MICROBORE LC/MS

THE COMBINATION OF MICROBORE LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY

By

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ABSTRACT

An inexpensive method was developed for the conversion of a high performance liquid chromatography (HPLC) system for use with 1 mm I.D. microbore columns. Chromatographic performance of the system was tested under both isocratic and gradient elution conditions, using a standard mixture of 16 polycyclic aromatic hydrocarbons (PAH).

The microbore column HPLC was also coupled to a mass spectrometer equipped with a moving belt interface. Chromatographic performance under isocratic and gradient elution and mass spectral performance under scanning and selected ion monitoring modes were tested using the PAH standard.

A marine sediment extract was subjected to qualitative and quantitative analysis for PAH. Qualitative results on the sample were obtained from a combination of retention indices, mass spectra, and retention times. Quantitation was performed by microbore column liquid chromatography-mass spectrometry (LC/MS) in the selected ion monitoring mode of operation. The method of calibration used was external calibration.

The microbore column HPLC system exhibited good chromatographic behavior. Resolution, peak shape and short term retention time reproducibility were good, although, long term retention time

fluctuations, due to changing mobile phase flow rates, were noted.

The combination of microbore column HPLC with a moving belt interface and mass spectrometer gave excellent results. Problems commonly encountered with conventional column (4.6 mm I.D.) LC/MS, such as backstreaming, droplet formation, and splattering were greatly reduced, resulting in no apparent loss of chromatographic integrity and stable mass spectrometer operating conditions. These operating conditions proved to be most advantageous in the quantitative analysis of the marine sediment extract by selected ion monitoring.

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1. INTRODUCTION

This thesis presents the results of an investigation of the use of combined liquid chromatography/mass spectrometry (LC/MS) for the analysis of polycyclic aromatic hydrocarbons (PAH) in environmental samples. Part of the study involved the development and testing of a microbore column high performance liquid chromatography (HPLC) system in order to improve interfacing with a mass spectrometer. This introduction will first review the methods used in the field of organic analytical chemistry and then discuss the objectives of the research work.

1.1 ORGANIC ANALYTICAL CHEMISTRY

The detection, identification and quantitation of organic substances has always presented a challenge to the analytical chemist. From the early days of petroleum research, it was clear that very powerful instrumental methods of analysis would be required to deal with organic samples. Unlike traditional inorganic analytical chemistry, which encompasses a finite number of natural elements and compounds, organic analytical chemistry faces the problem of an infinite number of possible compounds. This can lead to extremely complex samples containing thousands of different compounds. Even for a given elemental composition, the number of possible isomers can be staggering. Isomeric variations may range from distinct functional group differences to

subtle stereochemical differences (1). In recent years, the demands for organic analytical methods have increased with the need to solve pressing problems in other fields such as biomedical and environmental studies (2). In such cases, the analytical challenge is even more complicated by the need for analysis of trace level analytes or minute sample sizes or even both!

The principal tools of the organic analytical chemist include gas and liquid chromatographies, mass spectrometry, nuclear magnetic resonance spectroscopy, and infrared, ultraviolet-visible and fluorescence spectrophotometries. With the complexity of mixtures and trace levels of individual species encountered in environmental and biomedical samples, combined chromatography-mass spectrometry methods are currently the most successful and widely used. These will be reviewed in the following sections.

1.2 CHROMATOGRAPHIC METHODS

Chromatography is a technique that provides the separation of mixtures of compounds by differential migration through a porous medium the migration being produced by electrical potential or by flow of liquid or gas (3). Chromatography by itself is not an analytical technique. For analysis, some method must be provided for detecting the separated compounds, whether it be the visual detection of coloured species, the weight of eluted material or the spectroscopic detection of species. Modern instrumental chromatography is based on the combination

of a chromatographic system with an on-line detector that yields a signal in response to an eluting substance.

The most popular chromatographic method of analysis, since its invention in 1941 by Martin and Synge, is gas chromatography (4). It provides the advantages of high resolving power, high sensitivity, rapid analysis and a wide variety of detectors, both selective and general. The most common detectors employed are the thermal conductivity (TCD), flame ionization (FID) and electron capture (ECD) detectors, all of which give high sensitivity with relatively simple design. Unfortunately, GC is restricted to the analysis of volatile and thermally stable compounds, which account for only 20% of all known compounds (5). Application of chemical derivatization methods to increase the volatility and thermal stability of polar compounds has only partially succeeded in extending the range of compounds amenable to analysis by GC. An additional shortcoming of GC, although not usually of concern to the analyst, is its poor capability for preparative scale separations.

Traditionally, liquid chromatography (LC) was very time and labour intensive, nonquantitative and irreproducible, thereby limiting its analytical applications (6). The development of small diameter particulate packings (3-10 um), chemically bonded stationary phases, and high pressure solvent delivery systems, led to high column efficiencies and excellent reproducibility characteristic of modern instrumental high performance liquid chromatography (HPLC) (7). This technique has the

capability of analyzing a wide range of compounds independent of their volatility and thermal stability. Despite all these advantages, HPLC lacks one important factor - a sensitive general detector, such as the FID in GC. The most common detectors are the UV-VIS absorption detector, which has very high sensitivities for those compounds with a chromophore and the refractive index detector, which is universal, but not very sensitive. Overall, HPLC has become a technique complementary to GC.

Recently, a great deal of interest has arisen in the use of microbore columns for liquid chromatography (8,9,10). Microbore column liquid chromatography differs from conventional HPLC in two respects. First, microbore column liquid chromatography uses narrow bore columns, usually 1 to 2 mm I.D. packed with small diameter particulate packing, 10 um or less. Secondly, a high pressure pumping system, able to accurately deliver flows of 10 to 50 uL/min, is used. In comparison, conventional HPLC employs columns with diameters of 4 to 5 mm I.D. and solvent flow rates in the range of 1 to 2 mL/min. With these changes, microbore column liquid chromatography has three basic advantages over its conventional counterpart. A primary advantage for many laboratories is solvent and packing material economy. This economy translates into lower operating costs and allow the use of exotic stationary and mobile phases (i.e., deuterated and chiral phases). The second important attribute is microbore column liquid chromatography's increased sensitivity with concentration-dependent detectors (e.g., UV-VIS absorption): a result of reduced dilution of the analyte in the effluent due to reduced flow rates. The third advantage is less obvious but is

still very important. Microbore column liquid chromatography columns have, because of their size, a low heat capacity, resulting in only minor temperature gradients throughout the length of the column. In addition, correction of any thermal gradients present in the column, through the use of thermostatted column sleeves, can be easily achieved. This results in highly reproducible retention data (8,11). There is also a fourth advantage that researchers are just beginning to utilize. Low flow rates in microbore column liquid chromatography allow the use of unusual detectors, particularly mass rate dependent detectors, that cannot tolerate large flow rates of mobile phase. Examples of such detectors include the flame ionization detector and the mass spectrometer. As discussed below, the combination of HPLC and mass spectrometry is a very powerful analytical tool.

