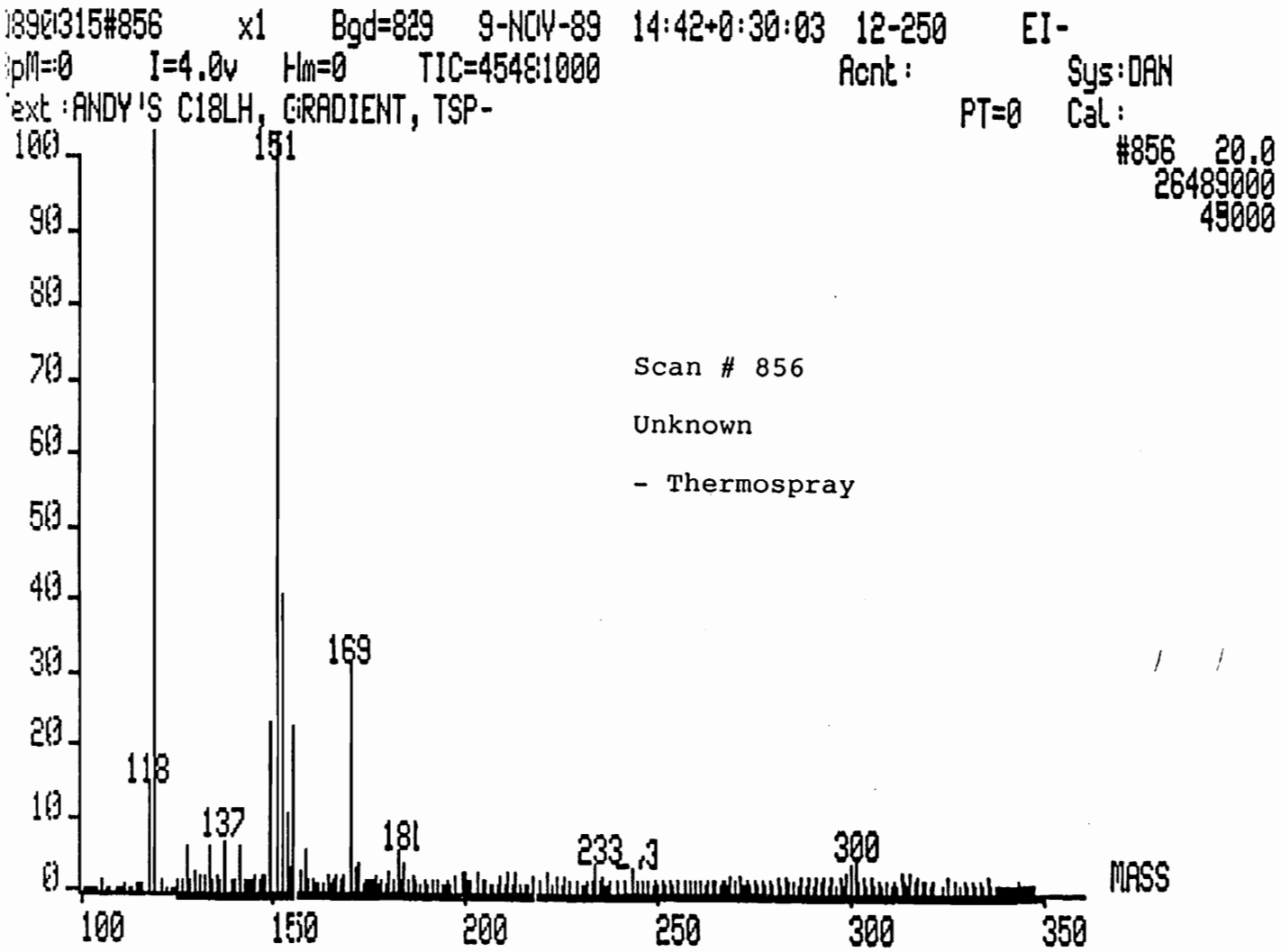

8890315#521 x1 Bgd=513 9-NOV-89 14:42+0:18:18 12-250 EI-
SpM=0 I=7.2v Hm=0 TIC=70153000 Acnt: Sys:DAN
Text:ANDY'S C18LH, GRADIENT, TSP- PT=0 Cal:
#521 10.0
46942000
53000
119
151
Scan # 521
Unknown
- Thermospray
166
196
226
256
286
MASS
100
150
200
250
300
350

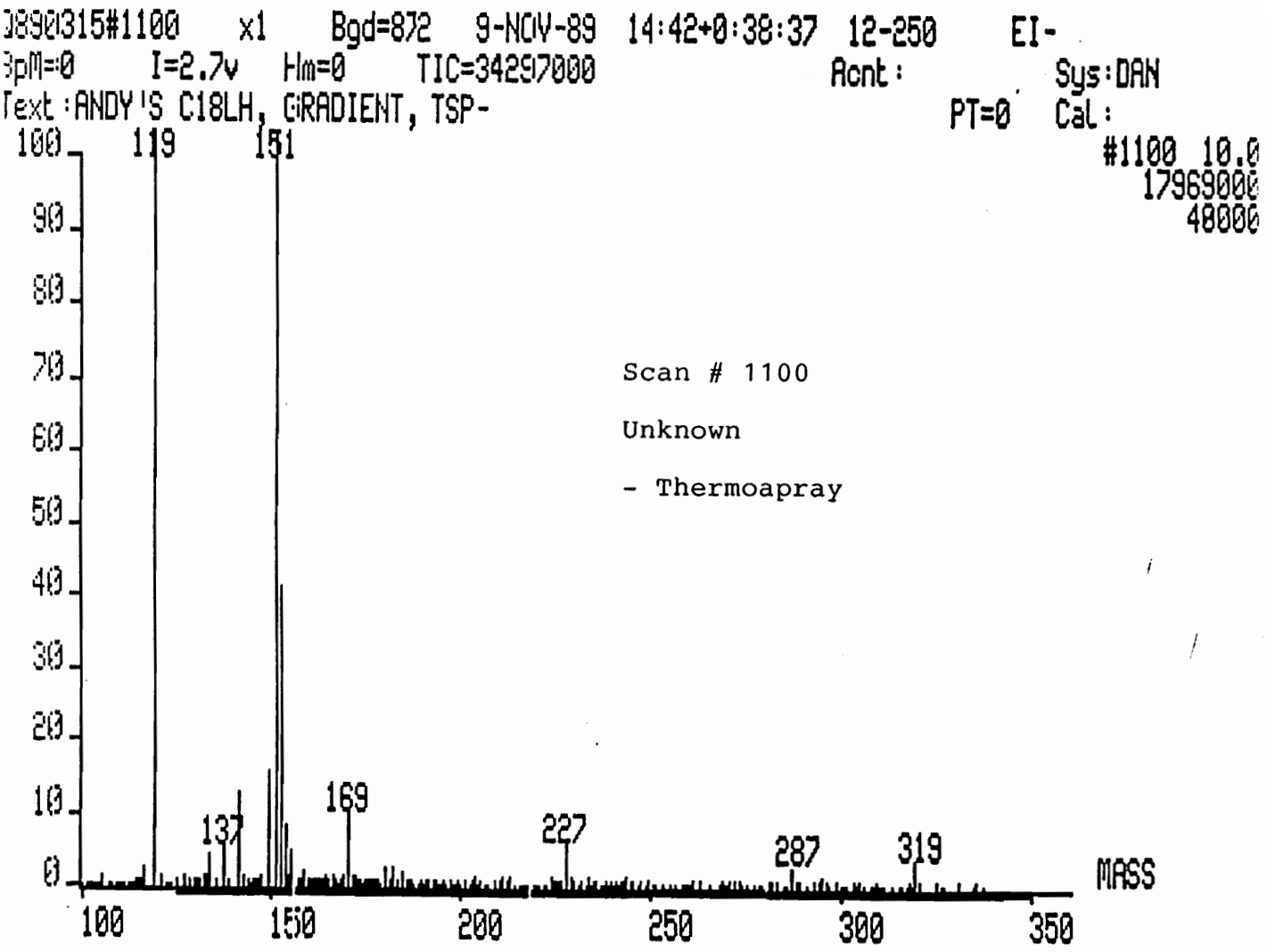

3890315#581 x1 Bgd=575 9-NOV-89 14:42+0:20:24 12-250 EI-
3pM=0 I=5.6v Hm=0 TIC=6362:1000 Acnt: Sys:DAN
Text:ANDY'S C18LH, GRADIENT, TSP- PT=0 Cal:
#581 10.0
36962000
10000
Scan # 581
Unknown
- Thermospray
100
90
80
70
60
50
40
30
20
10
0
119
151
169
211
225
238
287
MASS
100
150
200
250
300
350


D890315#639 x1 Bgd=630 9-NOV-89 14:42+0:22:26 12-250 EI-
BpM=0 I=6.1v Hm=0 TIC=5668:4000 Acnt: Sys:DAN
Text:ANDY'S C18LH, GRADIENT, TSP- PT=0 Cal:
#639 10.0
39663000
87000
Scan # 639
Unknown
- Thermospray
151
120
249
MASS
100
90
80
70
60
50
40
30
20
10
0
100
150
200
250
300
350


D890315#739 x1 Bgd=724 9-NOV-89 14:42+0:25:57 12-250 EI-
BpM=0 I=5.9v Hm=0 TIC=6186000 Acnt: Sys:DAN
Text:ANDY'S C18LH, GRADIENT, TSP- PT=0 Cal:
#739 10.0
38456000
68000
Scan # 739
Unknown
- Thermospray
100
90
80
70
60
50
40
30
20
10
0
119
151
169
195
219
MASS
100
150
200
250
300
350

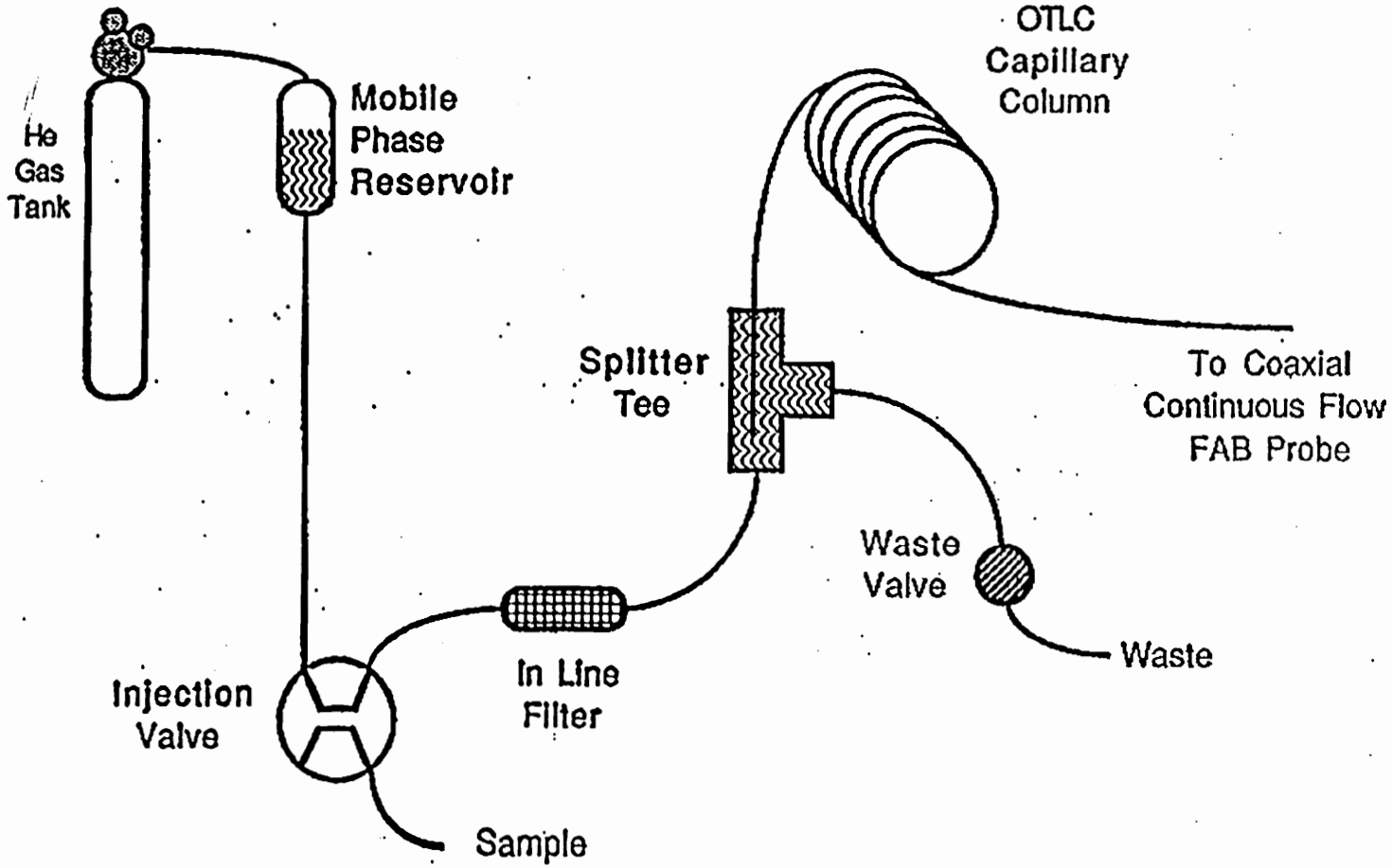

)890315#815 x1 Bgd=808 9-NOV-89 14:42+0:28:37 12-250 EI-
3pM=0 I=4.4v Hm=0 TIC=50909000 Acnt: Sys:DAN
Text:ANDY'S C18LH, GRADIENT, TSP- PT=0 Cal:
#815 10.0
29009000
55000
Scan # 815
Unknown
- Thermospray
MASS
100
90
80
70
60
50
40
30
20
10
0
119
151
169
197
233
257
317
100
150
200
250
300
350

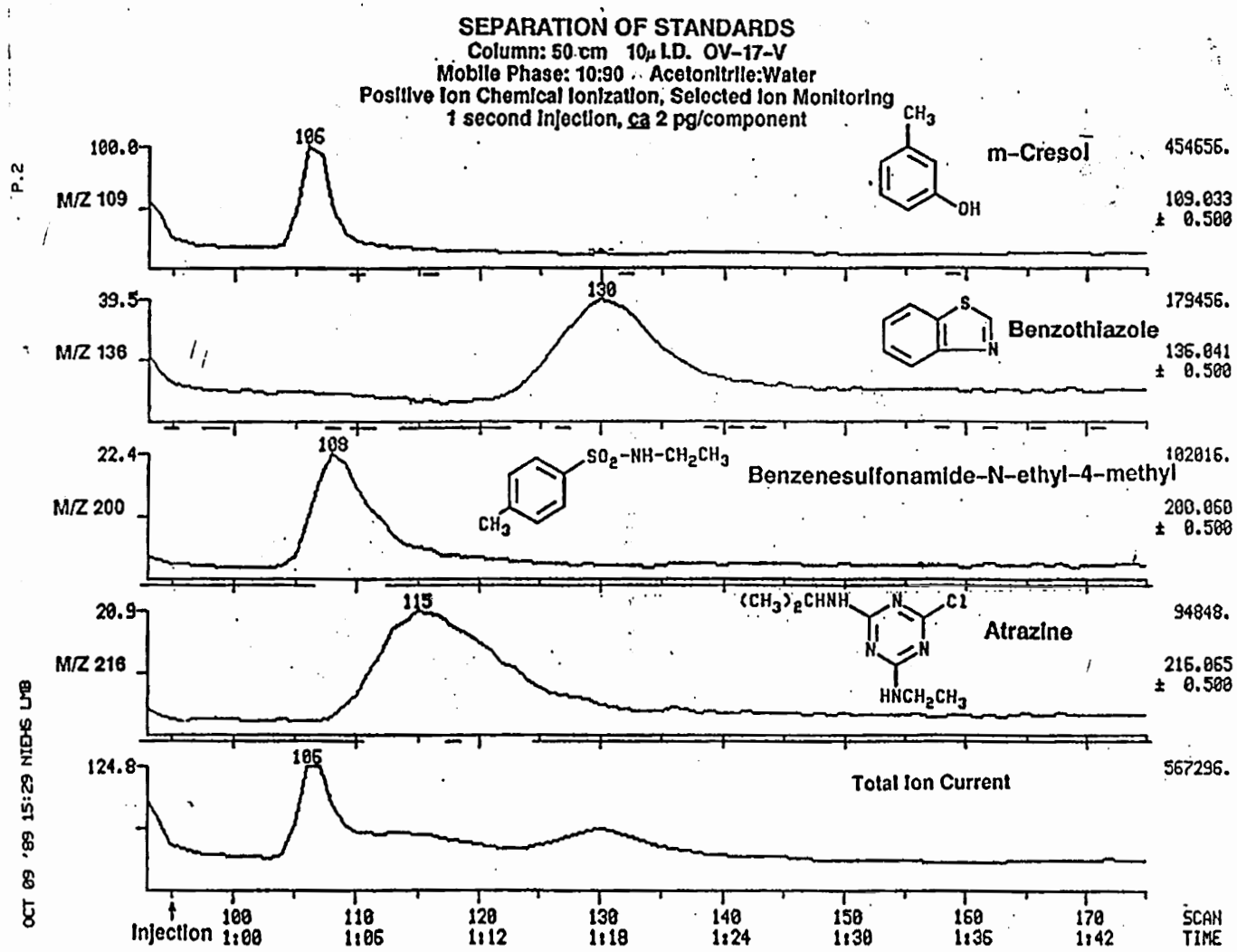

)890315#856 x1 Bgd=829 9-NOV-89 14:42+0:30:03 12-250 EI-
pM=0 I=4.0v Hm=0 TIC=4548:1000 Acnt: Sys:DAN
ext:ANDY'S C18LH, GRADIENT, TSP- PT=0 Cal:
#856 20.0
26489000
45000
Scan # 856
Unknown
- Thermospray
151
169
118
137
181
233
300
MASS
100
150
200
250
300
350


D890315#1100 x1 Bgd=872 9-NOV-89 14:42+0:38:37 12-250 EI-
BpM=0 I=2.7v Hm=0 TIC=34297000 Acnt: Sys:DAN
Text:ANDY'S C18LH, GRADIENT, TSP- PT=0 Cal:
#1100 10.0
17969000
40000
Scan # 1100
Unknown
- Thermoapray
100
90
80
70
60
50
40
30
20
10
0
119
151
137
169
227
287
319
MASS
100
150
200
250
300
350

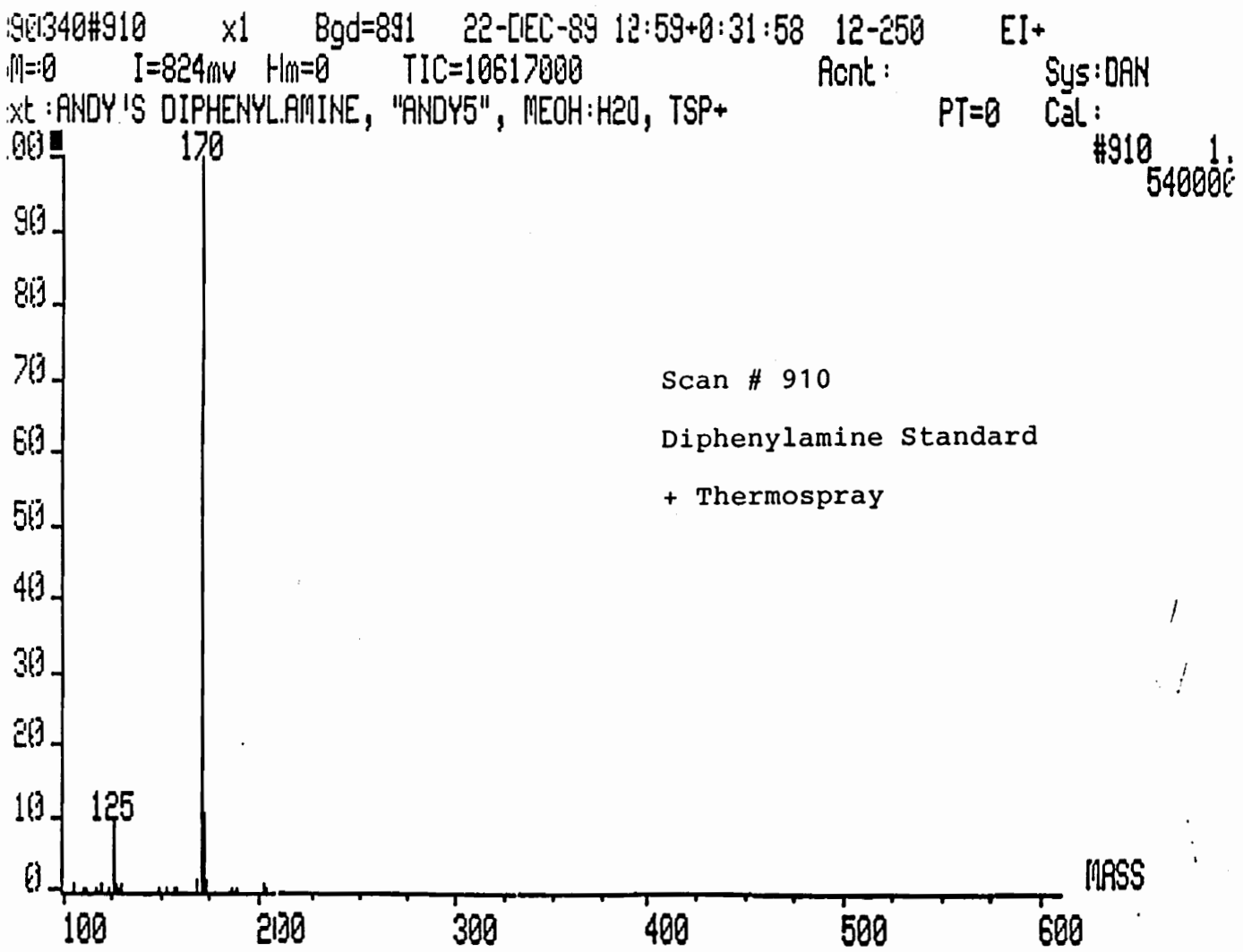
Appendix C. OTLC Schematic and Chromatogram of Standards