Microbore column liquid chromatography is, however, not without disadvantages. The microbore column's small size greatly reduces loading capacities (5 to 100 ug per individual compound) and injection volumes (1 uL). In addition, special equipment is required for the accurate and precise delivery of flow rates in the 50 uL/min range with both isocratic and gradient elution and with a minimum of internal dead volume (Figure 1). Modification of conventional HPLC systems is necessary to allow the use of microbore columns. This not only includes solvent delivery systems but also detector cells, injectors and all connecting tubing (8).

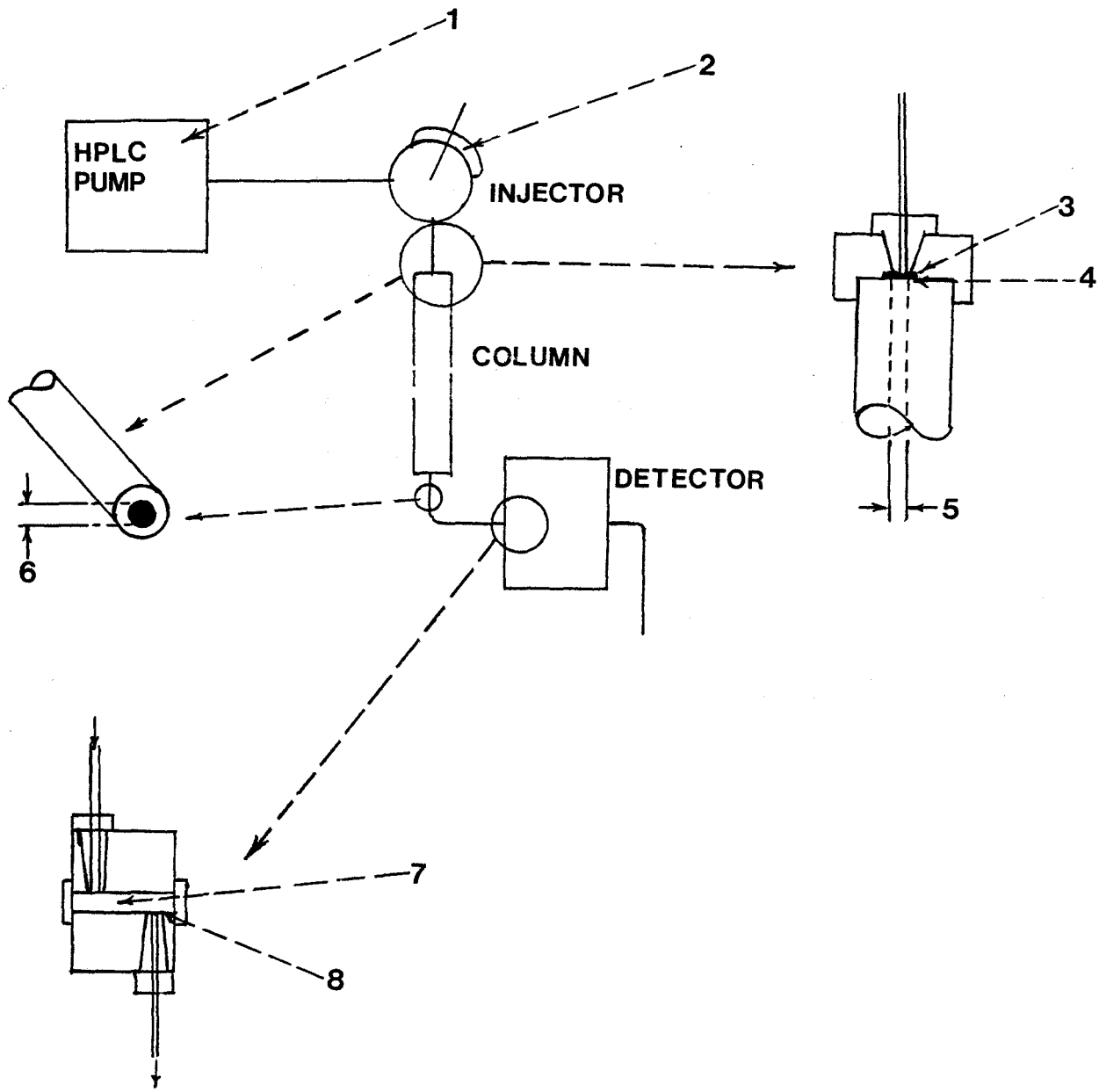


Figure 1: Schematic diagram showing areas of concern in a microbore column liquid chromatography system: 1) accurate metering of mobile phase at low flow rates (30 to 100 uL/min); 2) accurate low internal volume injector (1 uL); 3) zero dead volume fittings; 4) ultra low volume column frits (1.5 mm O.D.); 5) narrow internal column diameter (1 mm I.D.); 6) narrow bore stainless steel tubing (0.13 mm I.D.); 7) low volume detector cells (1uL); and 8) zero dead volume fittings.

The first objective of this research project is to modify a conventional HPLC system for use with microbore columns. The modified system will then be used in association with an ultra-violet absorption detector and a mass spectrometer equipped with a moving belt interface. System optimization and evaluation will then be carried out with the analysis of polycyclic aromatic hydrocarbons (PAH).

1.3 COMBINED LC/MS

A multitude of chromatographic detectors have been developed but none compare with the mass spectrometer in terms of being a selective and sensitive universal detector. The mass spectrometer not only has the ability to detect minute quantities of analyte, but, because of its spectroscopic capabilities, it can yield information pertaining to the compound's structure, particularly its molecular weight and elemental composition. In addition, the deconvolution of coeluted species, on the basis of differing mass-to-charge ratios, is possible with the mass spectrometer and modern data systems (12,13).

The coupling of gas chromatography and mass spectrometry (GC/MS) effectively integrates the advantages of GC, high resolution and speed, with the aforementioned advantages of mass spectrometry. The physical coupling of these two instruments is easily accomplished with the use of fused silica capillary columns by means of directly inserting the column into the mass spectrometer source. This creates no problems since the pumping capacity of the mass spectrometer can easily handle the 1 mL/min

of He introduced from the GC column.

The combination of HPLC and MS has been highly desired for many years because of the advantages of the mass spectrometer as a sensitive, general detector which is lacking for HPLC. The implementation of this combined technique would extend the range of compounds that can be analyzed over those amenable to GC/MS (12). In addition, with the advent of ionization techniques not applicable with GC/MS, such as fast atom bombardment (FAB) and thermospray, the range of applicable samples would be further broadened with LC/MS. However, the direct union of conventional HPLC and MS is impractical: the mass spectrometer requires high vacuum, whereas HPLC is performed at atmospheric pressure with condensed liquid phases. This basic incompatibility becomes obvious when one considers that a modern mass spectrometer can only accommodate 3-5 mL/min of gas and HPLC effluent at 1 mL/min results in 300 to 1300 mL/min of solvent vapour, depending on the type of solvent. Therefore an interface between the two instruments is necessary to remove the large volumes of mobile phase before it reaches the mass spectrometer.