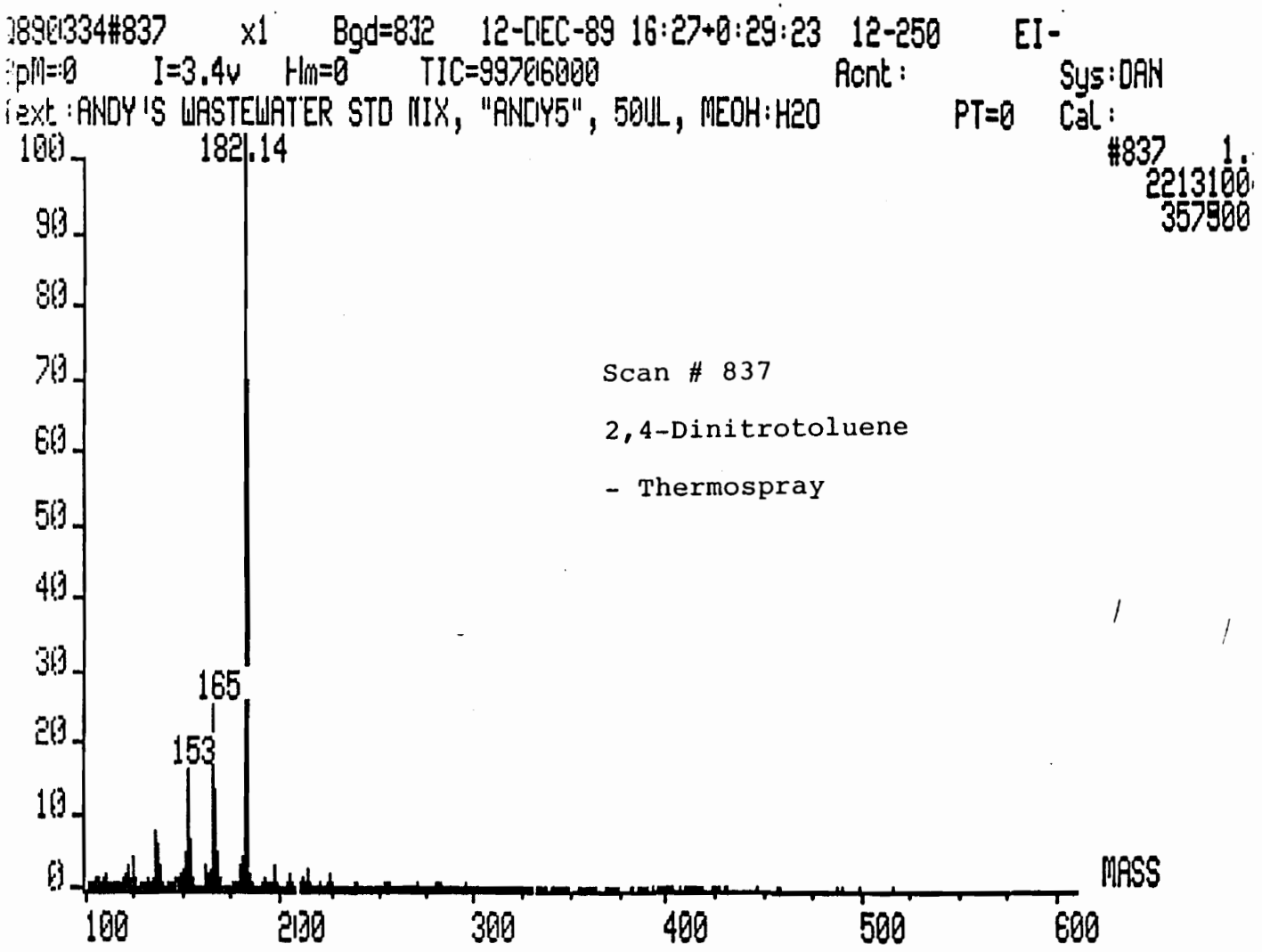
OTLC Schematic

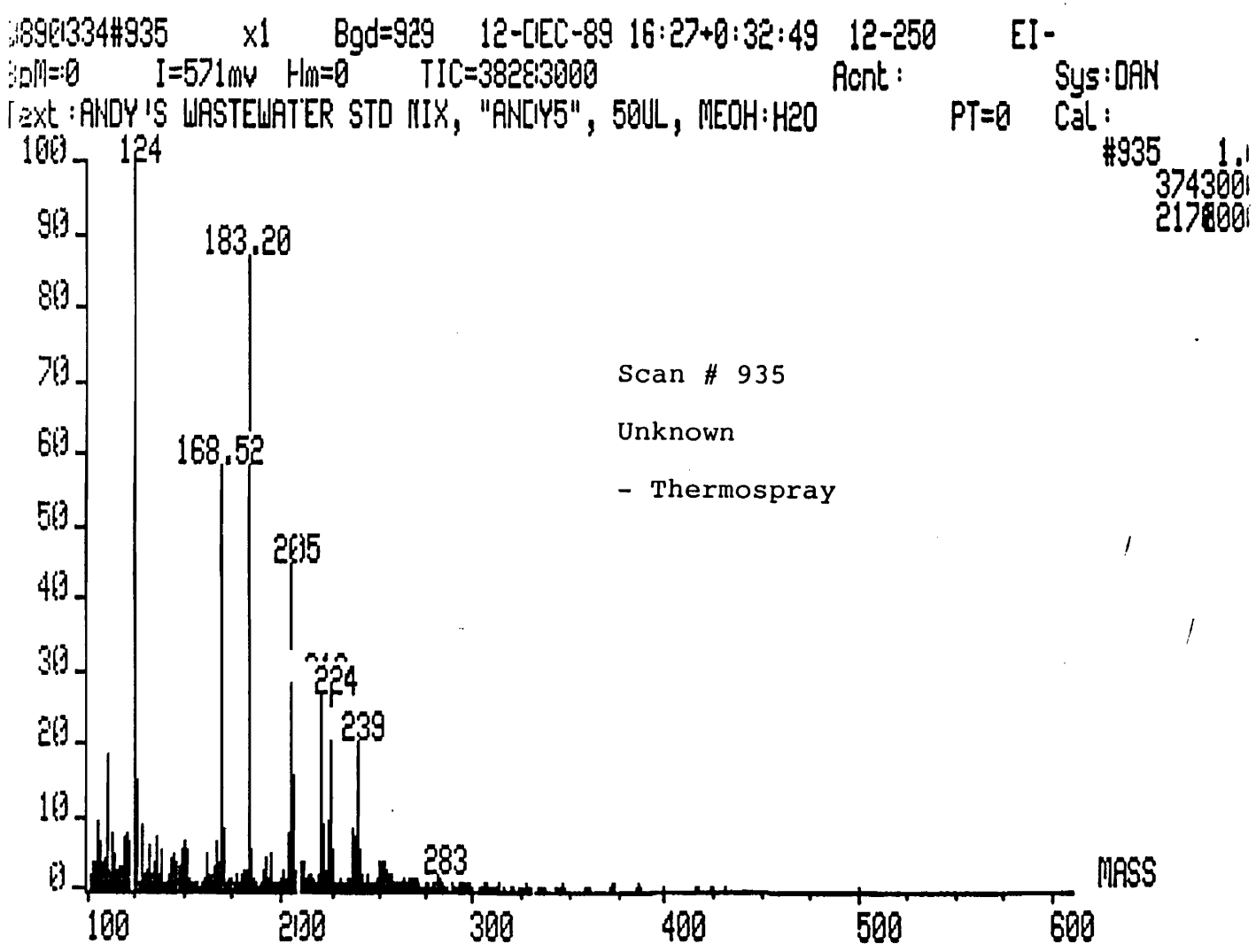


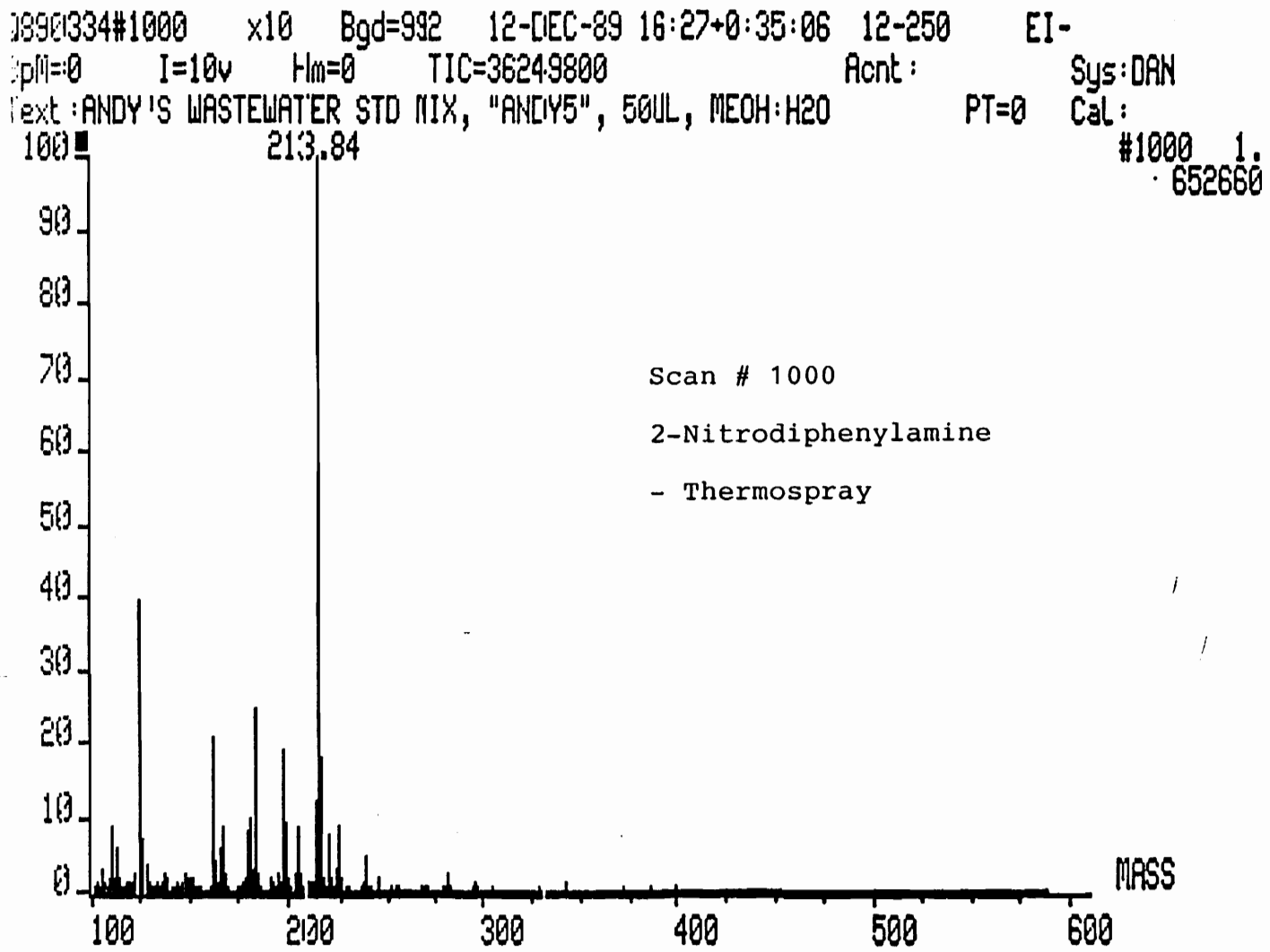

SEPARATION OF STANDARDS
Column: 50 cm 10μ I.D. OV-17-V
Mobile Phase: 10:90 Acetonitrile:Water
Positive Ion Chemical Ionization, Selected Ion Monitoring
1 second injection, ca 2 pg/component
P.2
100.0
M/Z 109
106
m-Cresol
454656.
109.033
± 0.500
39.5
M/Z 136
130
Benzothiazole
179456.
136.041
± 0.500
22.4
M/Z 200
108
Benzenesulfonamide-N-ethyl-4-methyl
102016.
200.060
± 0.500
20.9
M/Z 216
115
Atrazine
94848.
216.065
± 0.500
124.8
106
Total Ion Current
567296.
OCT 09 '89 15:29 NIDHS LMB
Injection
100 1:00
110 1:06
120 1:12
130 1:18
140 1:24
150 1:30
160 1:36
170 1:42
SCAN TIME

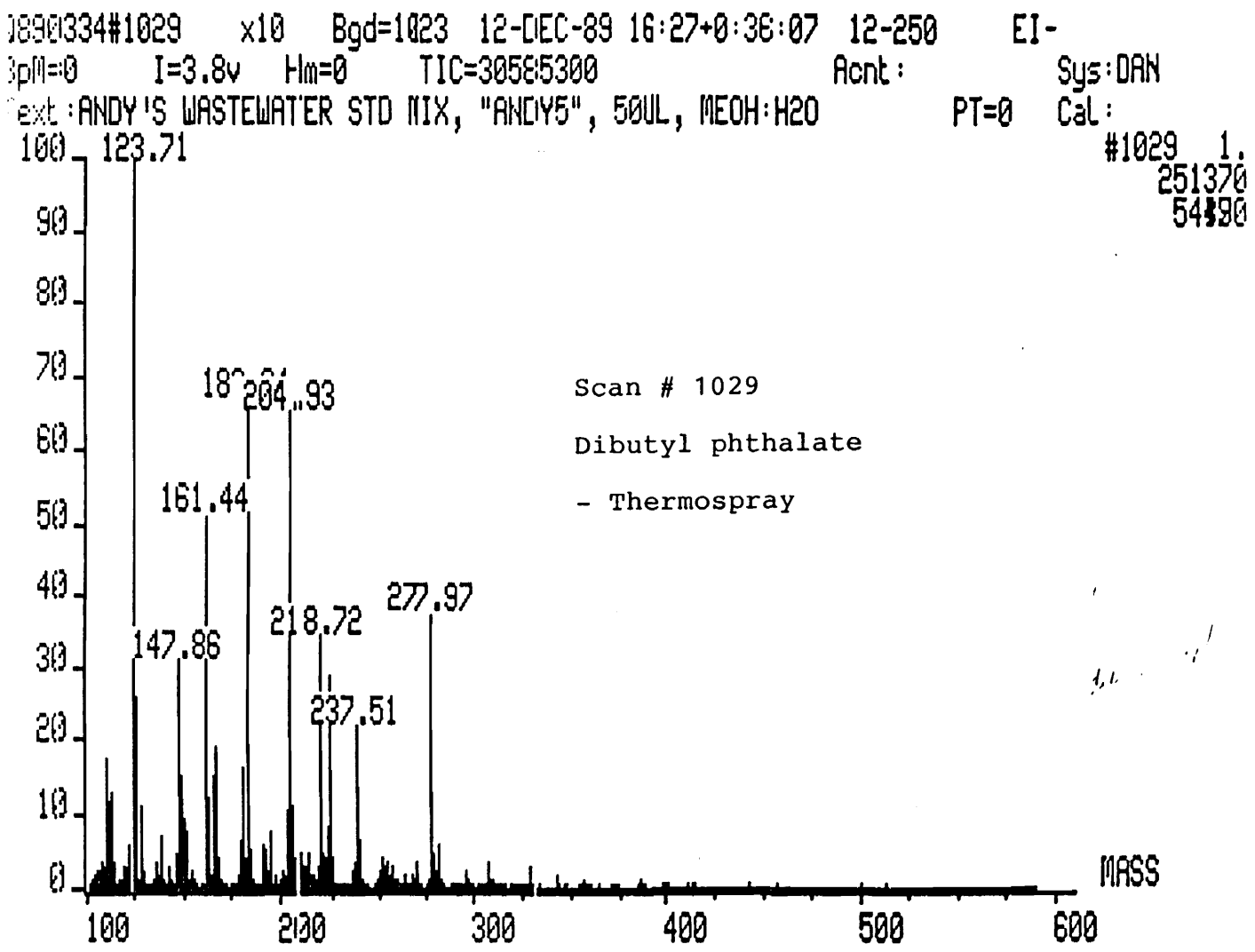
Appendix D. Thermospray LC/MS Spectra of Wastewater Standards (Table 13)


:90340#910 x1 Bgd=891 22-DEC-89 12:59+0:31:58 12-250 EI+
M=0 I=824mv Hm=0 TIC=10617000 Acnt: Sys:DAN
xt:ANDY'S DIPHENYLAMINE, "ANDY5", MEOH:H20, TSP+ PT=0 Cal:
#910 1.
540000
.00
170
90
80
70
60
50
40
30
20
10
125
0
MASS
100
200
300
400
500
600
Scan # 910
Diphenylamine Standard
+ Thermospray

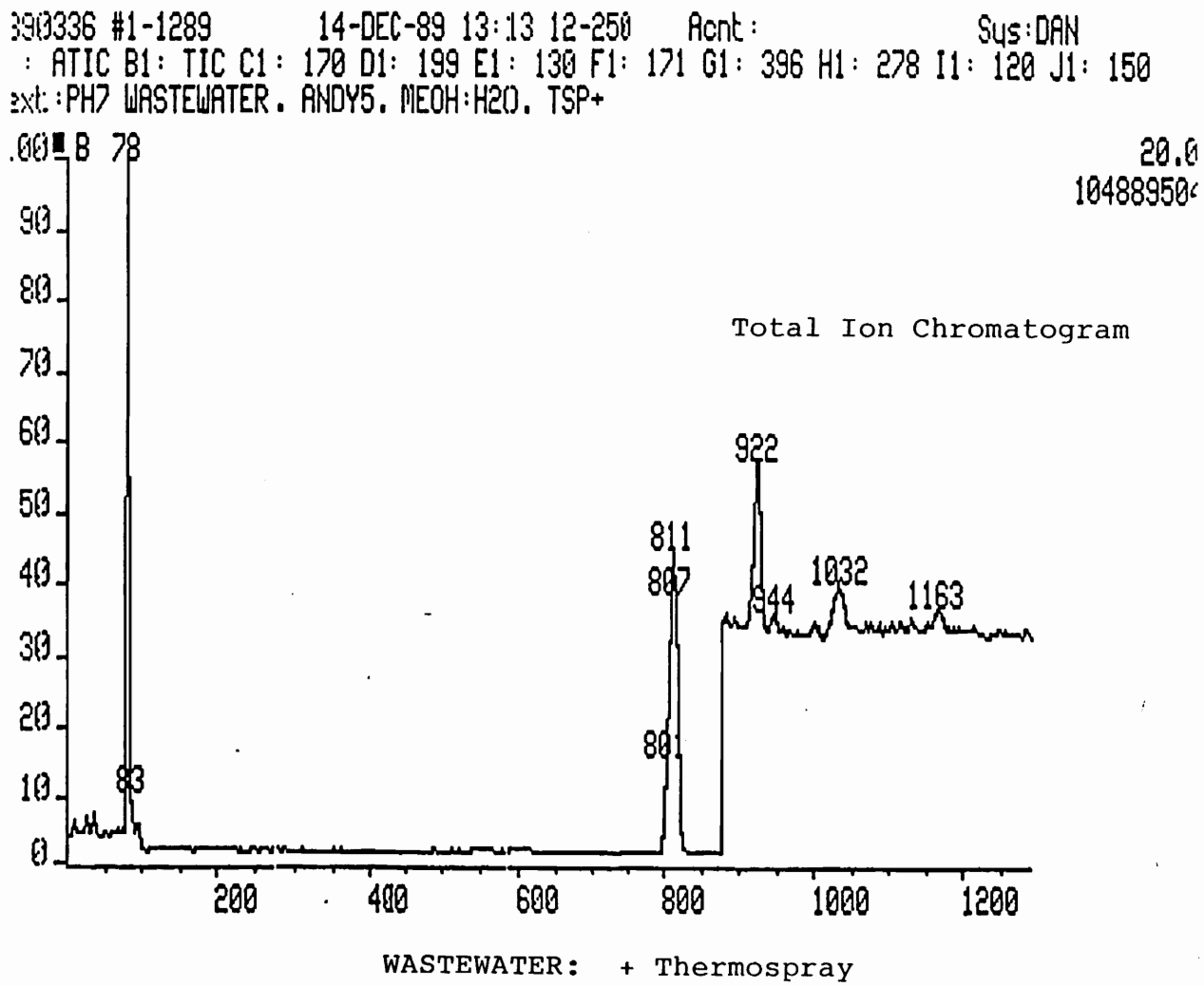

0890334#837 x1 Bgd=832 12-DEC-89 16:27+0:29:23 12-250 EI-
pM=0 I=3.4v Hm=0 TIC=99706000 Acnt: Sys:DAN
ext:ANDY'S WASTEWATER STD MIX, "ANDY5", 50UL, MEOH:H2O PT=0 Cal:
#837 1.
2213100
357500
182.14
165
153
Scan # 837
2,4-Dinitrotoluene
- Thermospray
MASS
100
90
80
70
60
50
40
30
20
10
0
100
200
300
400
500
600

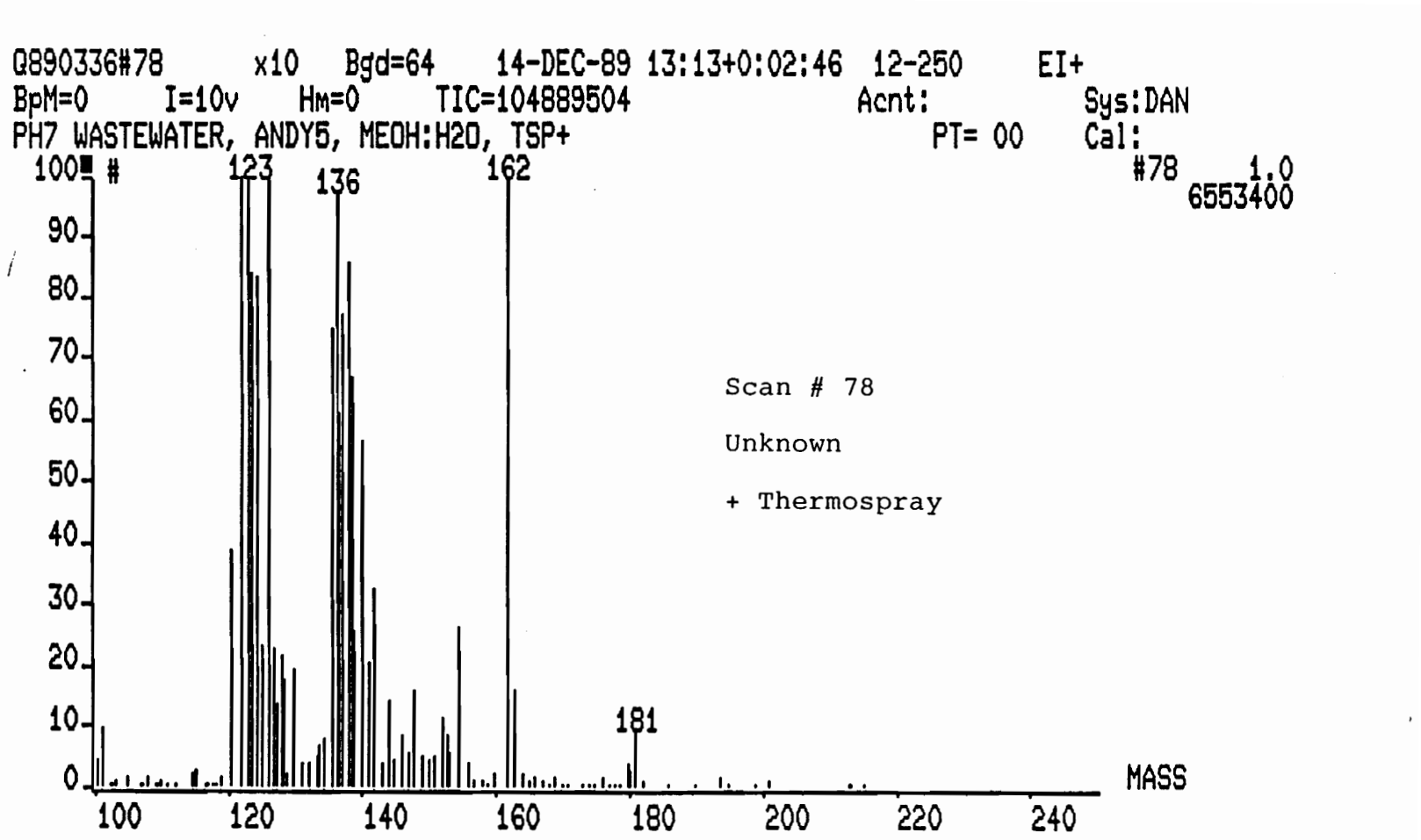

8890334#935 x1 Bgd=929 12-DEC-89 16:27+0:32:49 12-250 EI-
BPM=0 I=571mv Hm=0 TIC=3828000 Acnt: Sys:DAN
Text:ANDY'S WASTEWATER STD MIX, "ANDY5", 50UL, MEOH:H2O PT=0 Cal:
#935
Scan # 935
Unknown
- Thermospray
124
183.20
168.52
205
224
239
283
MASS
100
90
80
70
60
50
40
30
20
10
0
100
200
300
400
500
600

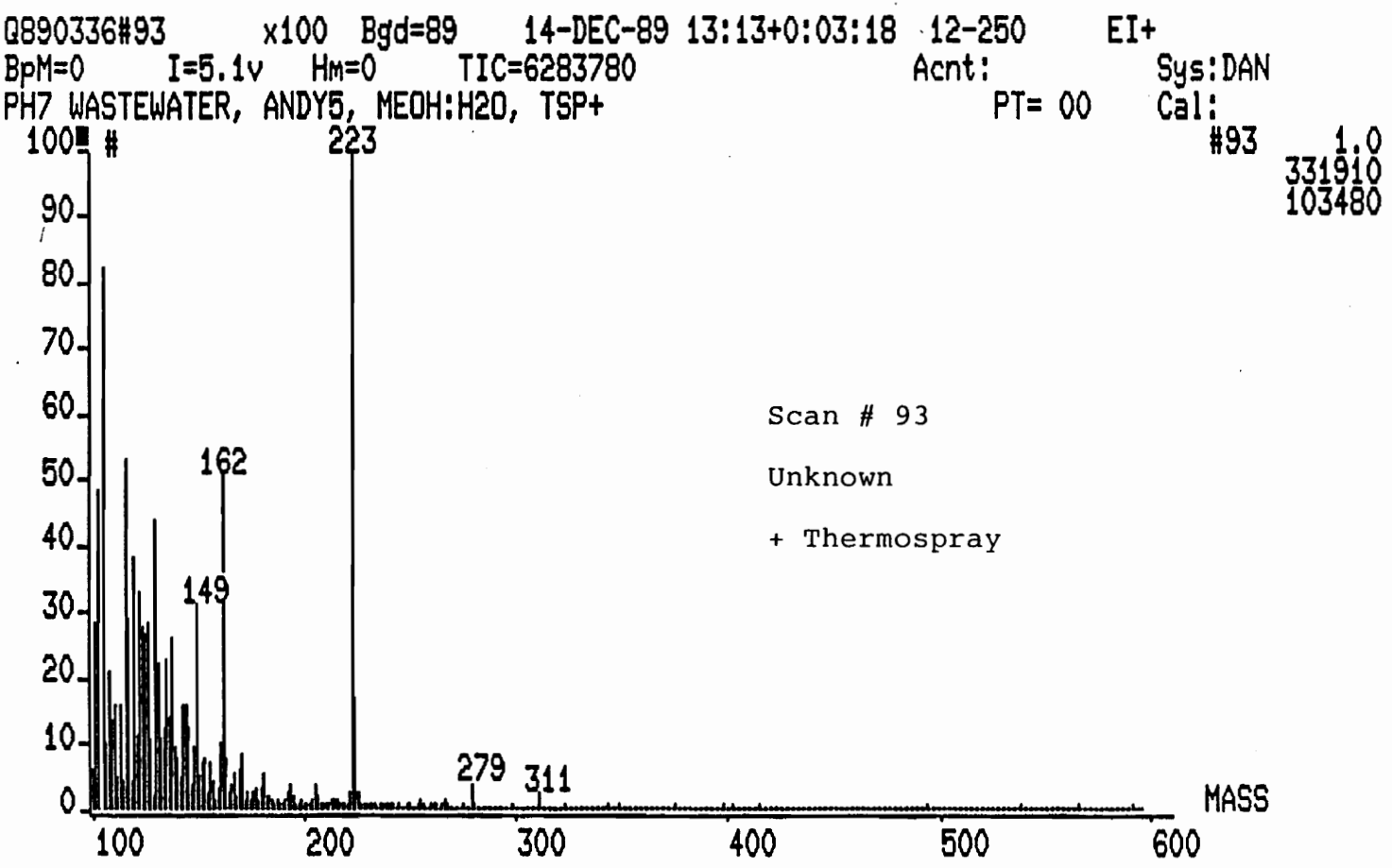

0890334#1000 x10 Bgd=992 12-DEC-89 16:27+0:35:06 12-250 EI-
pM=0 I=10v Hm=0 TIC=36249800 Acnt: Sys:DAN
Text:ANDY'S WASTEWATER STD MIX, "ANDY5", 50UL, MEOH:H2O PT=0 Cal:
#1000 1.
652660
213.84
Scan # 1000
2-Nitrodiphenylamine
- Thermospray
MASS
100
90
80
70
60
50
40
30
20
10
0
100
200
300
400
500
600

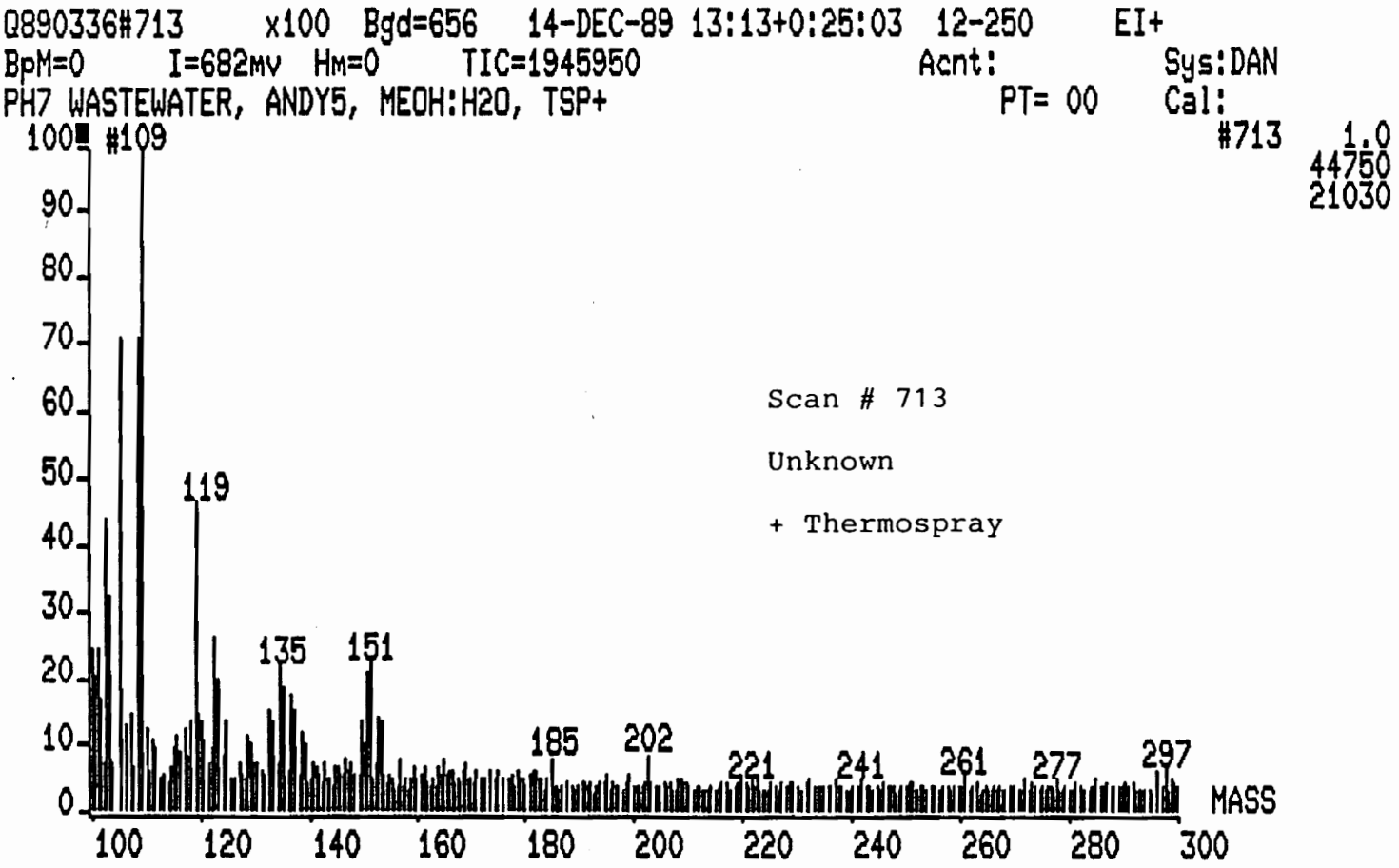

)890334#1029 x10 Bgd=1023 12-DEC-89 16:27+0:38:07 12-250 EI-
3pM=0 I=3.8v Hm=0 TIC=3058:5300 Acnt: Sys:DAN
ext:ANDY'S WASTEWATER STD MIX, "ANDY5", 50UL, MEOH:H2O PT=0 Cal:
#1029 1.
251370
54590
123.71
204.93
161.44
147.86
218.72
277.97
237.51
Scan # 1029
Dibutyl phthalate
- Thermospray
MASS
100
90
80
70
60
50
40
30
20
10
0
100
200
300
400
500
600

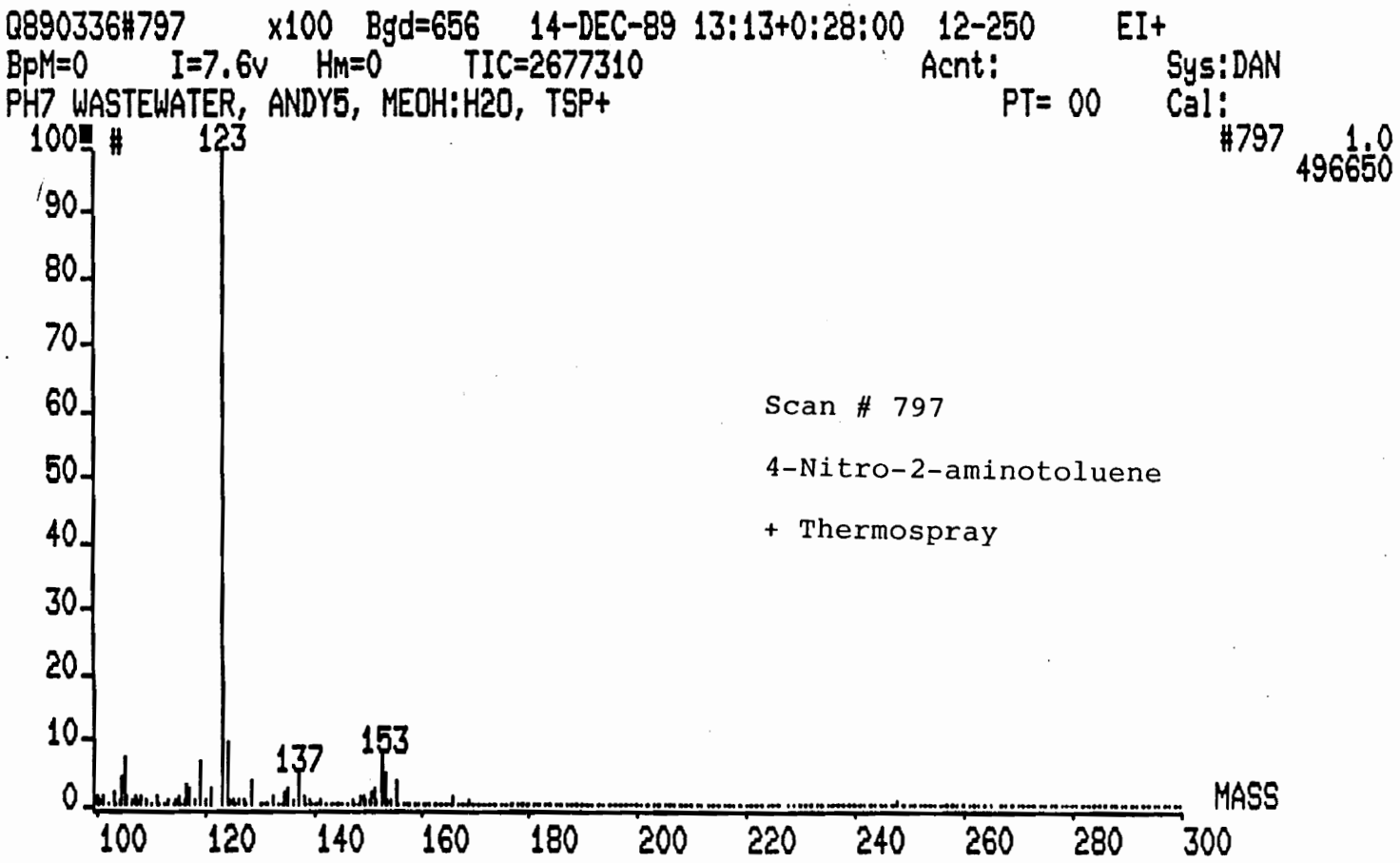
Appendix E. Thermospray LC/MS Spectra of Solid Phase Extracted Wastewater Samples (Table 14)

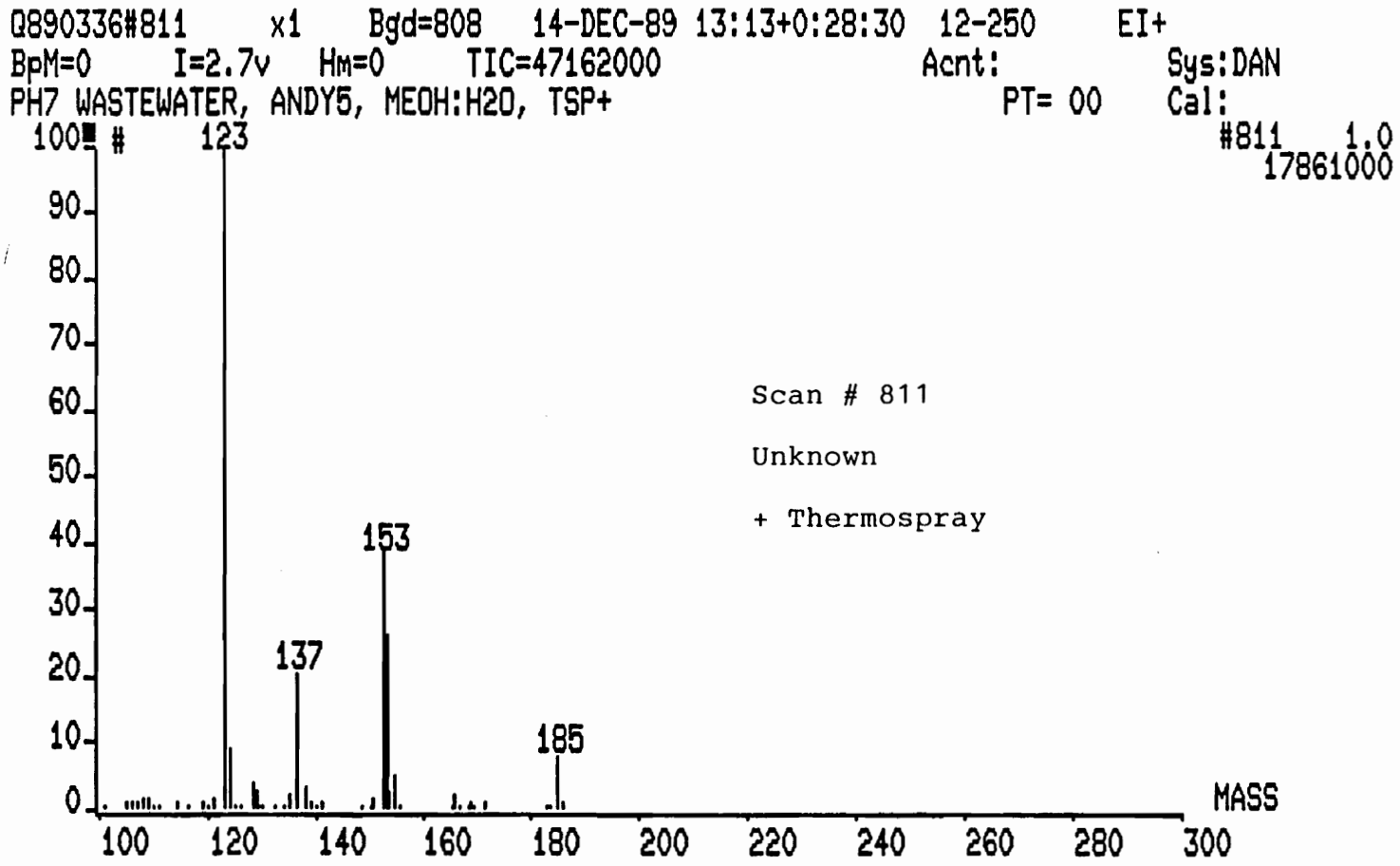

390336 #1-1289 14-DEC-89 13:13 12-250 Acnt: Sys:DAN
: ATIC B1: TIC C1: 170 D1: 199 E1: 130 F1: 171 G1: 396 H1: 278 I1: 120 J1: 150
ext:PH7 WASTEWATER. ANDY5. MEOH:H2O. TSP+
.00 B 78
20.0
10488950
90
80
70
60
50
40
30
20
10
0
Total Ion Chromatogram
922
811
807
1032
944
1163
801
83
200
400
600
800
1000
1200
WASTEWATER: + Thermospray