To avoid the many difficulties associated with directly combining HPLC and MS, many researchers turned to off-line LC/MS. In this method the HPLC effluent is collected in discrete samples and then analyzed by direct probe inlet mass spectrometry. The advantages gained by this method are many fold. The complete separation of HPLC and MS circumvents the use of expensive interfaces and allows for a wider variety of ionization techniques to be used (e.g., field desorption

(FD)). In this way off-line LC/MS affords a quick and inexpensive way to perform qualitative analysis on routine samples. However, this method has some serious limitations (12,13). Quantitation is very difficult via direct probe insertion. Complex samples pose a problem in that the collection and evaporation of each fraction becomes tedious and time consuming. Compounds invisible to the on-line HPLC detector can be missed because continuous mass spectral detection is not being performed (12,13). In addition, the ability to deconvolute coeluting compounds on the basis of mass is also lost. The use of on-line LC/MS techniques overcomes these problems. Due to the perseverence of early workers, LC/MS has developed into a viable technique and in many cases the method of choice.

As previously mentioned, because of the basic incompatibilities of HPLC and MS, an interface must be used to mate these two techniques. To ensure that neither instrument's operations are hindered and that high quality results are obtained, the interface should conform to a number of criteria. With respect to the operation of the liquid chromatograph, the interface must tolerate a wide variety of solvents at varying flow rates and should also maintain low mixing volumes in order to ensure chromatographic integrity. With respect to the operation of the mass spectrometer, the interface should be compatible with a wide range of operating conditions such as various ionization modes or levels of resolution and sensitivity (12,13). With respect to the transfer of solute between the two instruments, the interface should be as efficient as possible, constant and independent of the chemical and physical

nature of the solutes or solvents. Lastly, the solute should not be chemically modified by the interface in a uncontrolled manner (12,13).

Presently, there are four available types of LC/MS interfaces that accomodate most of these requirements, namely:

(a) Atmospheric pressure ionization (API)

(b) Direct liquid injection (DLI)

(c) Jet interfaces

(d) The moving belt interface

Atmospheric pressure ionization (API), the earliest LC/MS interface design directly introduces the effluent into an external ionization chamber where a corona discharge or radioactive emitter ionizes the solute and solvent. The ionized gas is then sampled by the mass analyzer through a pinhole positioned in front of the accelerating plates. This system typically handles 1 to 2 mL/min of solvent without detriment to the mass spectrometer; however, solvent choice is restricted to solvents with low proton or electron affinities. This design is very sensitive to any solvent impurities, resulting in complex ion-molecule reactions. Only spectra resembling chemical ionization (CI) spectra are possible and transfer efficiences are poor (12,14,15).

Direct liquid injection (DLI) involves the introduction of the HPLC effluent through a pinhole orifice or semi-permeable membrane restrictor. The restrictor causes a fine axial jet to be formed that is ionized by a conventional electron ionization (EI) filament. This results in CI-like conditions, due to the fact that the mobile phase

acts as a reagent gas. This commercially available design is very simple and easy to operate. It has very good chromatographic integrity and can accept a wider variety of solvents than API. Transfer efficiencies vary depending on the type of column used in the chromatographic process. Conventional columns require an effluent split resulting in transfer efficiencies of 1%; however, if microbore columns are used in conjunction with a cryogenic pumping system, direct coupling can be accomplished, resulting in extremely high transfer efficiencies. Major problems associated with DLI are frequent clogging of the membrane or orifice, the restriction of performing only in CI mode and the restriction of using mobile phases that can act as a suitable reagent gas for ionization in the mass spectrometer (12,14,15).

Jet interfaces are still in the developmental stages but they do show great promise. The two most popular types of jet interfaces are thermospray and electrospray. The operating principle of these two types of jet interfaces are much the same as the API interface except that instead of ionizing all of the effluent, thermospray and electrospray produce a fine stream of charged droplets by means of a strong thermal or electrical field gradient at tip of the nebulizing nozzle. This produces a fine axial spray of droplets from which charged species are ejected through a pinhole orifice into the mass analyzer (16). This occurs as a result of the droplets decreasing in size and thus creating an increased charge density at the surface. This process continues until the mass analyzer's electric field is strong enough to desorb the ions from the liquid surface (16). These designs can easily

accomodate 1 to 2 mL/min of polar solvent. The chromatographic integrity is good and transfer efficiencies are compariable to or better than most interfaces. A limitation to these methods is their dependency on a buffered aqueous medium used to assist in the production of the charged species in the interface (12,13,14).

Currently, the moving belt interface is one of the most popular LC/MS interfaces. This popularity can be attributed to its commercial availability, adaptablity to a wide variety of mass spectrometers, ability to perform EI, CI and FAB, good chromatographic integrity and excellent transfer efficiencies (12,13,14,15). The heart of the moving belt interface is a moving polyimide or stainless steel belt, whose dimensions and transport speed permit the deposition of about 1 mL/min of non-polar or weakly polar HPLC eluent. After deposition of the eluent, the belt passes under an infrared heater to evaporate solvent and then through a series of vacuum locks that serve to reduce the pressure to levels acceptable to the mass spectrometer source. A heater at the tip of the interface evaporates the solute from the belt into the source where ionization occurs (see Figure 2). Despite their widespread use, moving belt interfaces have had serious drawbacks. Polar solvents, such as water, greatly restrict the interface's solvent handling and gradient elution capabilities. This has been attributed to the poor coating and evaporation characteristics of polar solvents (17,18). Volatile solutes evaporate along with the solvents leading to poor transfer efficiencies (14); involatile solutes are not readily desorbed from the belt into the source (12,18). In addition, excessive infrared