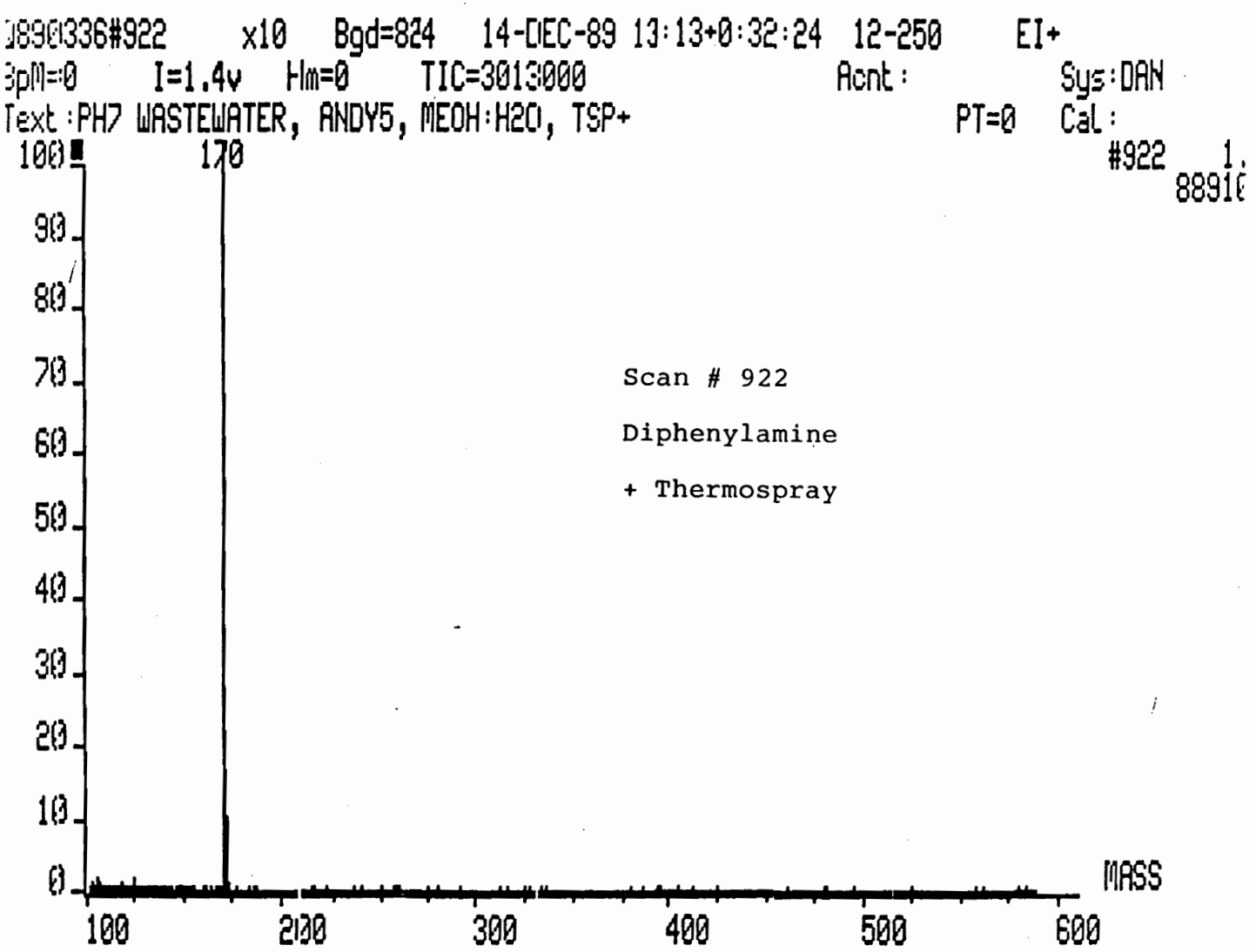

Q890336#78 x10 Bgd=64 14-DEC-89 13:13+0:02:46 12-250 EI+
BpM=0 I=10v Hm=0 TIC=104889504 Acnt: Sys:DAN
PH7 WASTEWATER, ANDY5, MEOH:H2O, TSP+ PT= 00 Cal:
#78 1.0
6553400
100 #
90
80
70
60
50
40
30
20
10
0
123
136
162
181
MASS
100
120
140
160
180
200
220
240
Scan # 78
Unknown
+ Thermospray

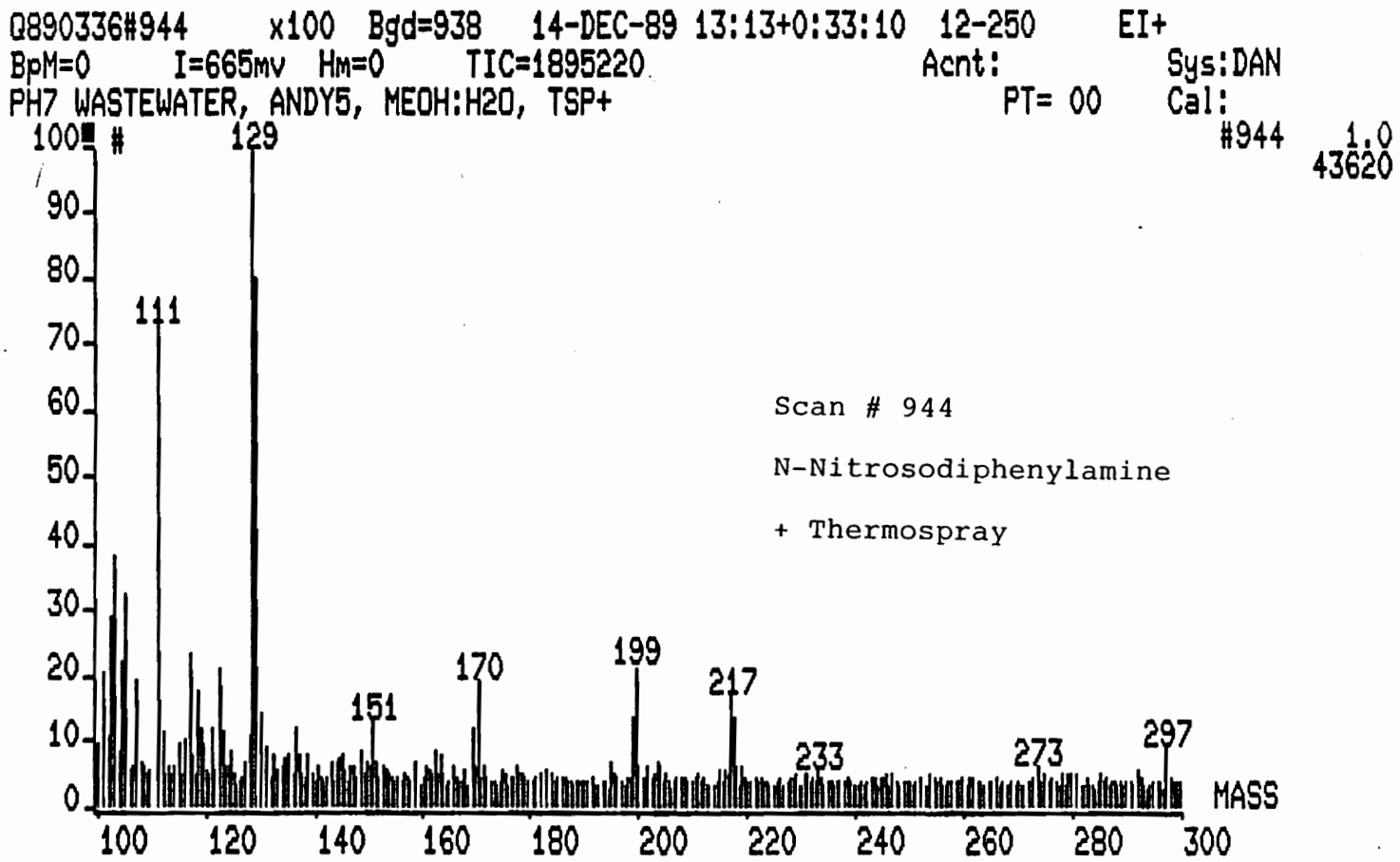

Q890336#93 x100 Bgd=89 14-DEC-89 13:13+0:03:18 12-250 EI+
BpM=0 I=5.1v Hm=0 TIC=6283780 Acnt: Sys:DAN
PH7 WASTEWATER, ANDY5, MEOH:H2O, TSP+ PT= 00 Cal:
#93 1.0
331910
103480
100
90
80
70
60
50
40
30
20
10
0
223
162
149
279
311
MASS
100
200
300
400
500
600
Scan # 93
Unknown
+ Thermospray

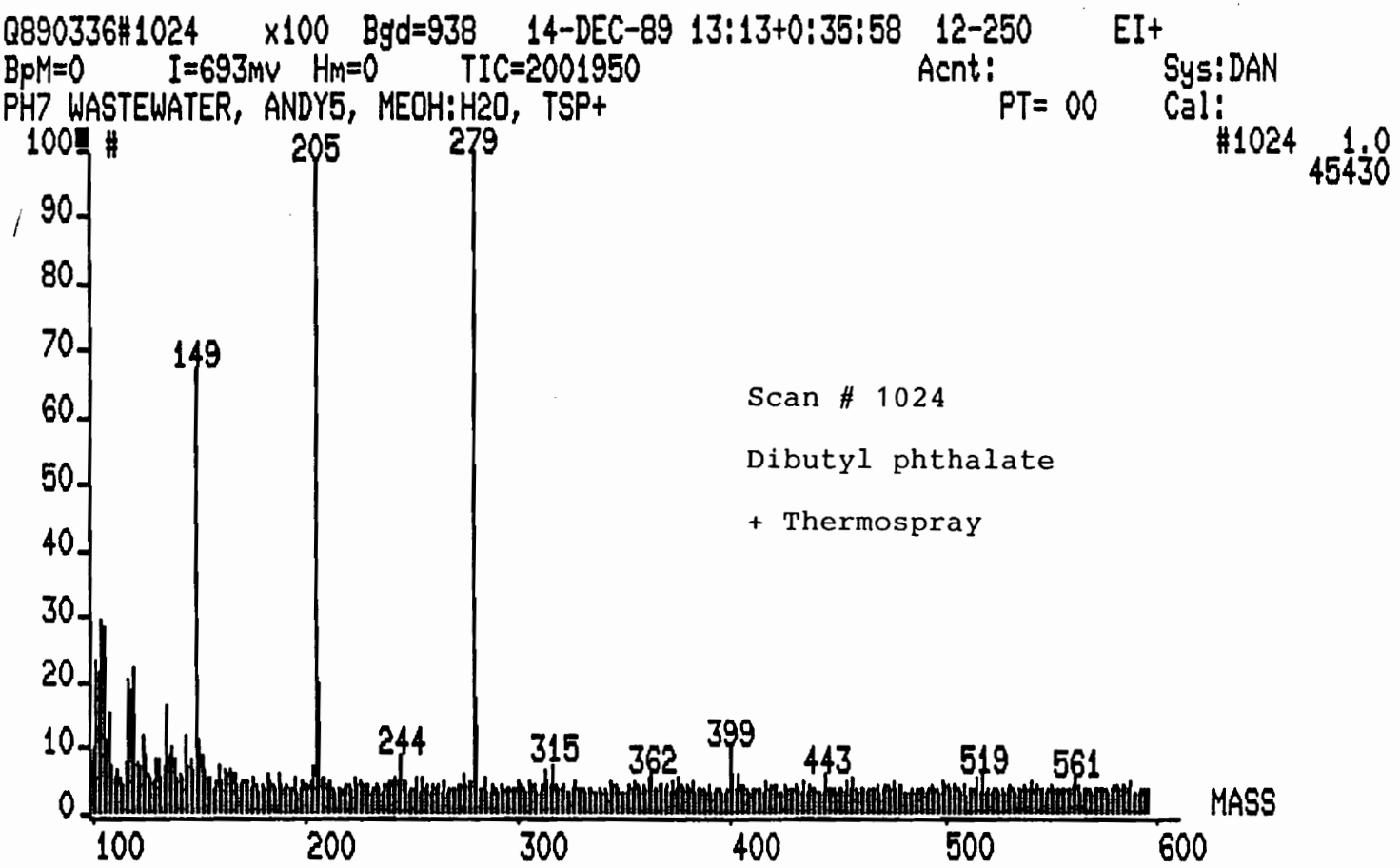

Q890336#713 x100 Bgd=656 14-DEC-89 13:13+0:25:03 12-250 EI+
BpM=0 I=682mv Hm=0 TIC=1945950 Acnt: Sys:DAN
PH7 WASTEWATER, ANDY5, MEOH:H2O, TSP+ PT= 00 Cal:
#713 1.0
44750
21030
#109
119
135
151
185
202
221
241
261
277
297
MASS
Scan # 713
Unknown
+ Thermospray

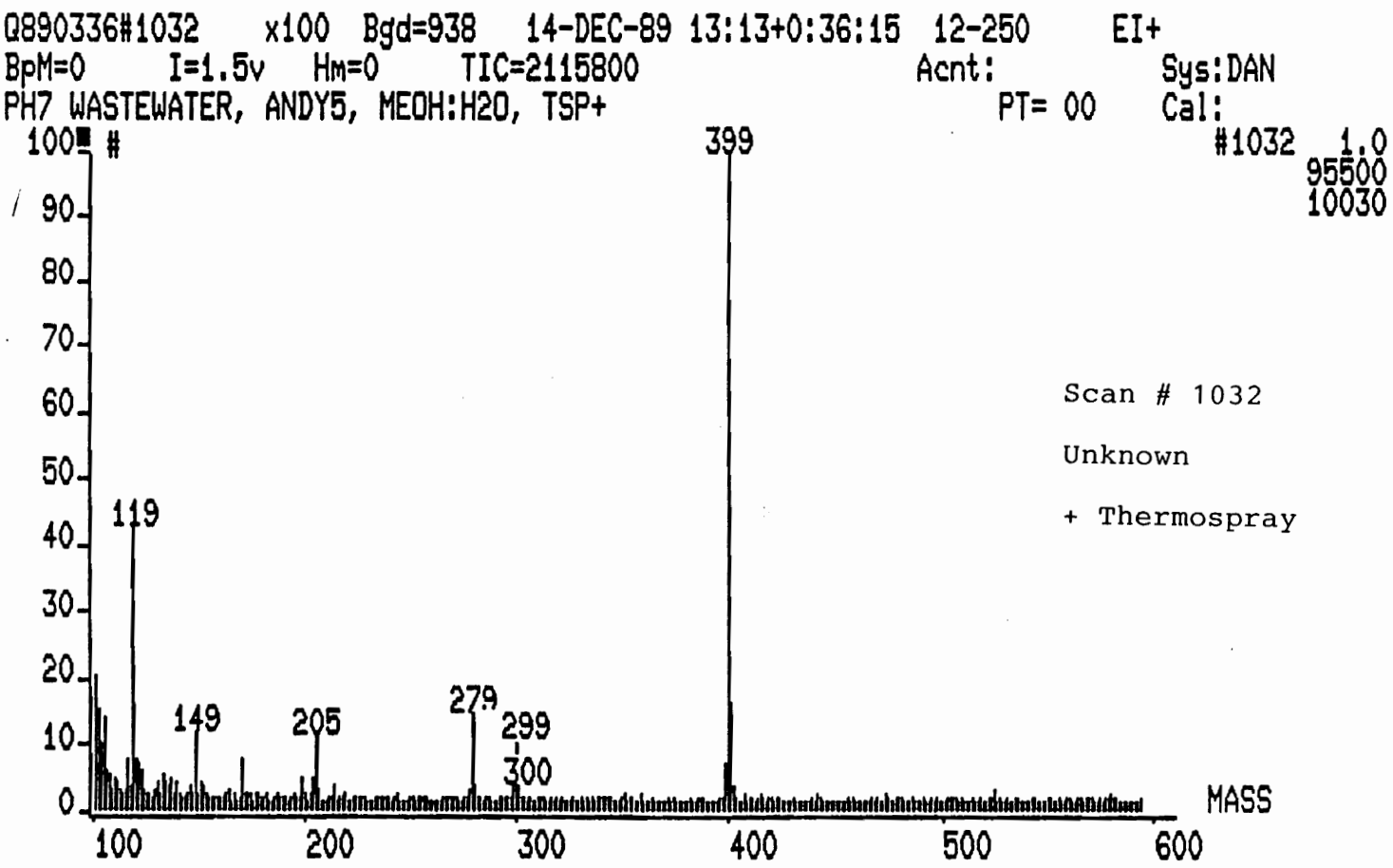

Q890336#797 x100 Bgd=656 14-DEC-89 13:13+0:28:00 12-250 EI+
BpM=0 I=7.6v Hm=0 TIC=2677310 Acnt: Sys:DAN
PH7 WASTEWATER, ANDY5, MEOH:H2O, TSP+ PT= 00 Cal:
#797 1.0
496650
100 #
123
90
80
70
60
50
40
30
20
10
0
137
153
MASS
100 120 140 160 180 200 220 240 260 280 300
Scan # 797
4-Nitro-2-aminotoluene
+ Thermospray

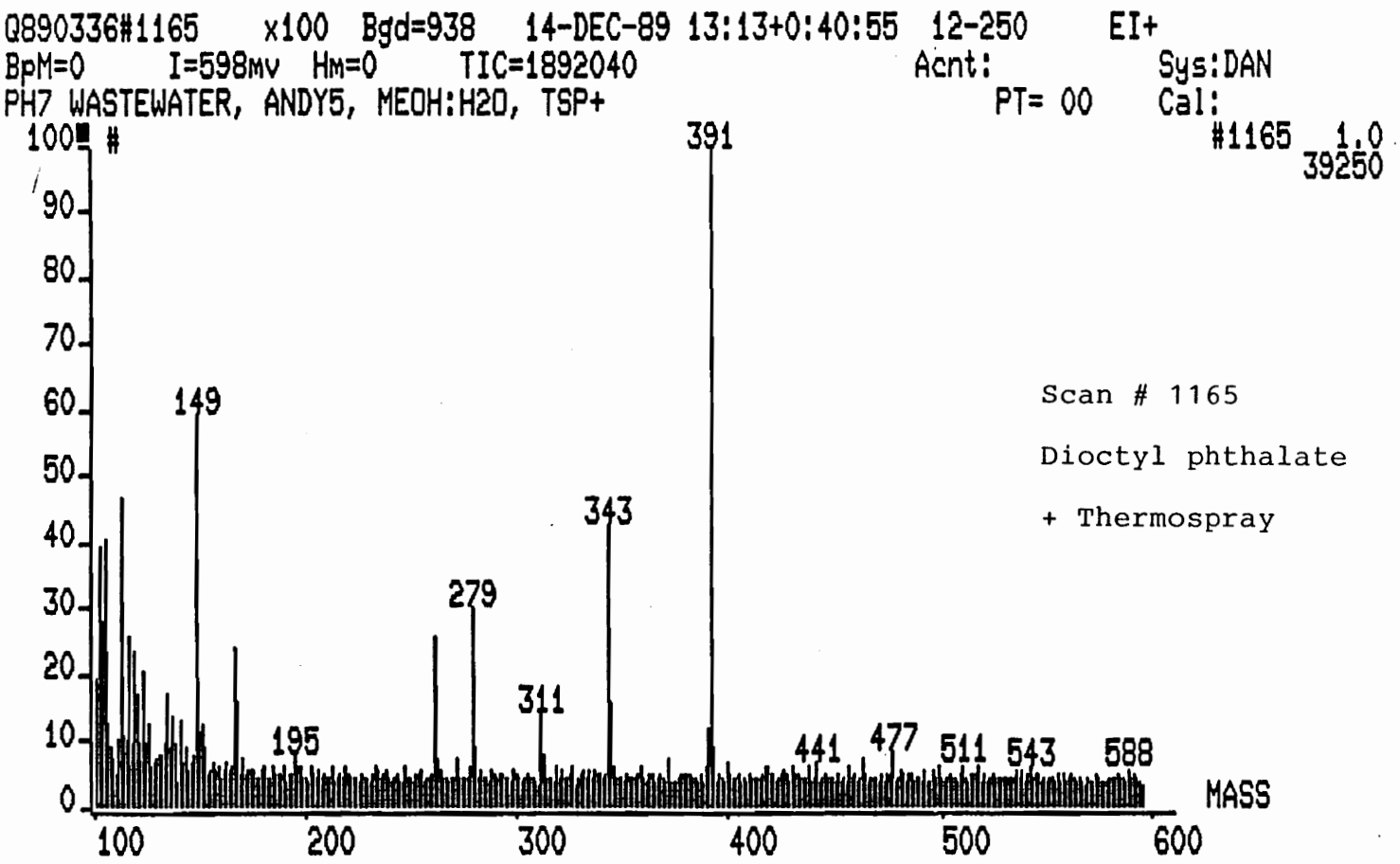

Q890336#811 x1 Bgd=808 14-DEC-89 13:13+0:28:30 12-250 EI+
BpM=0 I=2.7v Hm=0 TIC=47162000 Acnt: Sys:DAN
PH7 WASTEWATER, ANDY5, MEOH:H2O, TSP+ PT= 00 Cal:
#811 1.0
17861000
100 # 123
90
80
70
60
50
40
30
20
10
0
153
137
185
MASS
100 120 140 160 180 200 220 240 260 280 300
Scan # 811
Unknown
+ Thermospray

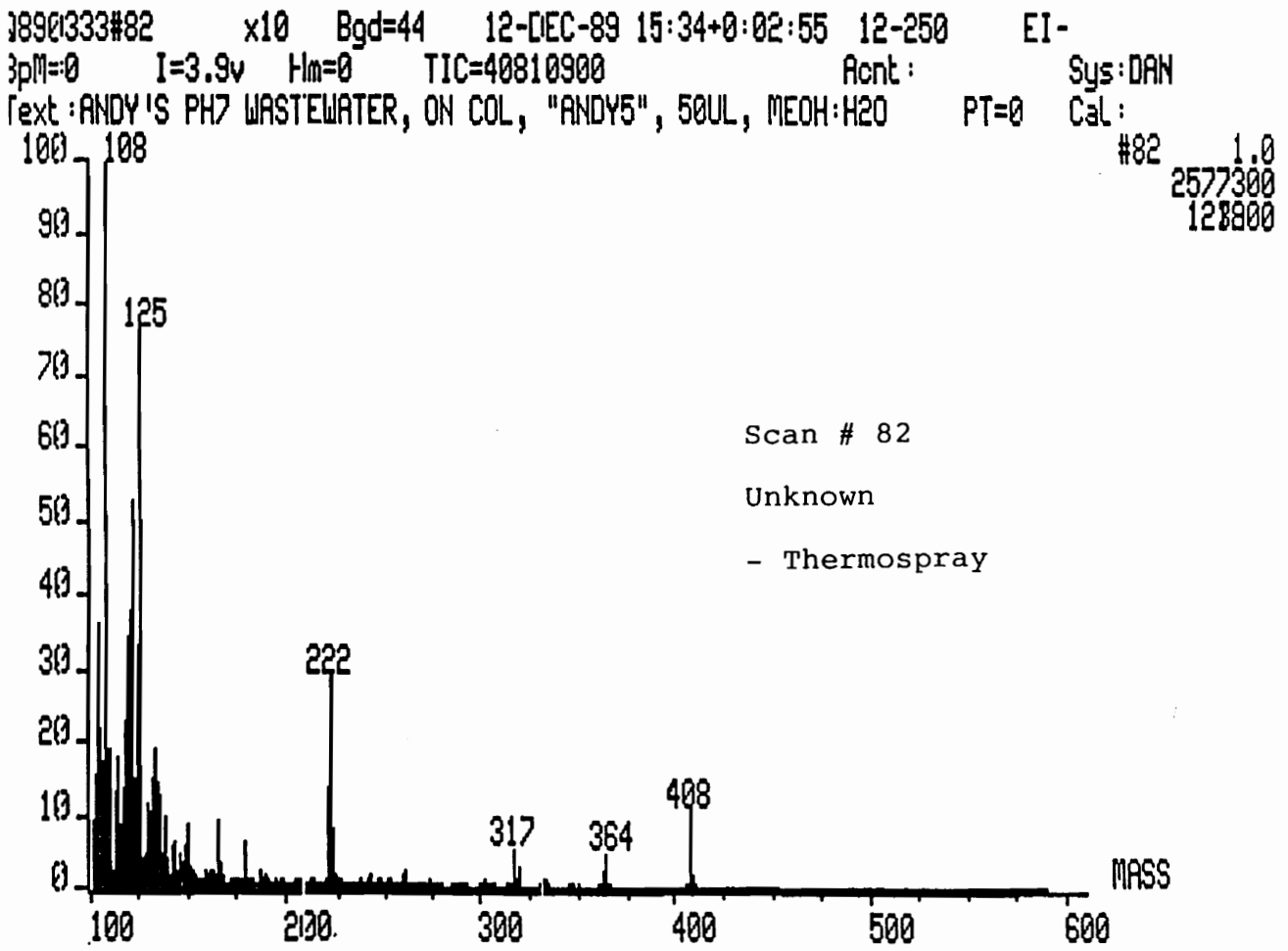

8890336#922 x10 Bgd=824 14-DEC-89 13:13+0:32:24 12-250 EI+
BpM=0 I=1.4v Hm=0 TIC=3013000 Acnt: Sys:DAN
Text:PH7 WASTEWATER, ANDY5, MEOH:H2O, TSP+ PT=0 Cal:
#922 1.
88910
170
Scan # 922
Diphenylamine
+ Thermospray
MASS
100
90
80
70
60
50
40
30
20
10
0
100
200
300
400
500
600

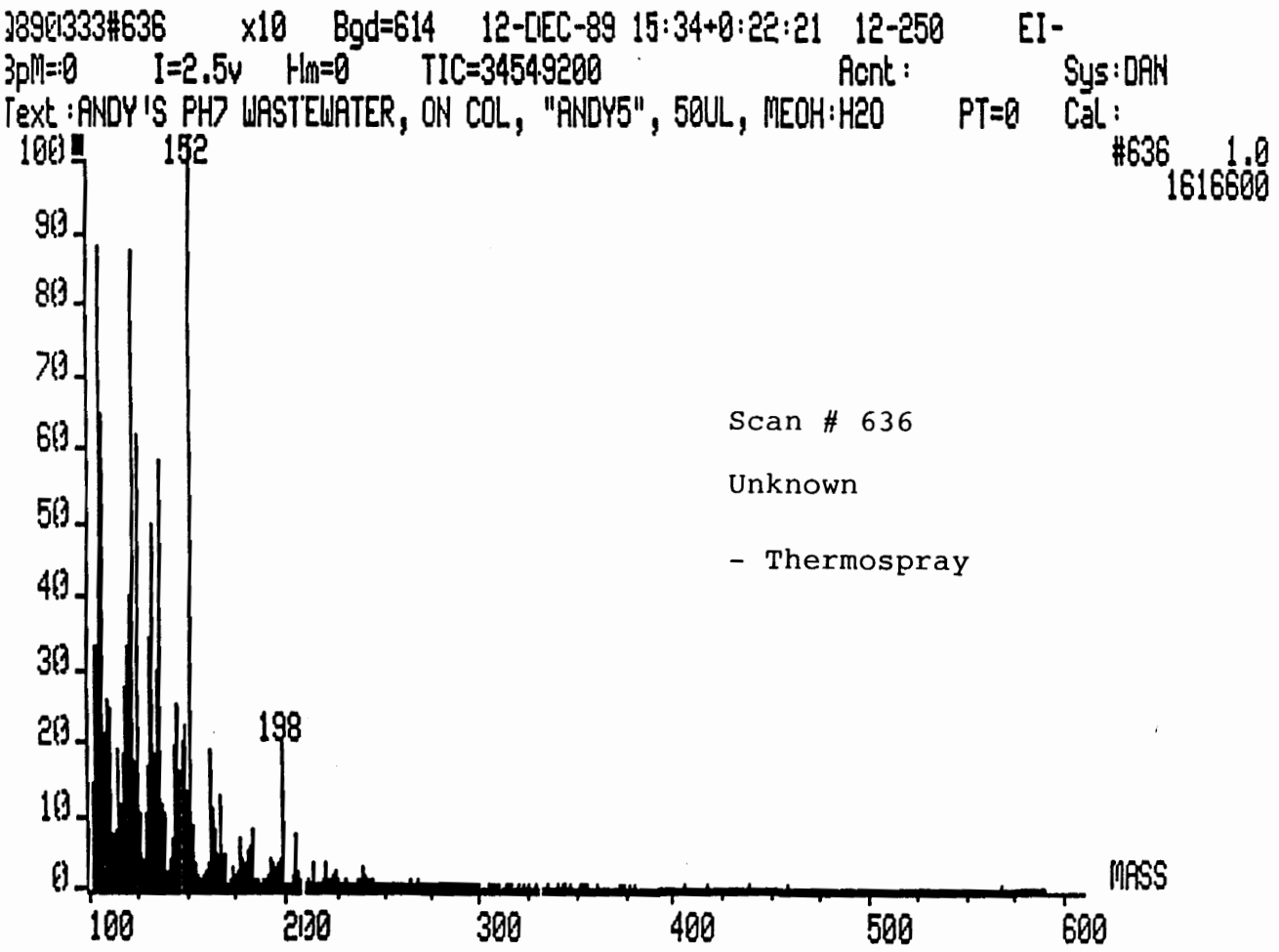

Q890336#944 x100 Bgd=938 14-DEC-89 13:13+0:33:10 12-250 EI+
BpM=0 I=665mv Hm=0 TIC=1895220 Acnt: Sys:DAN
PH7 WASTEWATER, ANDY5, MEOH:H2O, TSP+ PT= 00 Cal:
#944 1.0
43620
Scan # 944
N-Nitrosodiphenylamine
+ Thermospray
MASS

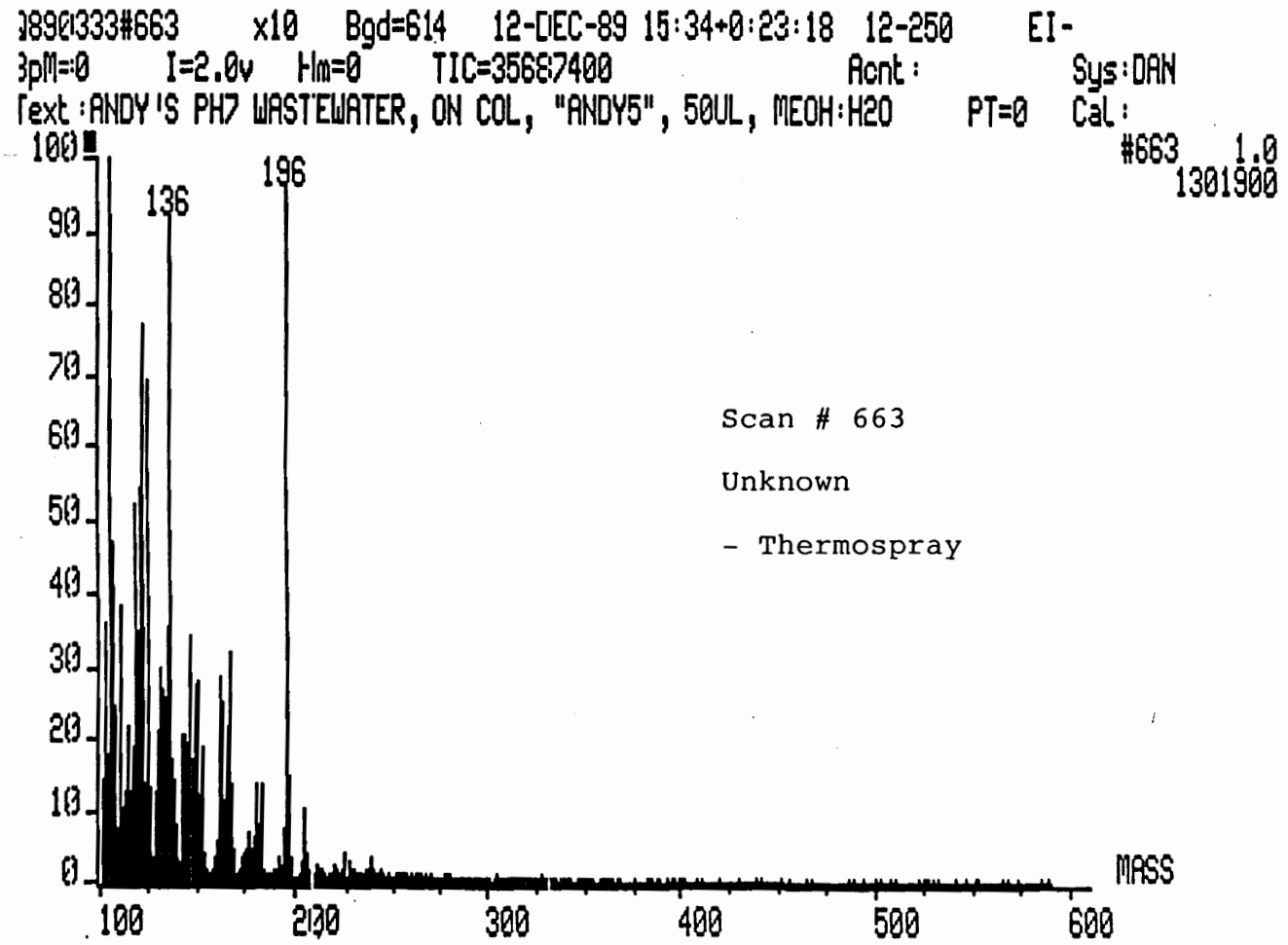

Q890336#1024 x100 Bgd=938 14-DEC-89 13:13+0:35:58 12-250 EI+
BpM=0 I=693mv Hm=0 TIC=2001950 Acnt: Sys:DAN
PH7 WASTEWATER, ANDY5, MEOH:H2O, TSP+ PT= 00 Cal:
#1024 1.0
45430
100 #
205
279
149
244
315
362
399
443
519
561
Scan # 1024
Dibutyl phthalate
+ Thermospray
MASS
100
200
300
400
500
600

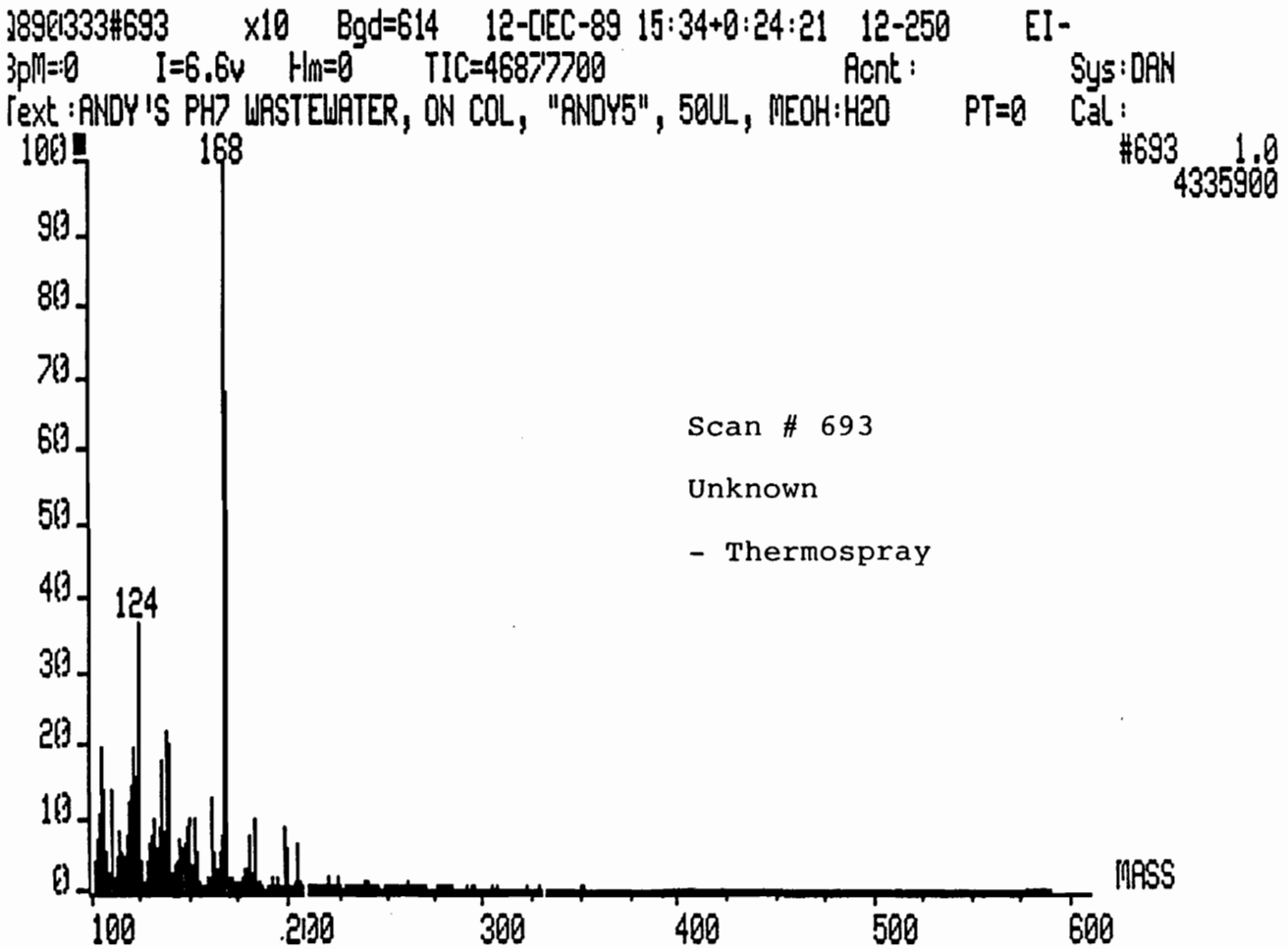

Q890336#1032 x100 Bgd=938 14-DEC-89 13:13+0:36:15 12-250 EI+
BpM=0 I=1.5v Hm=0 TIC=2115800 Acnt: Sys:DAN
PH7 WASTEWATER, ANDY5, MEOH:H2O, TSP+ PT= 00 Cal:
#1032 1.0
95500
10030
100 #
90
80
70
60
50
40
30
20
10
0
399
119
149
205
279
299
300
MASS
100
200
300
400
500
600
Scan # 1032
Unknown
+ Thermospray

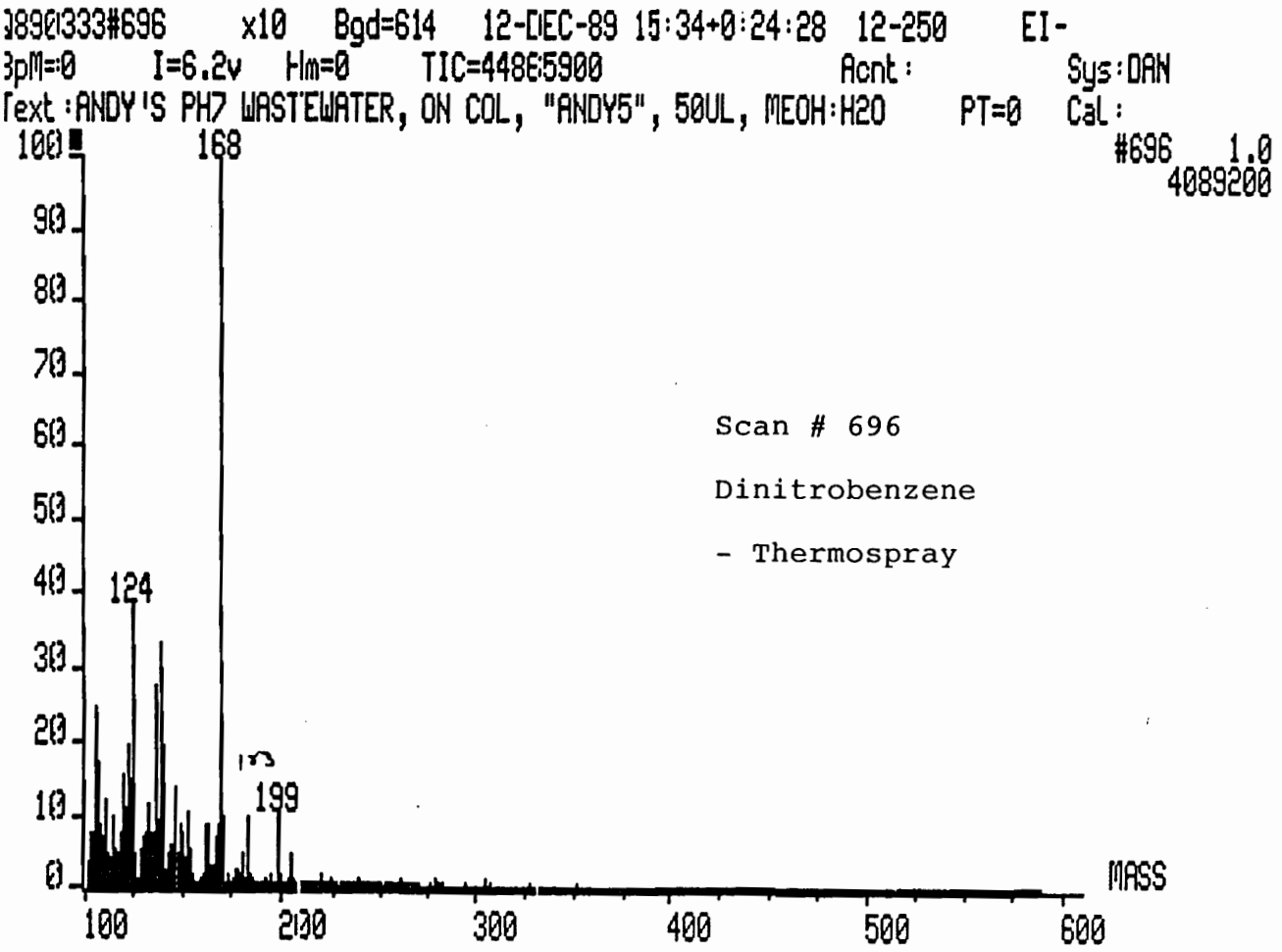

Q890336#1165 x100 Bgd=938 14-DEC-89 13:13+0:40:55 12-250 EI+
BpM=0 I=598mv Hm=0 TIC=1892040 Acnt: Sys:DAN
PH7 WASTEWATER, ANDY5, MEOH:H2O, TSP+ PT= 00 Cal:
#1165 1.0
39250
100 #
90
80
70
60
50
40
30
20
10
0
149
195
279
311
343
391
441
477
511
543
588
MASS
100
200
300
400
500
600
Scan # 1165
Dioctyl phthalate
+ Thermospray