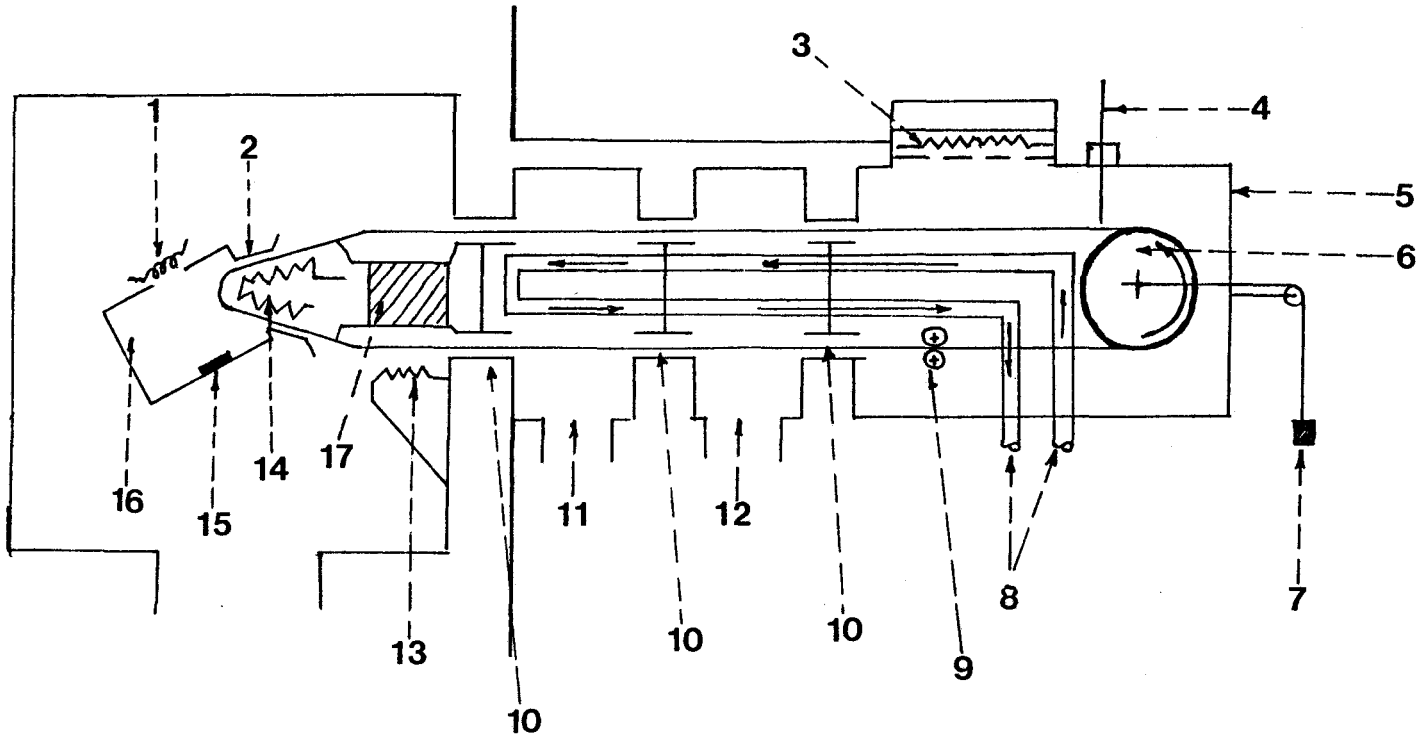


Figure 2: VG Micromass liquid chromatography-mass spectrometry moving belt interface:

1) electron ionization filament
2) source seal (for CI)
3) infrared heater
4) column effluent tube
5) belt interface housing
6) idler-wheel
7) weighted tension adjustment
8) cooling water lines
9) pinch roller and belt drive
10) tunnel seals
11) vacuum pump (0.1 to 0.5 torr)
12) vacuum pump (450 to 100 torr)
13) belt cleaning heater
14) sample heater
15) collector plate
16) ion chamber (0.5 torr)
17) ceramic insulator.

heating required for high flow rates and strongly polar solvents lead to the loss of analyte through evaporation. To help alleviate some of these problems, spray deposition systems can be used. This method involves the coating of the effluent onto the moving belt by means of a fine spray which serves to allow smoother coating of aqueous solvents and reduce problems associated with solvent evaporation. However, spray deposition is limited to only about 0.2 to 0.5 mL/min of aqueous effluents (19,20).

Recent work with microbore columns has shown that many problems associated with high solvent volumes of conventional column LC/MS can be eliminated (21,22,9). The reduced flow rates of microbore columns (10 to 100 uL/min) eliminate solvent spreading and evaporation problems, especially with polar solvents and facilitate the use of gradient elution. In addition, this reduces the need for solvent evaporation via the infrared heater thus improving transfer efficiencies. Microbore column LC/MS, with all its desirable qualities seems like the next logical step in the maturation process of LC/MS.

It is the objective of this research project to use our microbore column HPLC system in association with a mass spectrometer equipped with a moving belt LC/MS interface. System optimization and evaluation will be carried out using PAH standards.

1.4 POLYCYCLIC AROMATIC HYDROCARBONS

Polycyclic aromatic hydrocarbons (PAH) have been studied by analytical chemists for a number of years. As early as the 1950's, PAH concentration from exhaust fumes was studied by Kotin (23). Since that time their presence has been detected in a variety of environmental samples, including airborne particulates (24), waste water (25), animal tissues (26), and marine sediments (27). Recently, it has been demonstrated that many members of this class of compounds have mutagenic and carcinogenic activity (28). This point, in addition to their widespread appearance in the environment, has been a strong driving force for the increased interest in the analysis of PAH, as evidenced in the literature (27,29,30,31,32).

Many analyical techniques have been applied to the analysis of PAH in environmental samples, including gas, liquid and thin layer chromatographies, nuclear magnetic resonance spectroscopy, UV-VIS, Fourier transform infrared and fluorescence spectrophotometries, and mass spectrometry. Presently, the most successful and widely used methods are capillary column GC/MS and HPLC with selective UV-VIS and fluorescence detection. The preference for these two techniques can be attributed to a number of factors. The combination of extremely high resolution, excellent selectivity for many alkylated PAH and good sensitivity has given rise to GC/MS popularity. On the other hand, routine determination of ultra trace level PAH, superior separation selectivity for various PAH isomers, low cost of instrumentation, and

easy integration with bioassay procedures has proven HPLC with selective detection to be a technique of great power. However, a technique that could effectively and efficiently combine the advantages of both these powerful techniques, i.e., the structurally informative nature of GC/MS and the superior separation selectivity of HPLC, would be most desirable. For the reasons discussed earlier, we believe that microbore LC/MS is such a technique.

1.5 STANDARD SAMPLES

As new methods of analysis are developed or as established methods are routinely used, there is a need to test their accuracy. The best way to determine the performance of a procedure, in addition to the analysis of standards, is to analyze a reference sample. This type of sample has all the matrix effects present in actual samples, but in addition, it has been well characterized by at least two proven techniques. To date the American National Bureau of Standards has made available a reference standard of 16 PAH in acetonitrile and reference samples of urban dust and shale oil for which they have certified the concentrations of certain PAH (see Table 1). With the increasing use of marine resources, accurate analysis of marine materials is becoming important. To meet the need for marine standards the National Research Council of Canada (NRC) established the Marine Analytical Chemistry Standards Program. They have identified marine sediment as a sample for which certified reference materials are required. Currently, Dr. W.D. Jamieson's research group at NRC's Atlantic Regional Laboratory is conducting analyses of PAH in

Table 1: Target polycyclic aromatic hydrocarbons contained in the National Bureau of Standards sample SRM-1647.

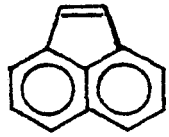
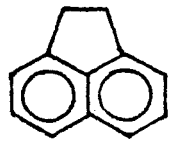
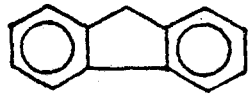
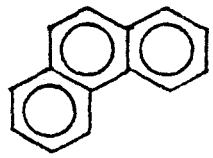
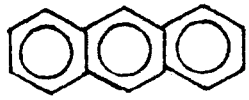
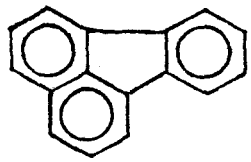
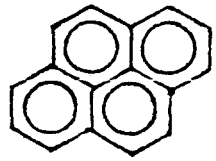
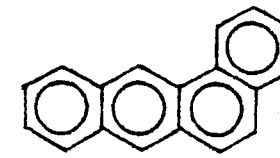
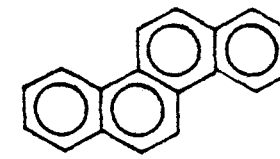
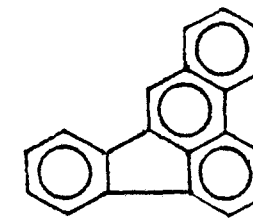
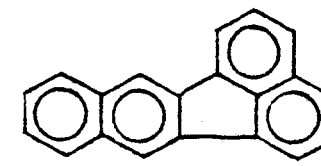
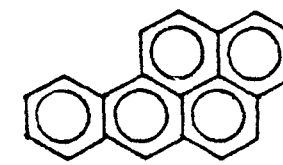
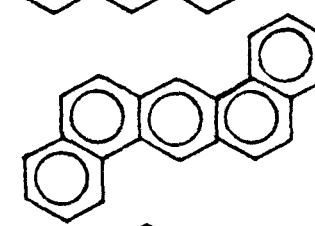
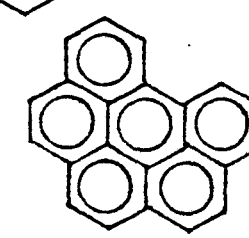
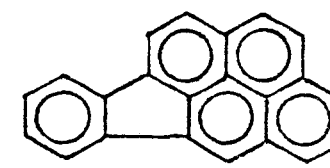
Compound Number	Mole. Wght.	NBS Std Conc'n (ng/uL)	Compound Name	Structure
1	128	22.5	Naphthalene	
2	152	19.1	Acenaphthylene	
3	154	21.0	Acenaphthene	
4	166	4.92	Fluorene	
5	178	5.06	Phenanthrene	
6	178	3.29	Anthracene	
7	202	10.1	Fluoranthene	
8	202	9.84	Pyrene	

Table 1 (continued)

9	228	5.03	Benz[a]anthracene	
10	228	4.68	Chrysene	
11	252	5.11	Benzo[b]fluoranthene	
12	252	5.02	Benzo[k]fluoranthene	
13	252	5.30	Benzo[a]pyrene	
14	278	3.68	Dibenz[ah]anthracene	
15	276	4.01	Benzo[ghi]perylene	
16	276	4.06	Indeno[1,2,3-cd]pyrene	

marine sediments extracts by capillary column GC/MS. As previously mentioned, this method is recognized as an excellent method for the determination of PAH. However for a sample to be certified it is essential that it be tested by at least two different analytical techniques. We believed that microbore column LC/MS would serve as a viable second technique for the certification of this sample. In addition, the analysis of this sample by our proposed system would allow us to directly compare the results obtained from our studies with those obtained by GC/MS. Ultimately this would give us an excellent marker by which we can gauge the performance of our microbore column LC/MS system. Therefore the last objective of this study is the analysis of the aforementioned marine sediment extract to allow us to properly evaluate the performance of our microbore column LC/MS system.

1.6 RESEARCH OBJECTIVES

The following objectives were developed with the ultimate aim being an increased knowledge of the performance and applicability of the combination of microbore column liquid chromatography with mass spectrometry and evaluation of this system for the eventual purchase of microbore column liquid chromatography equipment:

a) The modification of currently available HPLC instrumentation for use with microbore columns and the performance evaluation of such a system using PAH standards.

b) The application and performance evaluation of microbore column liquid chromatography for use with a mass spectrometer equipped with a moving belt LC/MS interface using PAH standards.

c) The qualitative and quantitative determination of PAH in a marine sediment extract using microbore column liquid chromatography-mass spectrmetry.

2. EXPERIMENTAL

2.1 REAGENTS

2.1.1 Solvents

HPLC grade methanol, acetonitrile, and methylene chloride were purchased from Caledon (Georgetown,Ontario). Distilled water was treated in a Millipore Milli-Q water (Millipore Corp., Bedford, MA) purification system (one ion-exchange, two carbon and one 0.22 um particulate filters).

2.1.2 Standards and Samples

The National Bureau of Standards (Washington, D.C.) standard solution of 16 PAHs in acetonitrile (SRM-1647) was purchased in sealed ampoules. The concentrations are given in Table 1. The ampoules were stored at room temperature in the dark and snapped open just before analysis. The contents were then distributed over three vials sealed with teflon-lined screw-caps. Aliquots were taken immediately for dilutions of 1:1, 1:5, and 1:10 in acetonitrile. Solutions were then stored in the refrigerator to minimize evaporation.

The National Research Council of Canada Atlantic Regional Laboratory (Halifax, N.S.) sediment extract (sediment code CASS-794) was

obtained in a sealed ampoule. The extract of 35 g of marine sediment had already been taken through a clean-up scheme (shown in Figure 3 and discussed in section 2.1.3) to isolate PAHs. The ampoule was then sealed as a hexane solution. The extract had considerable amounts of a yellow-brown precipitate (probably sulphur) in the ampoule. When the analysis was to be performed, the ampoules were snapped open and the contents were transferred quantitatively with methylene chloride washes to volumetric flasks and made to the mark with methylene chloride (10.00 mL). Aliquots of this solution were then taken for dilutions (usually in acetonitrile). If a solution had to be analyzed by HPLC without dilution, an aliquot of the solution was blown down to dryness with a nitrogen stream and then dissolved in the same volume of acetonitrile. A precipitate (probably sulphur) often formed in such situations and filtration through a 0.45 um teflon filter (Millipore Corp., Bedford, MA) was performed to prevent blockage of the columns.

2.1.3 Sample Extraction and Cleanup

The sample extraction and cleanup was performed by NRC personnel. The procedure used is illustrated in Figure 3. The sediment (35 g) was Soxhlet extracted with 150 mL hexane for 24 hr. After evaporation to approximately a 5 mL volume on a rotary evaporator, extracts were eluted through a column of silica and copper (7 g of 100/200 mesh silica gel activated at 150°C, slurry packed with hexane, covered with a 1 cm layer of copper (cleaned with rinses of 20% nitric acid, water, acetone and hexane) and washed with 40 mL ethyl ether) with 15 mL ethyl ether