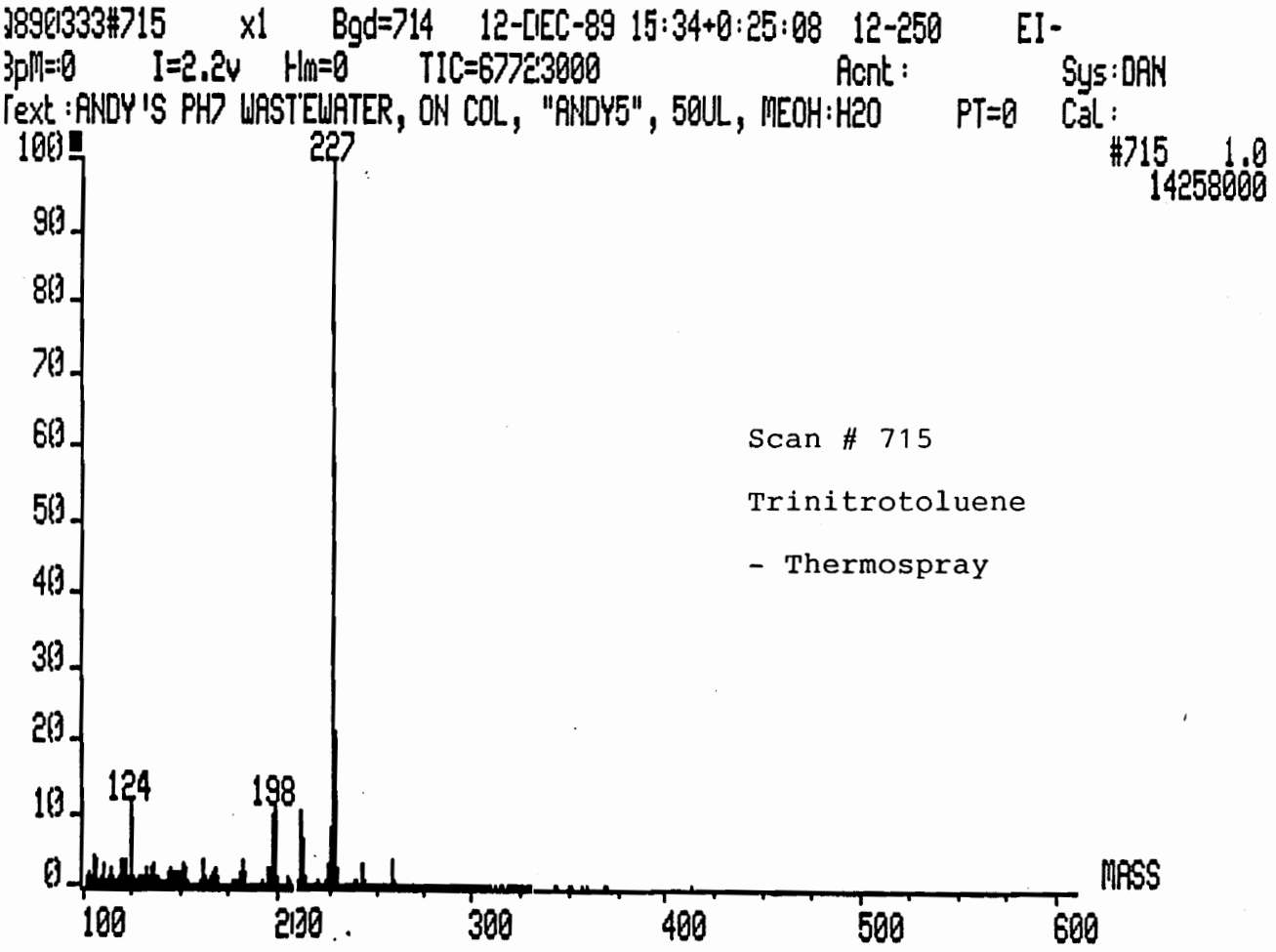

)890333#82 x10 Bgd=44 12-DEC-89 15:34+0:02:55 12-250 EI-
3pM=0 I=3.9v Hm=0 TIC=40810900 Acnt: Sys:DAN
Text:ANDY'S PH7 WASTEWATER, ON COL, "ANDY5", 50UL, MEOH:H2O PT=0 Cal:
#82 1.0
2577300
128800
108
125
222
317
364
408
Scan # 82
Unknown
- Thermospray
MASS
100
90
80
70
60
50
40
30
20
10
0
100
200
300
400
500
600

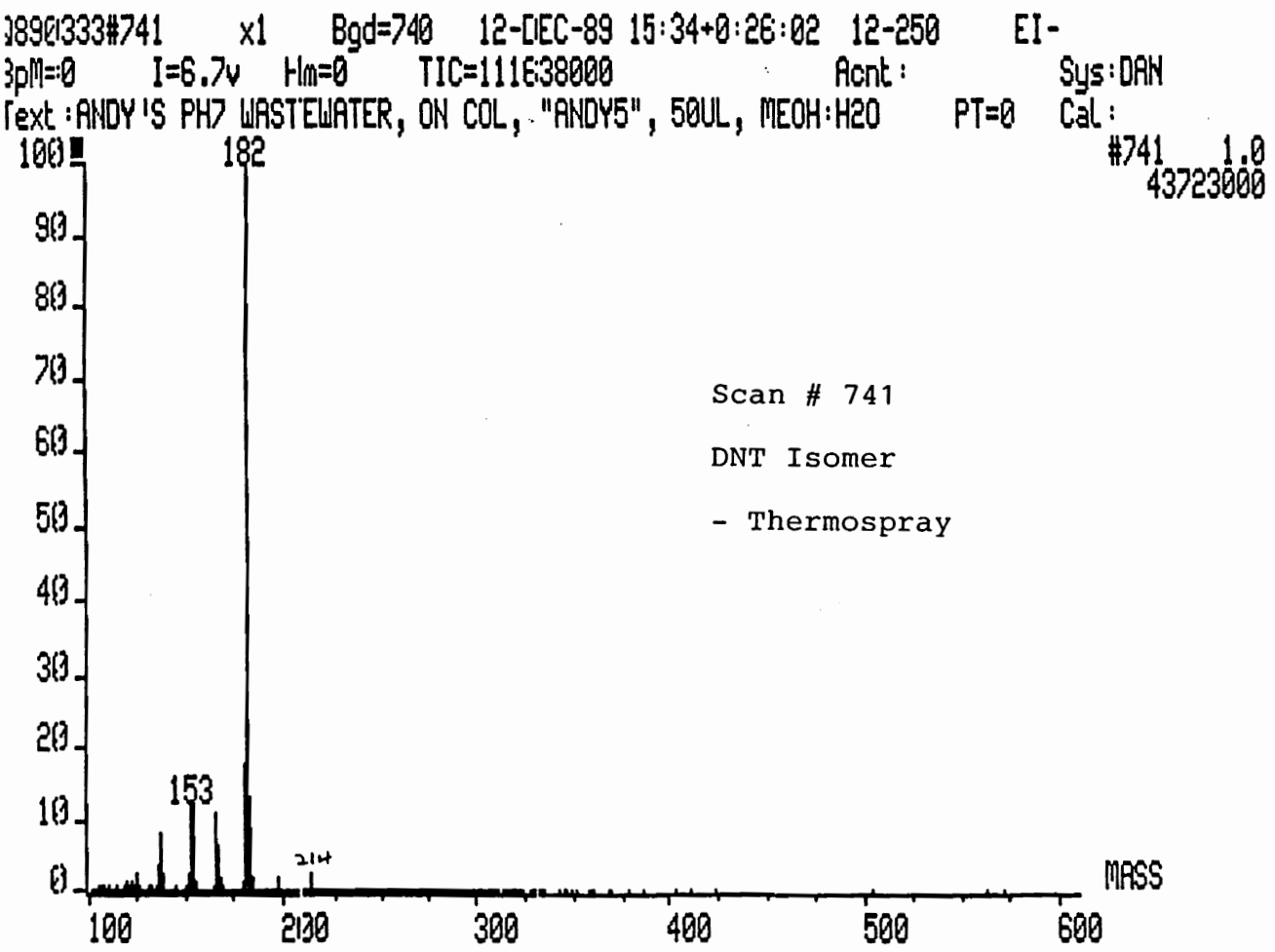

)890333#636 x10 Bgd=614 12-DEC-89 15:34+0:22:21 12-250 EI-
3pM=0 I=2.5v Hm=0 TIC=34549200 Acnt: Sys:DAN
Text:ANDY'S PH7 WASTEWATER, ON COL, "ANDY5", 50UL, MEOH:H2O PT=0 Cal:
#636 1.0
1616600
100
90
80
70
60
50
40
30
20
10
0
152
198
Scan # 636
Unknown
- Thermospray
MASS
100
200
300
400
500
600

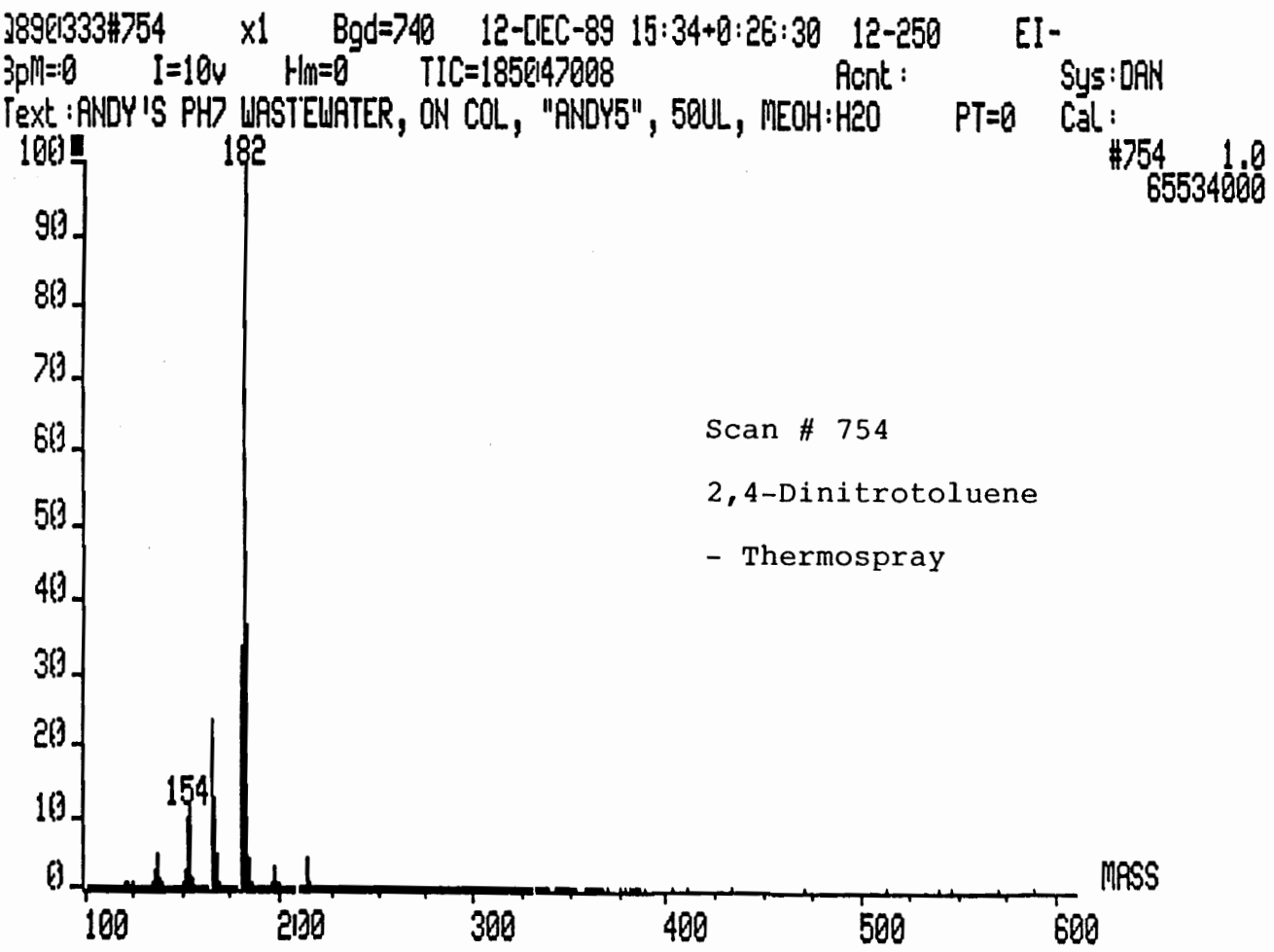

3890333#663 x10 Bgd=614 12-DEC-89 15:34+0:23:18 12-250 EI-
3pM=0 I=2.0v Hm=0 TIC=35687400 Acnt: Sys:DAN
Text:ANDY'S PH7 WASTEWATER, ON COL, "ANDY5", 50UL, MEOH:H2O PT=0 Cal:
#663 1.0
1301900
196
136
Scan # 663
Unknown
- Thermospray
MASS
100
90
80
70
60
50
40
30
20
10
0
100
200
300
400
500
600

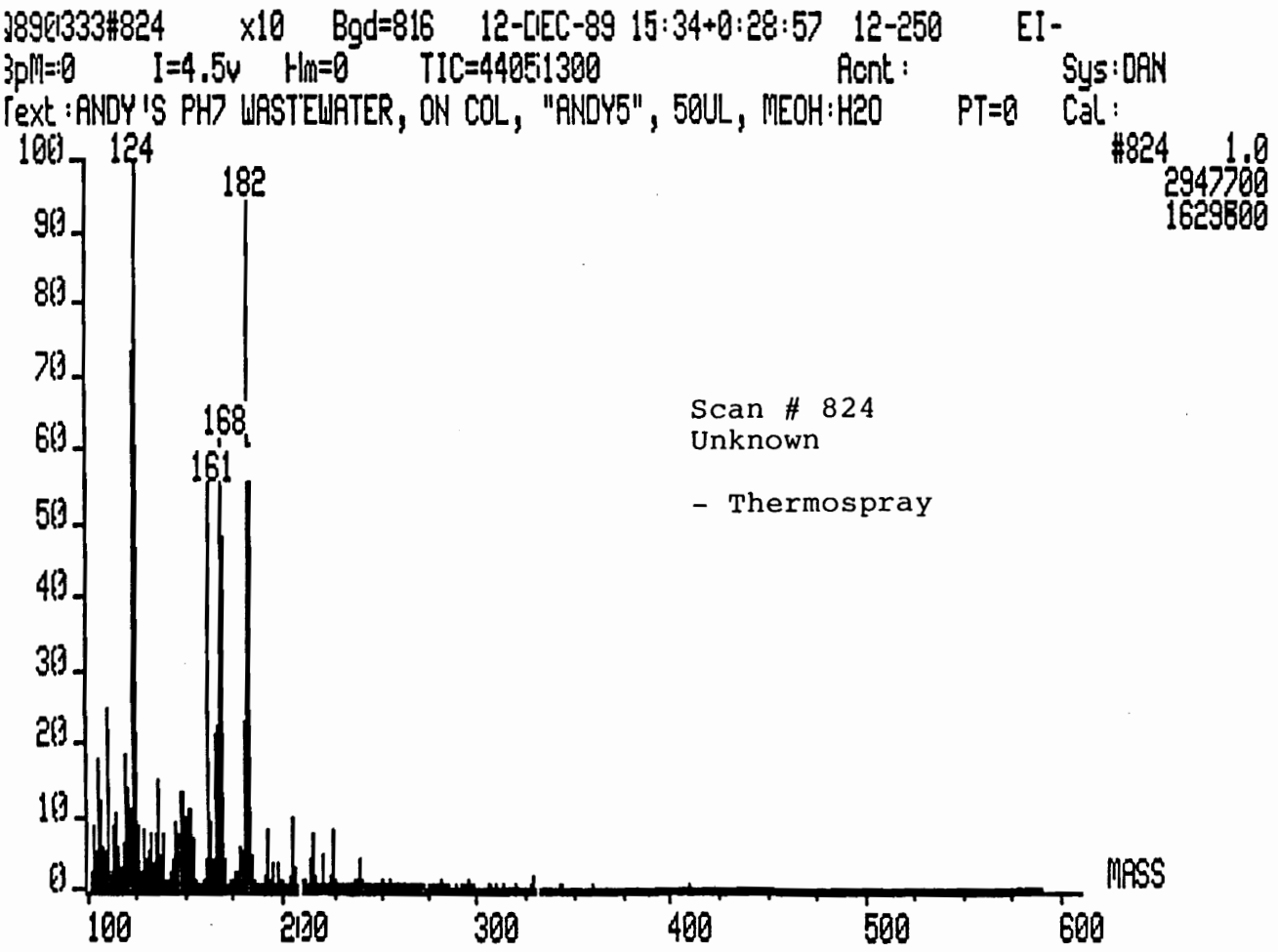

)890333#693 x10 Bgd=614 12-DEC-89 15:34+0:24:21 12-250 EI-
3pM=0 I=6.6v Hm=0 TIC=46877700 Acnt: Sys:DAN
Text:ANDY'S PH7 WASTEWATER, ON COL, "ANDY5", 50UL, MEOH:H2O PT=0 Cal:
#693 1.0
4335900
100
90
80
70
60
50
40
30
20
10
0
168
124
Scan # 693
Unknown
- Thermospray
MASS
100
200
300
400
500
600

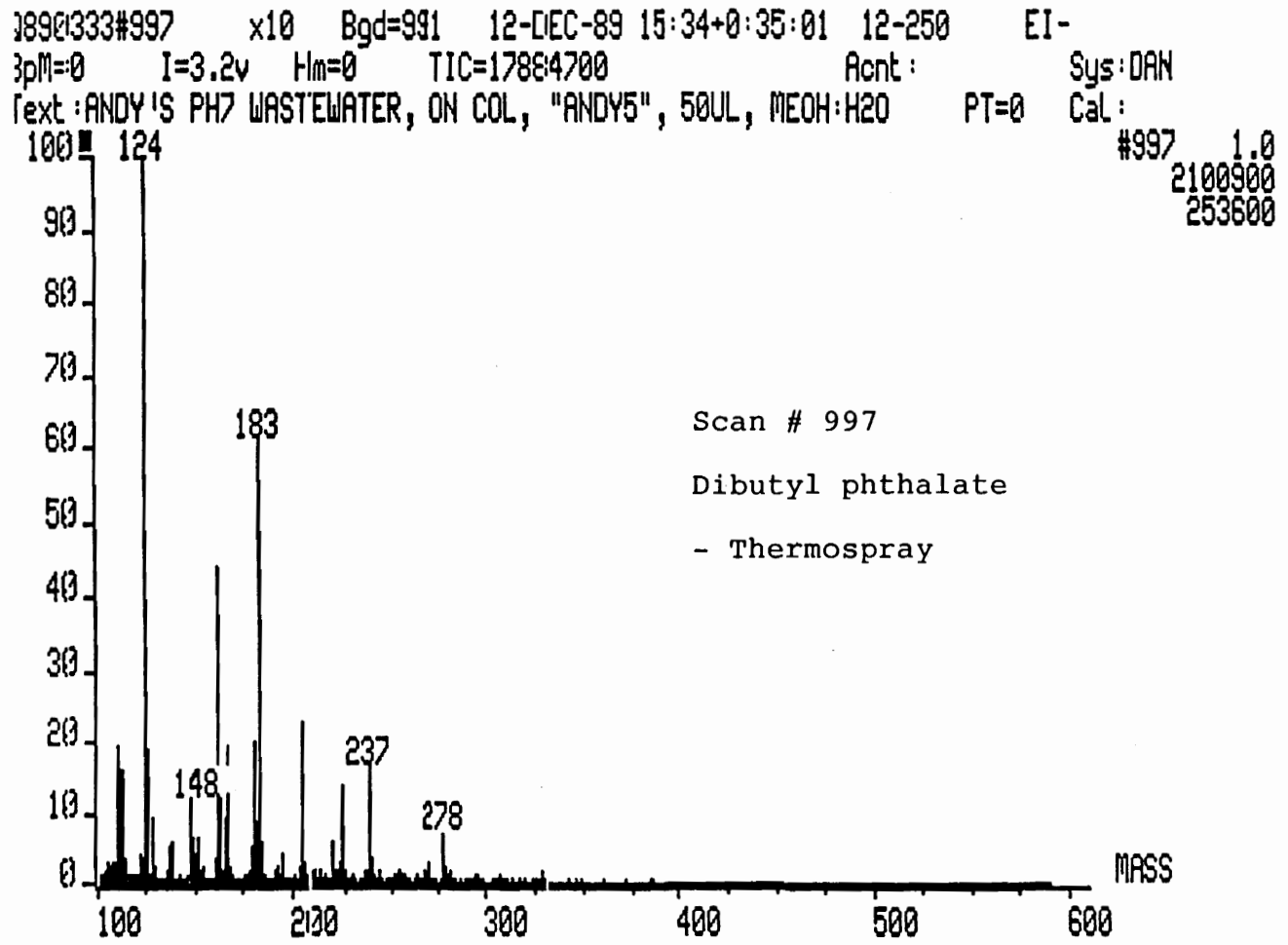

J890333#696 x10 Bgd=614 12-DEC-89 15:34+0:24:28 12-250 EI-
3pM=0 I=6.2v Hm=0 TIC=4486900 Acnt: Sys:DAN
Text:ANDY'S PH7 WASTEWATER, ON COL, "ANDY5", 50UL, MEOH:H2O PT=0 CaL:
#696 1.0
4089200
100
90
80
70
60
50
40
30
20
10
0
168
124
199
MASS
100
200
300
400
500
600
Scan # 696
Dinitrobenzene
- Thermospray