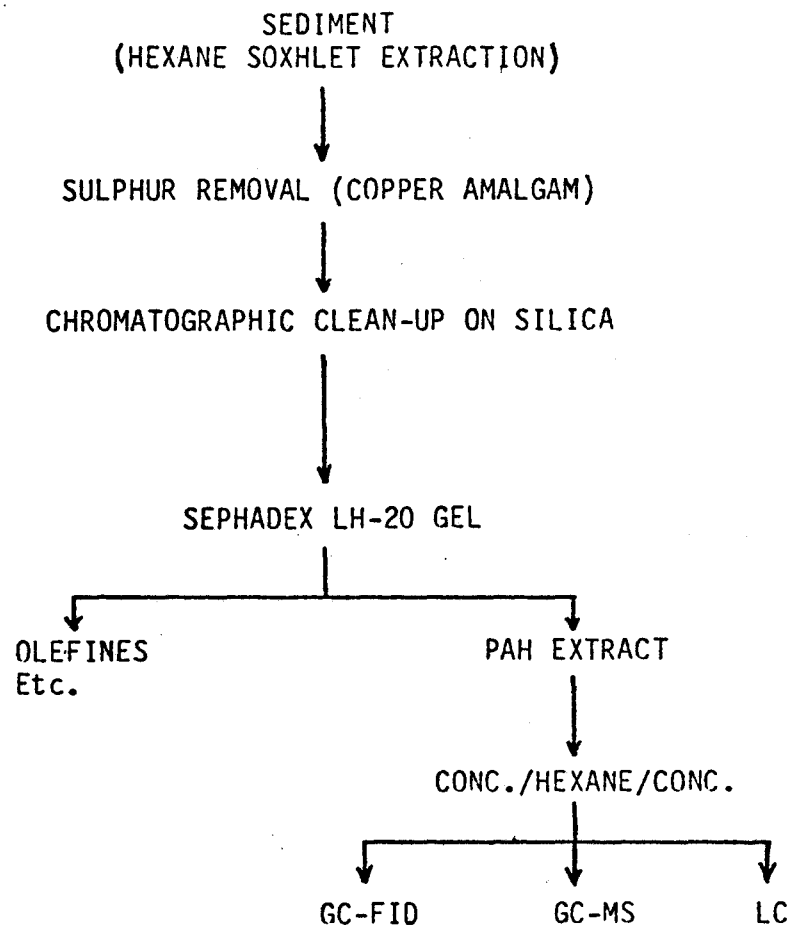


Figure 3: Clean-up scheme used by NRC for analysis of PAH in sediments.

followed by 3 mL 20% methylene chloride in ethyl ether and 20 mL of 40% methylene chloride in ethyl ether. The total eluate was taken over into hexane and evaporated to approximately 5 mL on a rotary evaporator. This extract was then eluted on a Sephadex LH-20 column (Pharmacia Fine Chemicals, Dorval, Quebec) (20 g of 25/100 um gel preswelled overnight and slurry packed into a 300 x 19 mm I.D. glass column) with 100 mL of cyclohexane/ methanol/methylene chloride (6/4/3) (The column was previuosly calibrated with azulene and perylene). Generally, the first 30 mL was discarded and the next 60 mL PAH fraction was collected. This fraction was reduced in volume, taken over into hexane, and sealed in ampoules.

2.2 INSTRUMENTATION

2.2.1 Microbore Liquid Chromatography

A Spectra Physics (Santa Clara, CA) model 8700 liquid chromatograph equipped with a Rheodyne model 1710 (Cotati, CA) 1 uL loop injector and a Spectra Physics (Santa Clara, CA) model 770 variable wavelength UV absorption detector (1 uL flow cell) were modified for use with microbore columns (1 to 2 mm I.D. x 25 cm.). Modifications are schematically shown in Figure 4. The flow from the SP8700 pumping system was split with a low dead volume union tee between the microbore column and the conventional column (flow restriction device). A Rheodyne model 1710 microloop (1uL) injector was placed after the splitter and connected to the microbore column with a short length of tubing. The

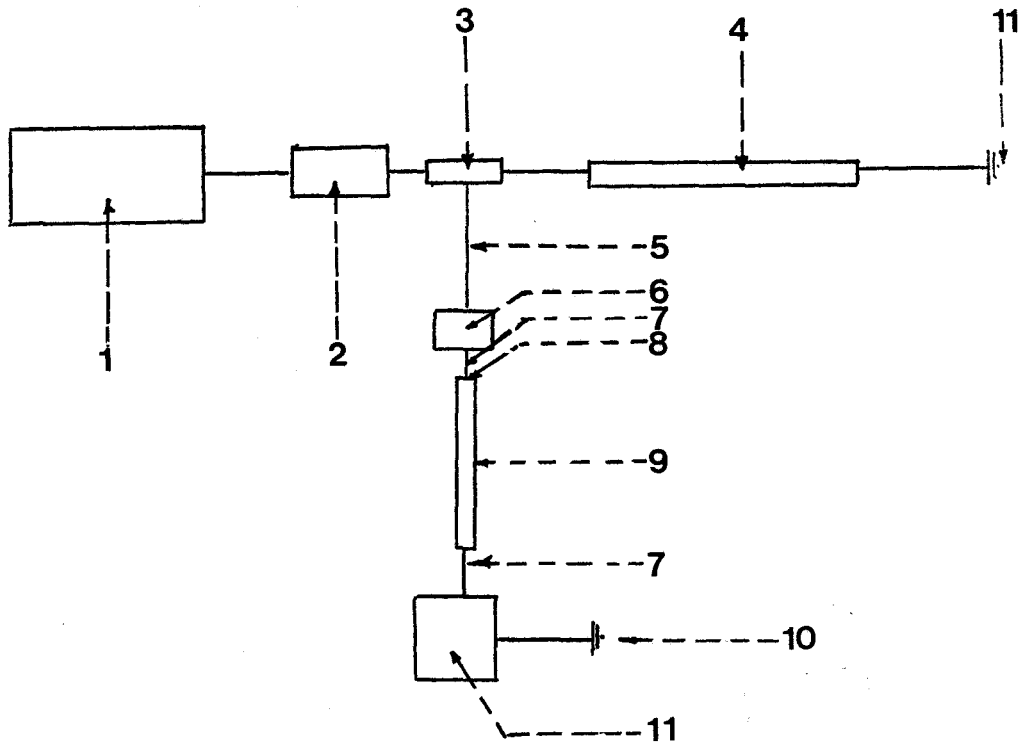


Figure 4: Schematic diagram of the effluent split microbore column liquid chromatograph: 1) Spectra Physics model 8700 liquid chromatograph; 2) solvent mixer;3) two way splitting tee 4) reverse phase (C-18) column (4.6 mm I.D. X 25 cm); 5) conventional liquid chromatography stainless steel tubing (0.50 mm I.D.);6) low volume injector (1 uL); 7) narrow bore stainless steel tubing (0.13 mm I.D.); 8) zero dead volume fitting; 9) reverse phase (C-18) microbore column (1 mm I.D. X 25 cm); 10) waste; and 11) ultra-violet absorption detector equipped with a 1 uL (1 mm path length) flow cell (with heat sink tubing by passed).

reverse phase microbore column used was 25 cm x 1 mm I.D. and was custom packed by Alltech (Alltech Assoc., Deerfield, Ill.) with 5 um Vydac 201-TP polymeric C-18 packing (Separations Group, Hesperia, CA.). The microbore column was then connected to a Spectra Physics model 770 variable wavelength UV-VIS absorption detector by another short length of teflon capillary tubing (5 cm x 0.18 mm I.D.). The normal 8 uL detector flow cell (10 mm pathlength) and heat sink tubing was replaced by a 1 uL micro-flowcell with a 1 mm pathlength.

Flow measurements were done by connecting a 50 uL precision micropipette via silicone tubing to the microbore column outlet or to the waste line of the detecter flow cell and measuring the time taken to fill the pipette. A plot of resultant flow rates versus pump pressures (at various pump flow rates from 0.5 to 2 mL/min) was used to determine microbore flow rates during analysis. The microbore system was tested for chromatographic performance with the NBS PAH standard. The column used for the PAH analysis was a custom packed microbore column (Alltech Associates, Deerfield, Ill.) (1 mm I.D. x 25 cm), packed with 5 um Vydac 201TP (Separations Group, Hesperia, CA). Chromatographic conditions utilized for this column were either a linear gradient of 60% ACN/H_2O to 100% ACN in 20 min and hold or isocratic elution at 80% ACN/H_2O both with a flow of approximately 40 uL/min at start.

2.2.2 Microbore Liquid Chromatography-Mass Spectrometry

Combined microbore liquid chromatography-mass spectrometry (LC-MS) was performed with a VG Micromass belt-transport LC-MS (Altrincham, U.K.) interface attached to a VG 7070F mass spectrometer (Altrincham, U.K.). Data acquisition and processing was performed on a VG 2035 data system (Altrincham, U.K.). The microbore column LC system (described in section 2.2.1) was attached to the LC-MS interface as shown in Figures 2, 5 and 6. The PAH standards and marine extract were chromatographed as described in Section 2.2.1.

Analysis using the full scan mode were performed with a scan range of 450 to 90 amu at 2 sec/dec with an exponential down scan and an interscan delay of 1 sec. Other parameters of importance are listed below. Selected ion monitoring was performed with accelerating voltage jumping using a dwell time of 100 msec. The masses monitored were 152, 154, 166, 178, 202, 252, 276, 278, and a lock mass of 281.

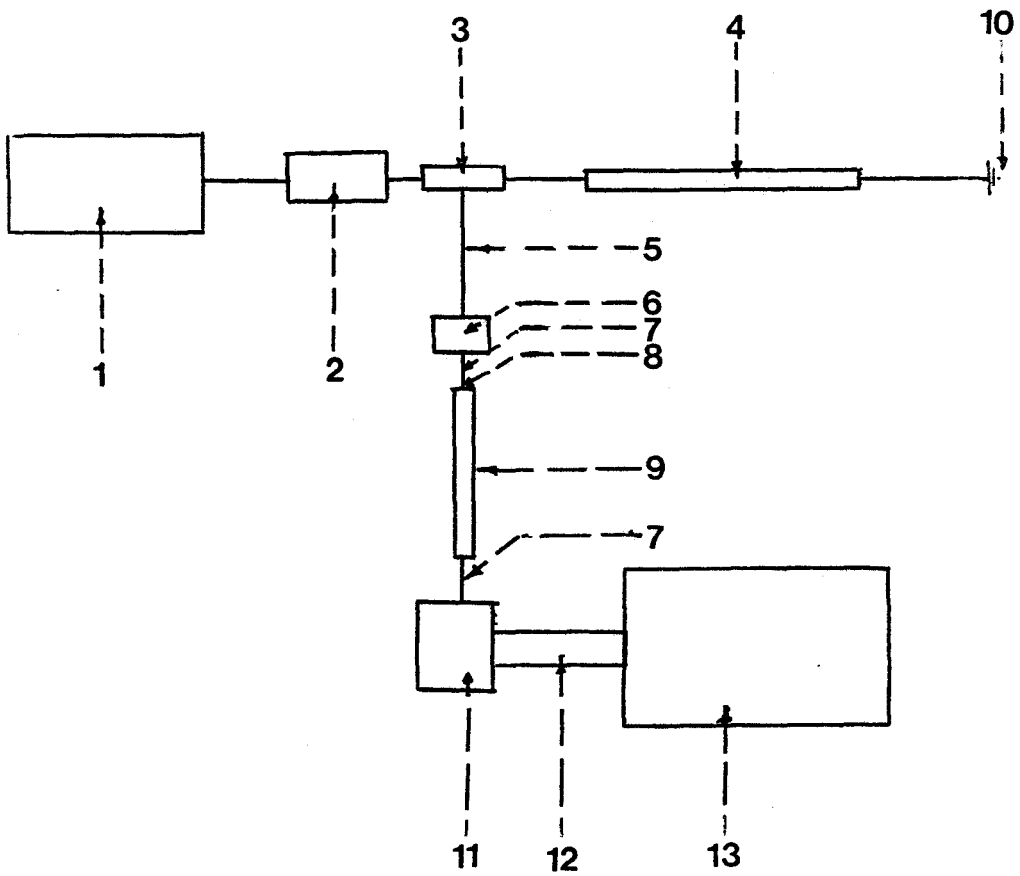


Figure 5: Schematic diagram of the microbore column liquid chromatography-mass spectrometry set-up: 1 to 9) same as on Figure 4; 10) waste; 11) liquid chromatography-mass spectrometer (LC/MS) moving belt interface housing; 12) LC/MS vacuum locks; and 13) VG 7070F mass spectrometer.

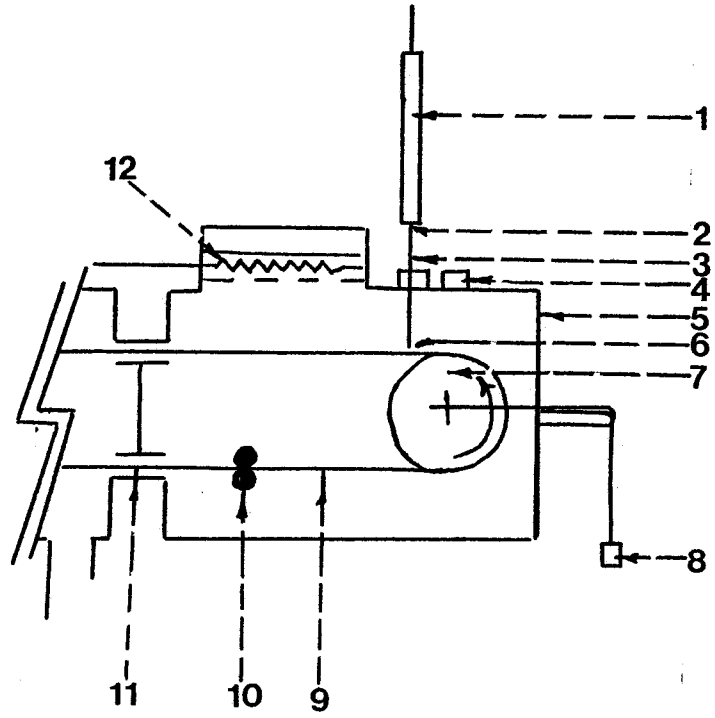


Figure 6: Detailed schematic diagram of the microbore column liquid chromatography - mass spectrometry moving belt interface: 1) microbore column (1 mm I.D. x 25 cm); 2) zero dead volume fitting; 3) narrow bore stainless steel tubing (0.18 mm I.D. x 6 cm); 4) syringe port; 5) liquid chromatography-mass spectrometer interface housing; 6) 1 mm gap between effluent tubing and moving belt; 7) idler wheel; 8) belt tension weight; 9) polyimide belt; 10) pinch roller and belt drive; 11) vacuum tunnel seal (50 to 100 torr); 12) infrared heater.