)890333#715 x1 Bgd=714 12-DEC-89 15:34+0:25:08 12-250 EI-
3pM=0 I=2.2v Hm=0 TIC=6772:3000 Acnt: Sys:DAN
Text:ANDY'S PH7 WASTEWATER, ON COL, "ANDY5", 50UL, MEOH:H2O PT=0 CaL:
#715 1.0
14258000
227
124
198
Scan # 715
Trinitrotoluene
- Thermospray
MASS
100
90
80
70
60
50
40
30
20
10
0
100
200
300
400
500
600

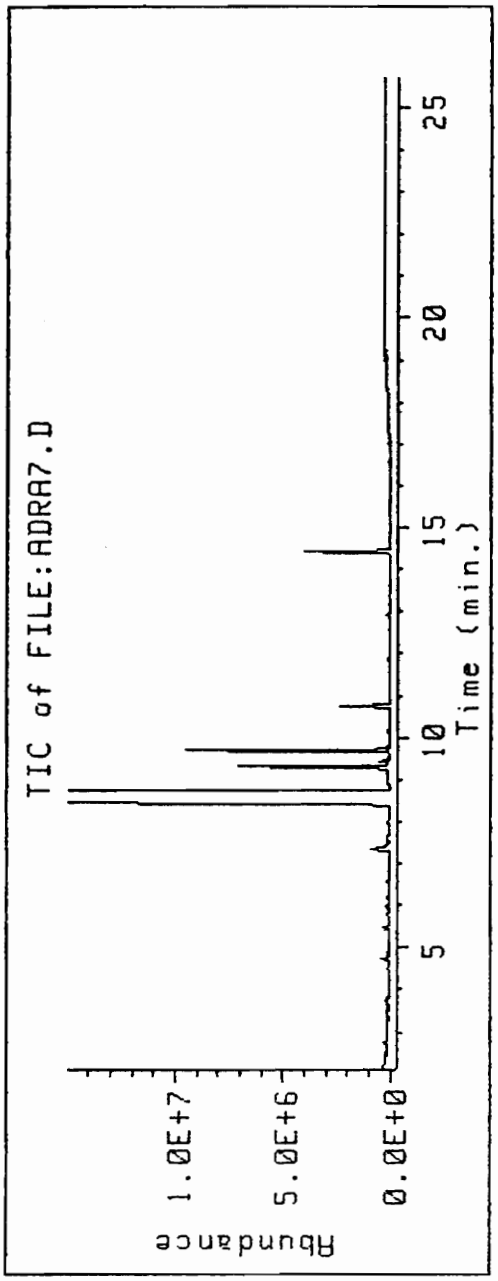

)890333#741 x1 Bgd=740 12-DEC-89 15:34+0:26:02 12-250 EI-
3pM=0 I=6.7v Hm=0 TIC=1116:38000 Acnt: Sys:DAN
Text:ANDY'S PH7 WASTEWATER, ON COL, "ANDY5", 50UL, MEOH:H2O PT=0 Cal:
#741 1.0
43723000
182
153
214
Scan # 741
DNT Isomer
- Thermospray
MASS
100
90
80
70
60
50
40
30
20
10
0
100
200
300
400
500
600


3890333#754 x1 Bgd=740 12-DEC-89 15:34+0:26:30 12-250 EI-
3pM=0 I=10v Hm=0 TIC=185047008 Acnt: Sys:DAN
Text:ANDY'S PH7 WASTEWATER, ON COL, "ANDY5", 50UL, MEOH:H2O PT=0 Cal:
#754 1.0
65534000
182
154
Scan # 754
2,4-Dinitrotoluene
- Thermospray
MASS
100
90
80
70
60
50
40
30
20
10
0
100
200
300
400
500
600


)890333#824 x10 Bgd=816 12-DEC-89 15:34+0:28:57 12-250 EI-
3pM=0 I=4.5v Hm=0 TIC=44051300 Acnt: Sys:DAN
Text:ANDY'S PH7 WASTEWATER, ON COL, "ANDY5", 50UL, MEOH:H2O PT=0 Cal:
#824 1.0
2947700
1629500
100
90
80
70
60
50
40
30
20
10
0
124
182
168
161
Scan # 824
Unknown
- Thermospray
MASS
100
200
300
400
500
600

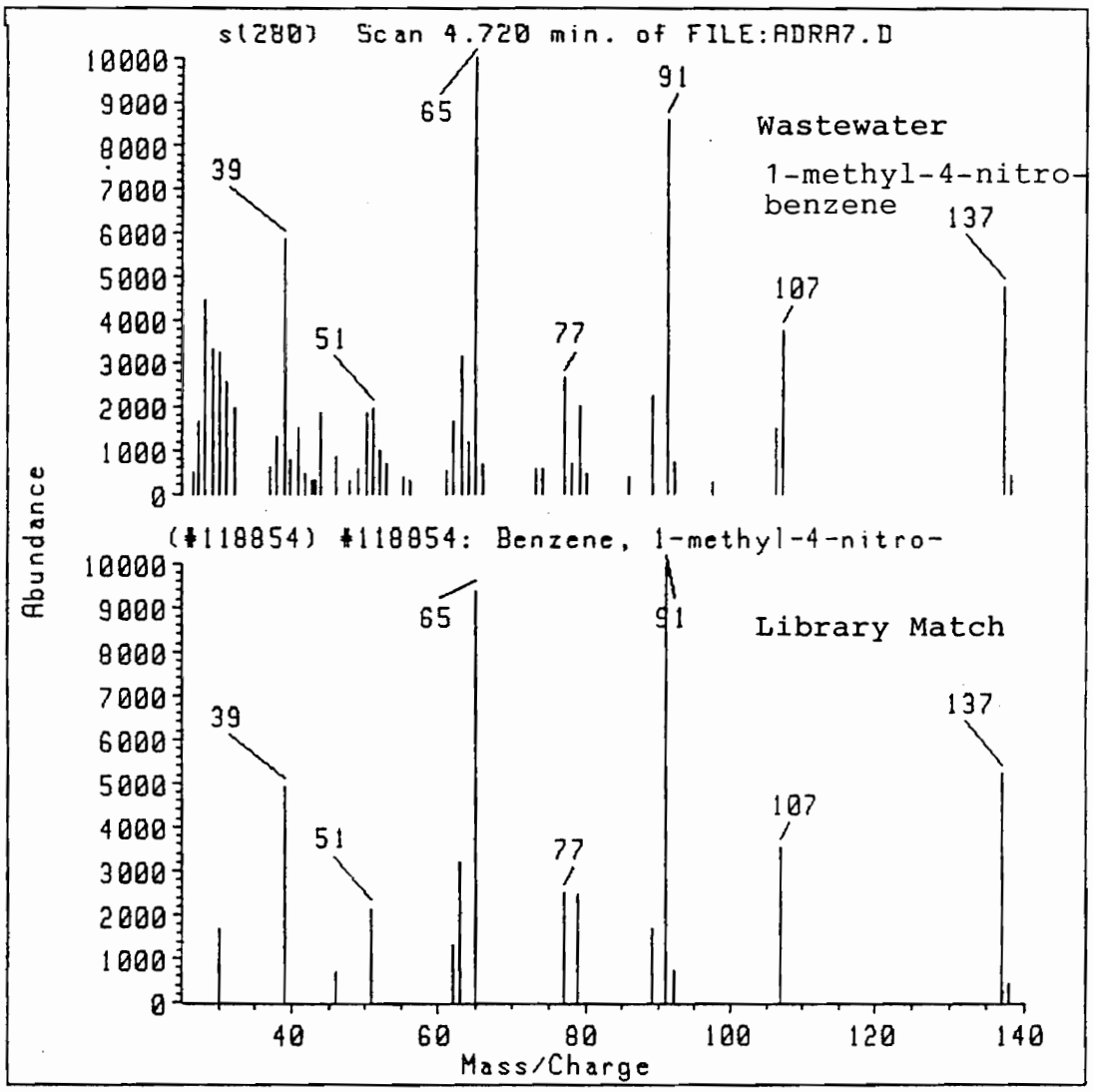

)8901333#997 x10 Bgd=991 12-DEC-89 15:34+0:35:01 12-250 EI-
3pM=0 I=3.2v Hm=0 TIC=1788:4700 Acnt: Sys:DAN
Text:ANDY'S PH7 WASTEWATER, ON COL, "ANDY5", 50UL, MEOH:H2O PT=0 Cal:
#997 1.0
2100900
253600
100
90
80
70
60
50
40
30
20
10
0
124
183
148
237
278
Scan # 997
Dibutyl phthalate
- Thermospray
MASS
100
200
300
400
500
600

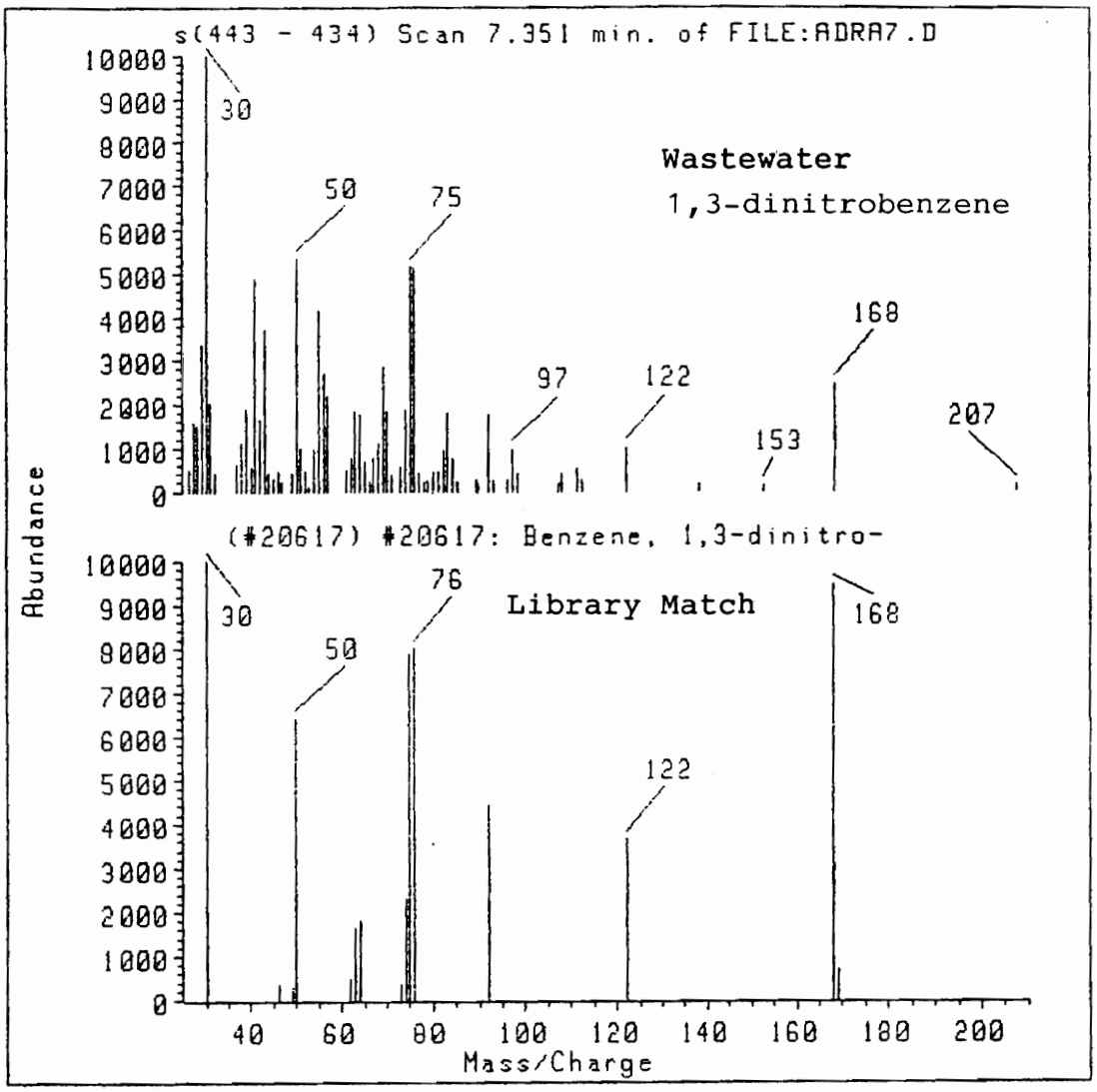
Appendix F. GC/MS Spectra of Solid Phase Extracted Wastewater Samples (Table 15)

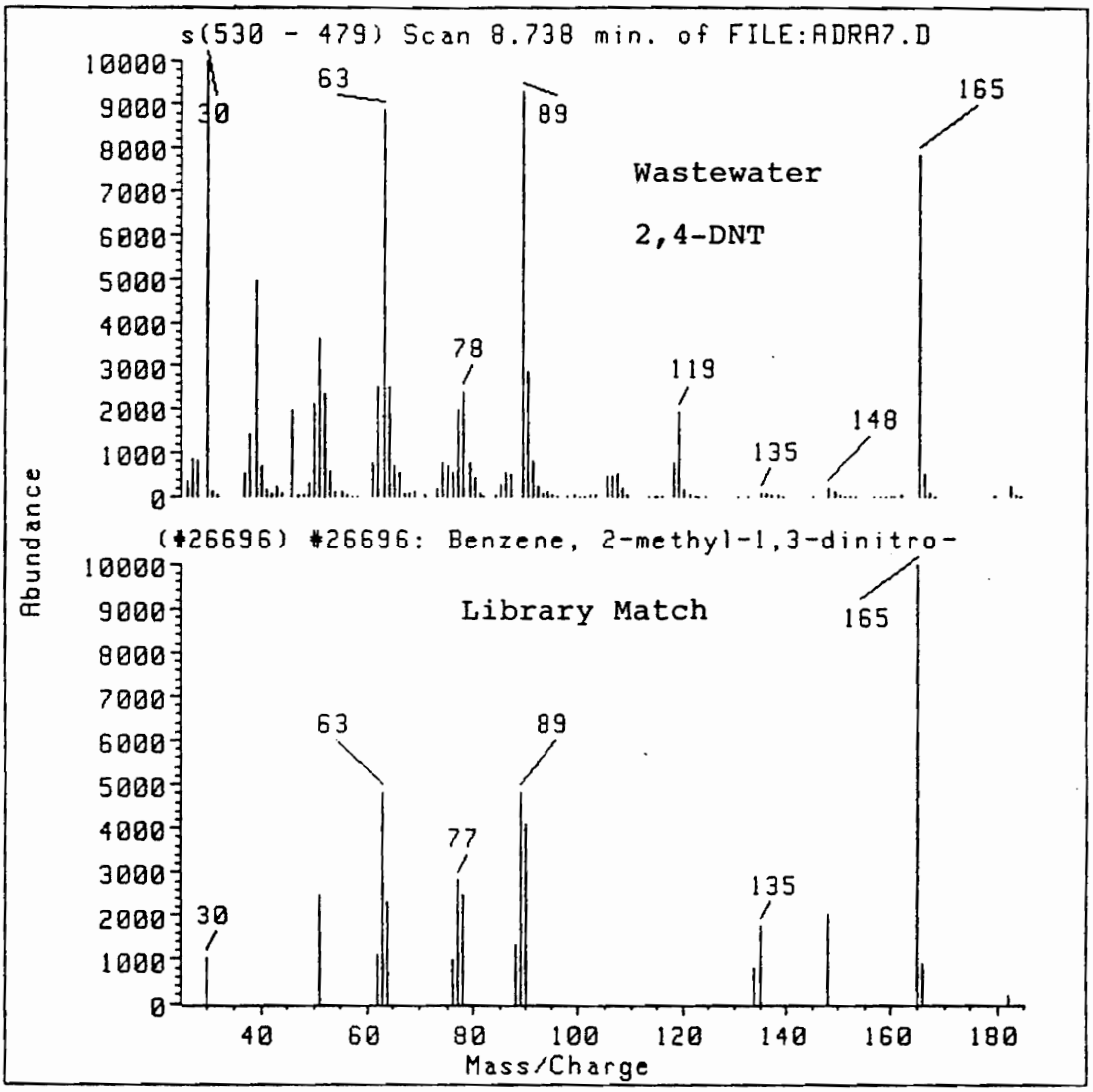


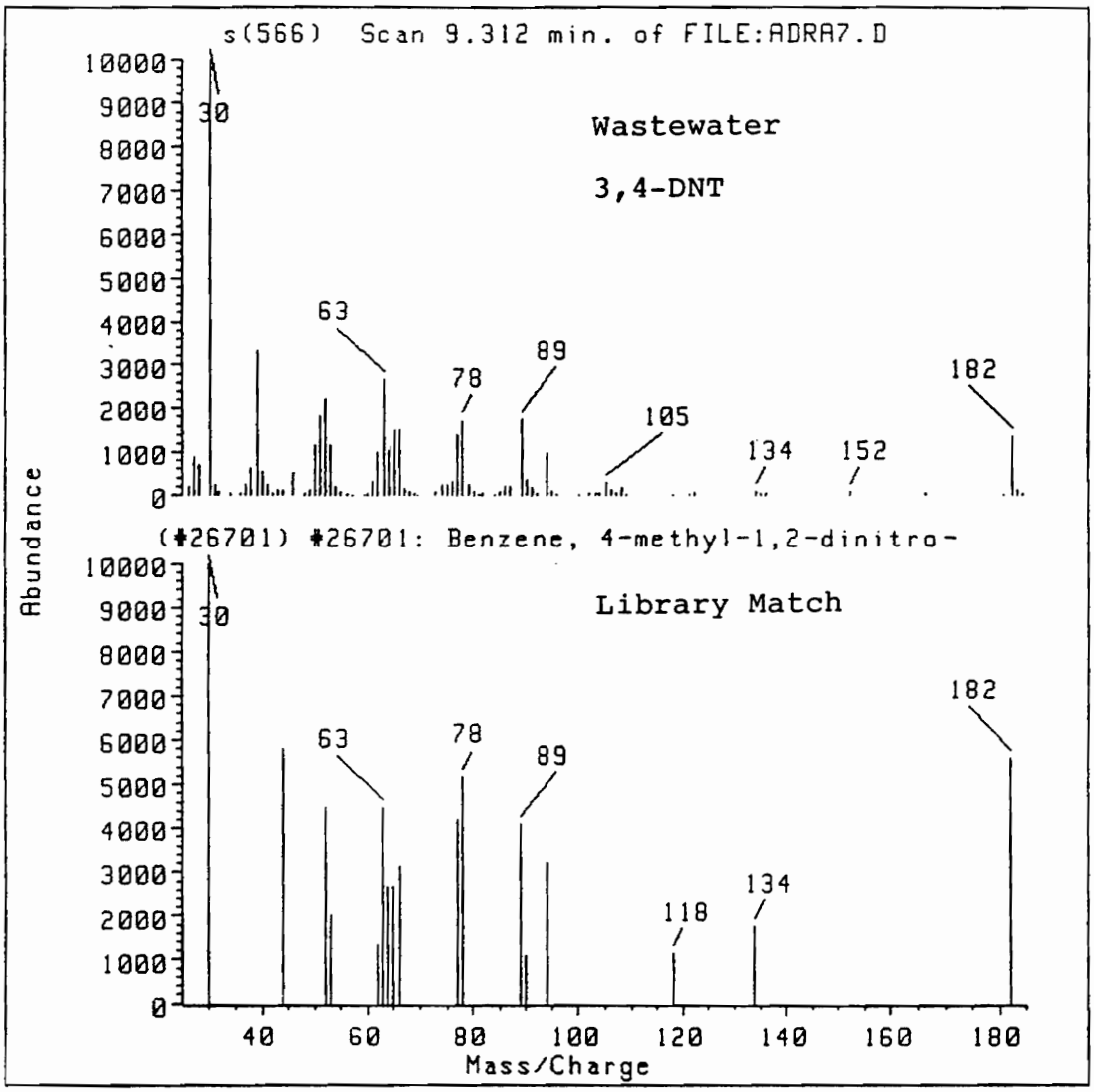
Total Ion Chromatogram of SPE Extracted

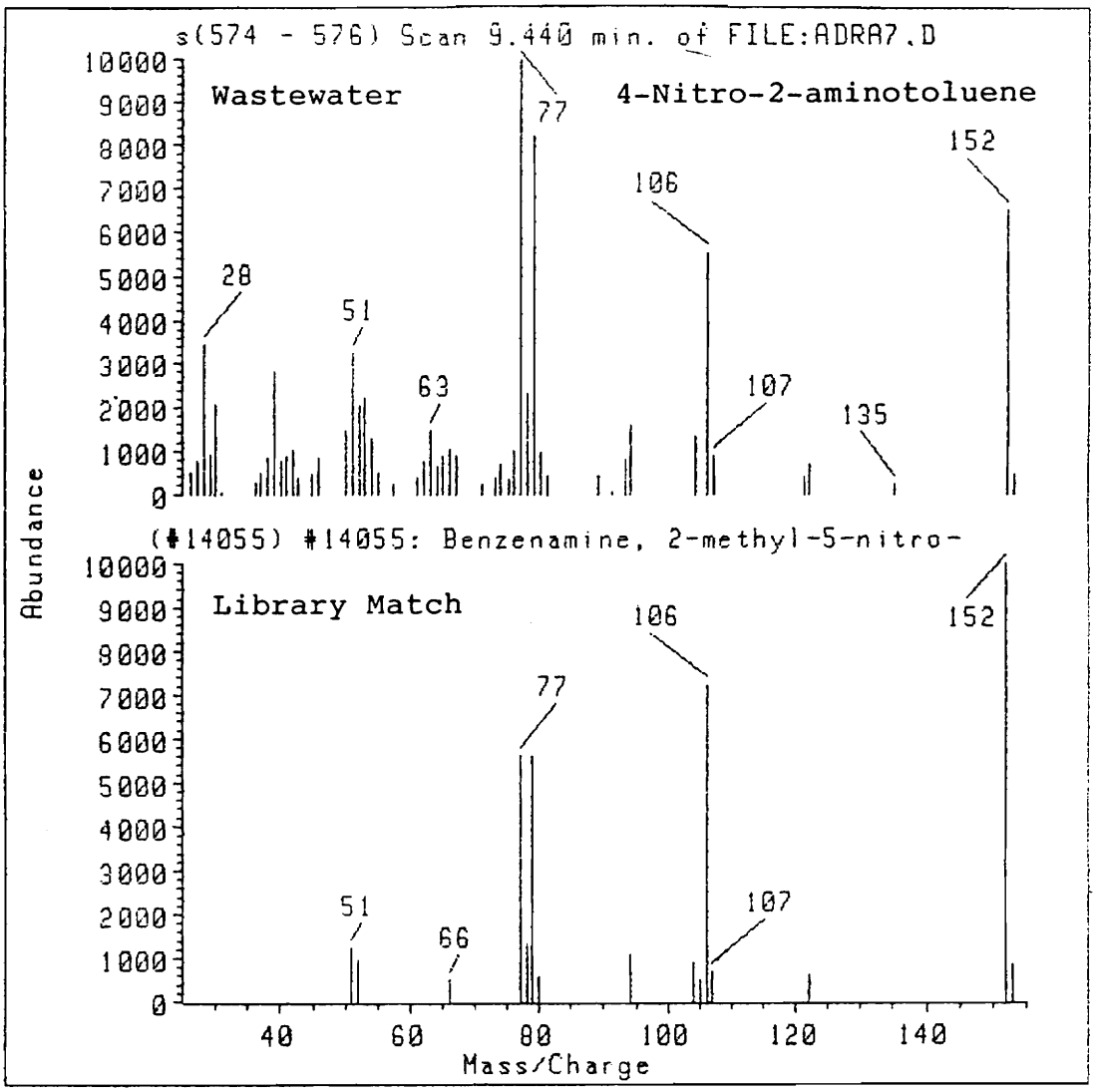
Industrial Wastewater

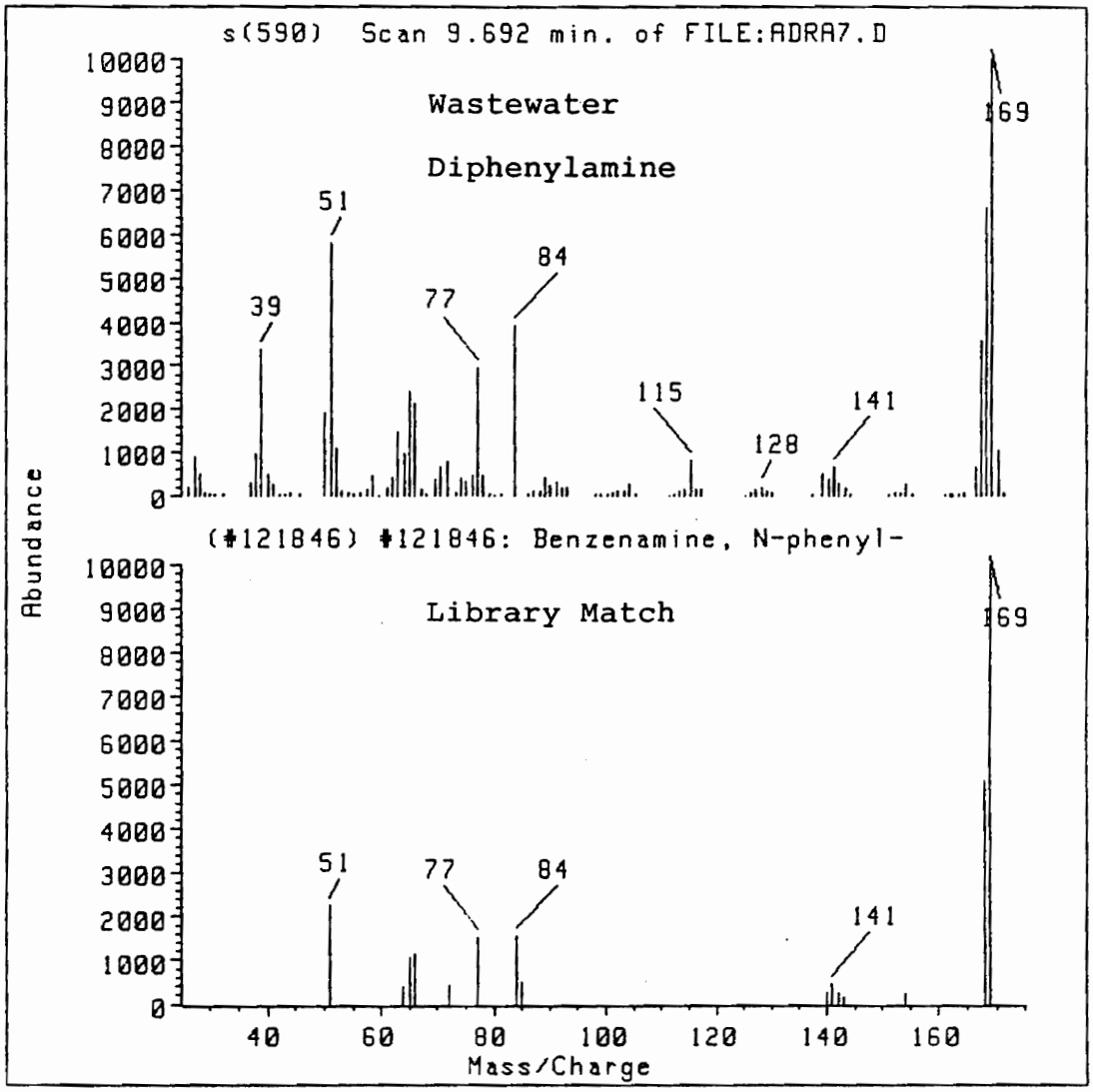

s(280) Scan 4.720 min. of FILE:ADRA7.D
Wastewater
1-methyl-4-nitro-benzene
(#118854) #118854: Benzene, 1-methyl-4-nitro-
Library Match
Abundance
Mass/Charge
39
51
65
77
91
107
137

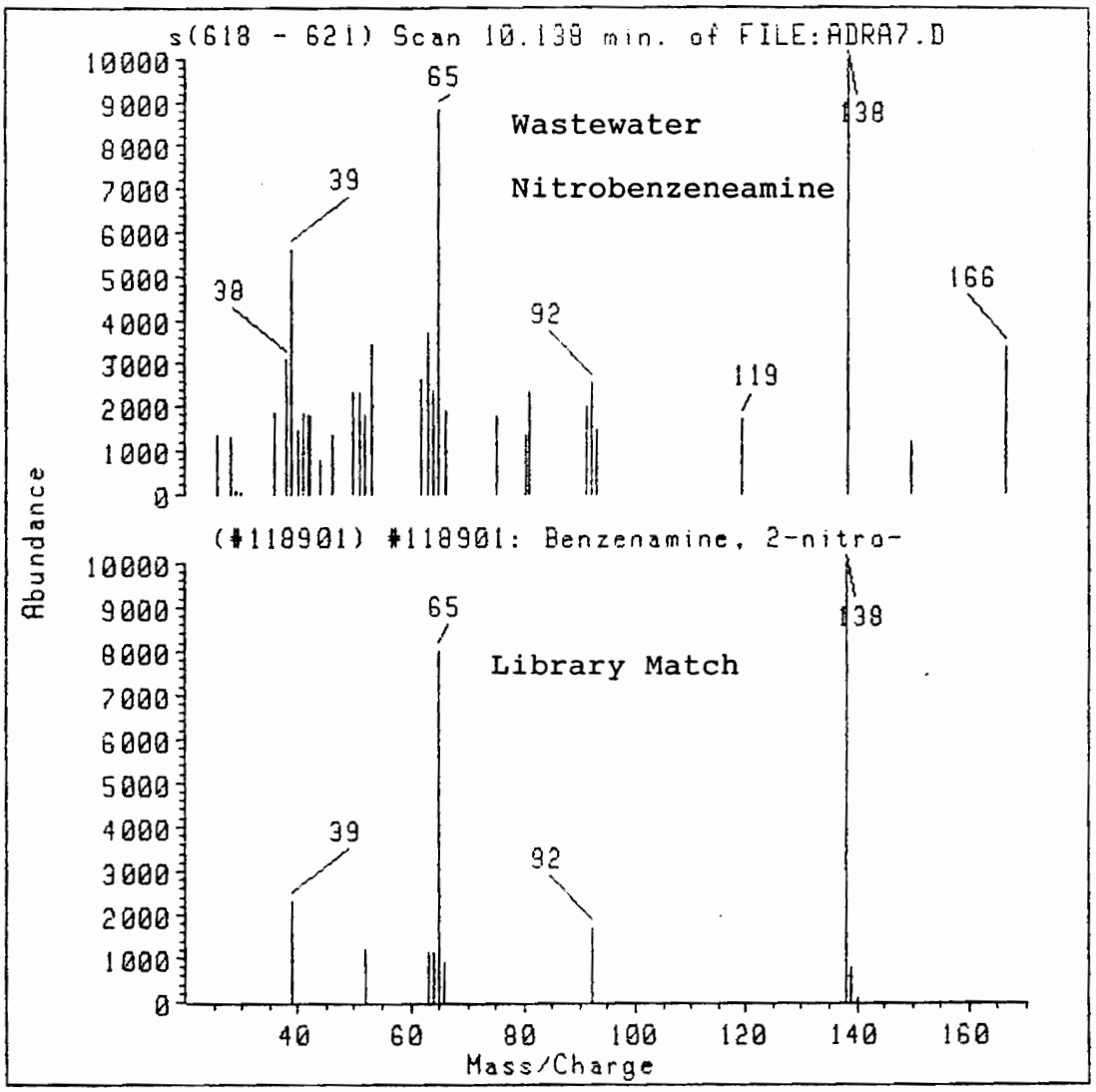

s(443 - 434) Scan 7.351 min. of FILE:ADRA7.D
Wastewater
1,3-dinitrobenzene
(#20617) #20617: Benzene, 1,3-dinitro-
Library Match
Abundance
Mass/Charge

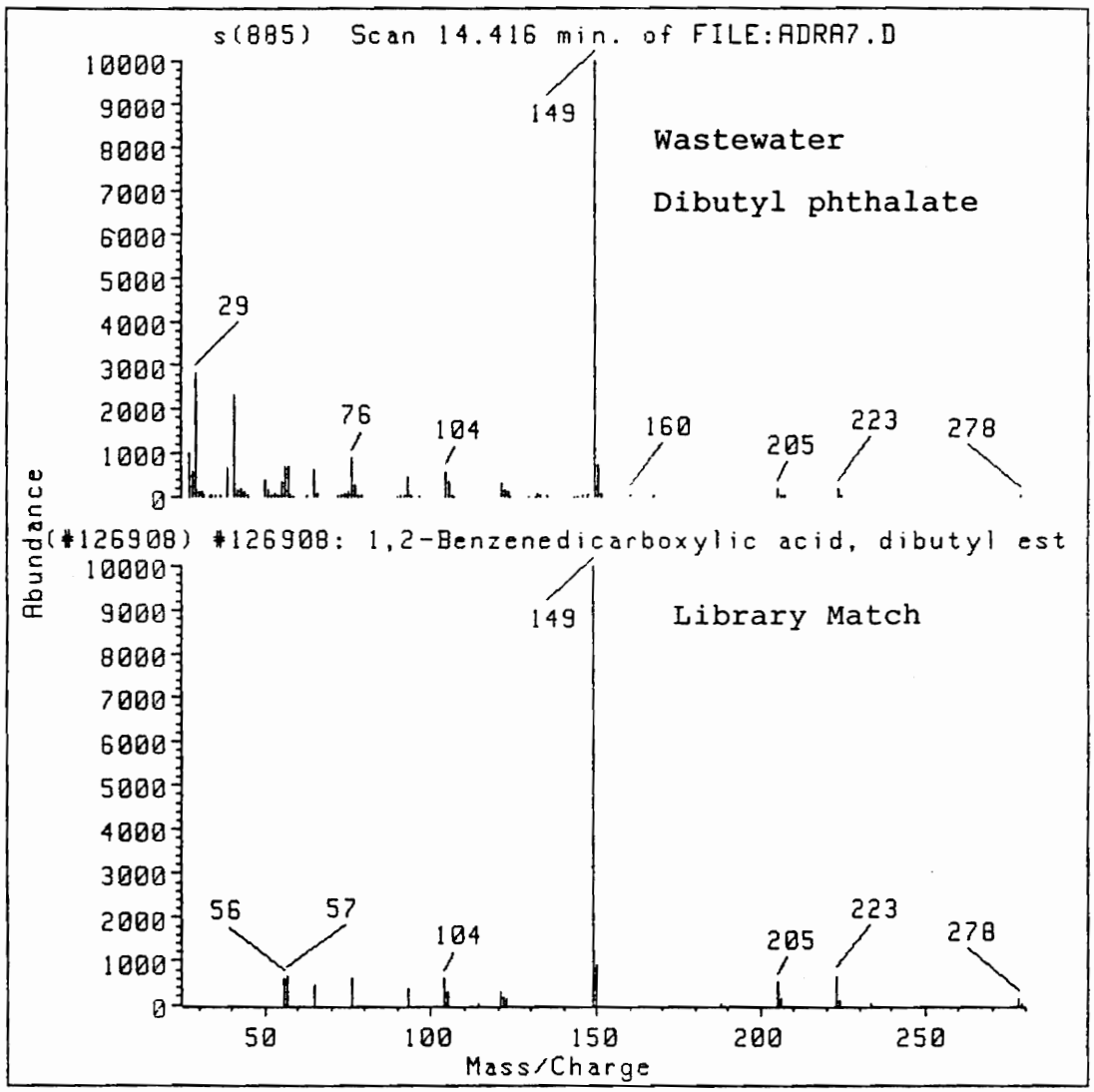

s(530 - 479) Scan 8.738 min. of FILE:ADRA7.D
Wastewater
2,4-DNT
Abundance
30
63
89
165
78
119
135
148
(#26696) #26696: Benzene, 2-methyl-1,3-dinitro-
Library Match
77
Mass/Charge
40
60
80
100
120
140
160
180


s(566) Scan 9.312 min. of FILE:ADRA7.D
Wastewater
3,4-DNT
(#26701) #26701: Benzene, 4-methyl-1,2-dinitro-
Library Match
Abundance
Mass/Charge
30
63
78
89
105
134
152
182
118


s(574 - 576) Scan 9.440 min. of FILE:ADRA7.D
Wastewater
4-Nitro-2-aminotoluene
(#14055) #14055: Benzenamine, 2-methyl-5-nitro-
Library Match
Abundance
Mass/Charge
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
28
51
63
77
106
107
135
152
66
40
60
80
100
120
140


s(590) Scan 9.692 min. of FILE:ADRA7.D
Wastewater
Diphenylamine
(#121846) #121846: Benzenamine, N-phenyl-
Library Match
Abundance
Mass/Charge
39
51
77
84
115
128
141
169


s(618 - 621) Scan 10.138 min. of FILE:ADRA7.D
Wastewater
Nitrobenzeneamine
(#118901) #118901: Benzenamine, 2-nitro-
Library Match
Abundance
Mass/Charge
65
39
38
92
119
138
166


s(885) Scan 14.416 min. of FILE:ADRA7.D
Wastewater
Dibutyl phthalate
(#126908) #126908: 1,2-Benzenedicarboxylic acid, dibutyl est
Library Match
Abundance
Mass/Charge
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
50
100
150
200
250
29
56
57
76
104
149
160
205
223
278

Appendix G. Raw Data for Linear Regression Analyses

Raw Data for 2,4-DNT Standard Curve and Linear Regression at 230 nm

Mass inj. ug	Area Units	Regression
2.1	4180	4198
3.15	6145	6121
3.15	6139	6121
3.15	5923	6121
6.3	11757	11888
6.3	11685	11888
6.3	11996	11888
6.3	12479	11888
9.45	17447	17656
9.45	17689	17656

Regression Output:

Constant		353
Std Err of Y Est		251
R Squared		0.99758
No. of Observations		10
Degrees of Freedom		8
X Coefficient(s)	1831	
Std Err of Coef.	32	

Raw Data for DPA Standard Curves and Linear Regressions

Mass inj. ug	Area at 230 nm	Area at 286 nm	Regression at 230 nm	Regression at 286 nm
15.75	12866	28394	12733	29168
15.75	12745	28220	12733	29168
10.5	8396	20595	8423	19827
10.5	8317	20451	8423	19827
6.3	4818	12846	4974	12354
6.3	4916	13065	4974	12354
5.25	4174	11228	4112	10486
5.25	4185	11257	4112	10486
3.15	2338	6545	2388	6750
3.15	2307	6469	2388	6750
0.79	550	1599	451	2551
0.79	550	1601	451	2551

Regression Output:		Regression Output:	
Constant	-197.848	Constant	1145.188
Std Err of Y Est	97.743	Std Err of Y Est	793.534
R Squared	0.99951	R Squared	0.99322
No. of Observations	12.000	No. of Observations	12.000
Degrees of Freedom	10.000	Degrees of Freedom	10.000
X Coefficient(s)	820.989	X Coefficient(s)	1779.202
Std Err of Coef.	5.725	Std Err of Coef.	46.479

Raw Data for DPA Standard Addition Linear Regression

Conc., mg/L	Area at 230 nm	Regression
0	377	355
0	393	355
1.05	464	523
1.05	464	523
2.1	672	691
2.1	768	691

Regression Output:

Constant		355
Std Err of Y Est		62
R Squared		0.88086
No. of Observations		6
Degrees of Freedom		4
X Coefficient(s)	160	
Std Err of Coef.	29	

Vita

Andrew Jay Danzig was born in Milford, CT on October 30, 1957. He lived two years in Milford, two years in Schenectedy, NY, four years in Beverly, MA and six years in DeWitt, NY. He completed his high school education in Fairfield, CT. After graduation, Andy lived for six months on a kibbutz in Israel. He returned to Israel two years later to spend his junior year at the Hebrew University of Jerusalem. Andy graduated from the University of Connecticut in May 1979 with a B.S. in Biology.

Andy served as a Peace Corps Volunteer in Niger, West Africa from 1980-1982. The most important event of his life occurred on March 6, 1983 with the birth of his daughter, Reva Rose Danzig. He spent four years in West Virginia and became an avid horticulturalist while working in the landscaping profession. He commenced his graduate studies at Virginia Tech in December 1987 and received his Master's degree in Environmental Engineering in May 1990.