Selected ion monitoring conditions used are listed below.

Optimum conditions for the microbore LC/MS analyses of PAHs were as follows:

Belt speed	0.5 cm/sec
Infrared heater	30%
Sample evaporator	80%
Inlet housing pressure	-0.8 barr
Ion source pressure	5×10^{-8} torr
Source temperature	250°C
Filament conditions	70 eV at 100 uA
Multiplier gain	2.5 KV
FA3 amplifier gain	1×10^{-7} Amps
Response time	0.3 msec (10 msec for SIM)

2.2.3 Gas Chromatography

Gas chromatography was performed on a Varian model 3700 gas chromatograph (Georgetown, Ontario) equipped with a cold on-column injector (J + W Scientific, Orangevale, CA) and a flame ionization detector (FID) at 300°C. The capillary column was a 30 meter narrowbore (.25 mm I.D.) fused silica DB-5 column (polymethyl + 5% phenyl siloxane, 0.1 mm phase thickness, J+W Scientific, Orangevale, CA). The column oven temperature was programmed from 60 to 300°C at 5°C/min and held for 40 min at 300°C. The helium carrier gas linear

velocity was set at 30 cm/sec.

2.2.4 Gas Chromatography - Mass Spectrometry

The selected ion monitoring GC/MS experiments were performed on a VG 7070F mass spectrometer equipped with a VG 2035 data system (Altrincham, U.K.). The selected ion monitoring conditions used were the same as those used for LC/MS analyses (see section 2.2.2). The fused silica column was directly connected to the ion source. The He carrier gas linear velocity was set at 50 cm/sec. All chromatographic conditions used are listed in section 2.2.3. The data was processed with the VG 2035 data system.

2.2.5 Chromatographic Data System

A 64K Apple II+ microcomputor (Apple Computor Inc., Cupertino, CA.) with an Interactive Microware "Adalab" interface card and "Ada-amp" amplifier (Interactive Microware Inc., State College, PA.) with multiplexing and programmable attenuation was used for chromatography data acquisition. A Hewlett Packard HP 7470A (Mississauga, Ont.) digital plotter was used for plotting data. All software for data acquisition, plotting and data analysis was written by M. Quilliam and R. Mann.

2.3 QUANTITIATION

Quantitiation of 16 PAH (listed in Table 1) was performed by microbore LC/MS-SIM using an external calibration method. Preparation

of the NBS standards and marine sediment extract is outlined in section 2.1.2. Three dilutions of the standard solution (1:1, 1:5, and 1:10) and undiluted extract were randomly injected (1 uL) in triplicate (in some cases, where time permitted, additional injections were made). Samples were then chromatographed as outlined in section 2.2.1. Selected ion monitoring conditions used are described in section 2.2.3. Both peak height and areas were measured using the VG 2035 data system interactive software. These values were then plotted against amount injected and subjected to a least squares linear fit. Relative weight responce (RWR) was then calculated by dividing peak heights or area by the dilution factor and the compound concentration.

3. RESULTS AND DISCUSSION

3.1 MICROBORE COLUMN HPLC SYSTEM

When we attempted to use a conventional HPLC system with microbore columns we found that flow settings between the range of 30 to 100 uL/min resulted in inaccurate and irreproducible flow rates and solvent composition in gradient operation. The next step was the modification of a conventional HPLC system to suit microbore work. The equipment that could be used was restricted to instruments available in our laboratory. For reasons of portability for use with the mass spectrometer, gradient capability and general accessibility to components for ease of modification, the SP8700 HPLC system was chosen.

The modification design chosen was a novel, inexpensive approach to the problem: operation of the conventional pumping system at normal flow rates (0.5 to 2 mL/min) in conjunction with a splitting system to control flow rates to the microbore column. In this way, it was hoped that accurate flow rates and gradients could be achieved. Obviously, with this approach, one advantage of microbore column HPLC is lost, i.e., solvent economy.

Figure 4 schematically illustrates the system that was constructed. The flow from the SP8700 pumping system was split with a low dead volume union tee between the microbore column and a flow

restriction device. The pressure drop across the restriction and the SP8700 pumping rate were adjusted to give the appropriate flow rate to the microbore column. Various restriction devices were studied. A simple needle valve and narrow bore tubing proved to be unsatisfactory. Reproducibility of flow control was not good. An old silica HPLC column (4.6 mm I.D. x 25 cm) was also used, but proved to give poor reproducibility in gradient operation (presumably due to slow equilibration of the silica with reverse phase solvents). Finally, it was found that an old reverse phase HPLC column (4.6 mm I.D. x 25 cm, 5 um Ultrasphere-ODS) gave excellent flow rate control and reproducibility. A Rheodyne model 1710 microloop (1 uL) injector was placed after the splitter and connected to the column with a short length of narrow bore (5 cm x 0.007 mm I.D.) stainless steel capillary tubing. The reverse phase microbore column used was 25 cm x 1 mm I.D. and was custom packed by Alltech with 5 um Vydac-201TP polymeric C-18 packing. This packing was selected for its excellent selectivity for PAH. The column was connected to a Spectra Physics model 770 variable wavelength UV-VIS absorption detector by another short length of teflon capillary tubing (5 cm x 0.018 mm I.D.). The normal 8 uL detector cell (pathlength 10 mm) and heat sink tubing was replaced with a 1 uL micro-flowcell with a 1 mm pathlength. This micro-flowcell was necessary to avoid band broadening, but the short pathlength did reduce the sensitivity of the system by 10-fold and defeated one of the advantages of microbore column HPLC, i.e., greater sensitivity with concentration-dependent detectors. Commercial systems are now available

with long pathlength micro-flowcells, but are quite expensive. For the purposes of testing our microbore HPLC system prior to LC/MS, this detection system was adequate.

The mobile phase flow rate through the microbore column was measured under various isocratic conditions, with a calibrated 50 uL microcapillary attached to the detector outlet with silicone tubing. Mobile phase composition and SP8700 pumping rates were adjusted. The results of these experiments are presented in Figures 7 and 8. Results showed that there existed a linear relationship between flow rate through the microbore column and pressure. This relationship allowed us to later determine or adjust the flow rate by simply monitoring the system pressure. Further testing of the reproducibility of this relationship, showed that although short term variations were minimal, long term reproducibility was somewhat poorer, possibly due to plugging of the column inlet frit by particulates. This situation was rectified by routine replacement of column frits or by using a Rheodyne model 1710 inline filter that could be frequently replaced when necessary. The system performance was also tested under gradient conditions. Figure 9 demonstrates that a linear relationship exists between solvent composition and flow rate. Thus flow rate changes during a gradient. The reproducibility of these results was similar to that of isocratic operation.

To test the entire system, a standard mixture of benzene, biphenyl and naphthalene was chromatographed under isocratic conditions. A

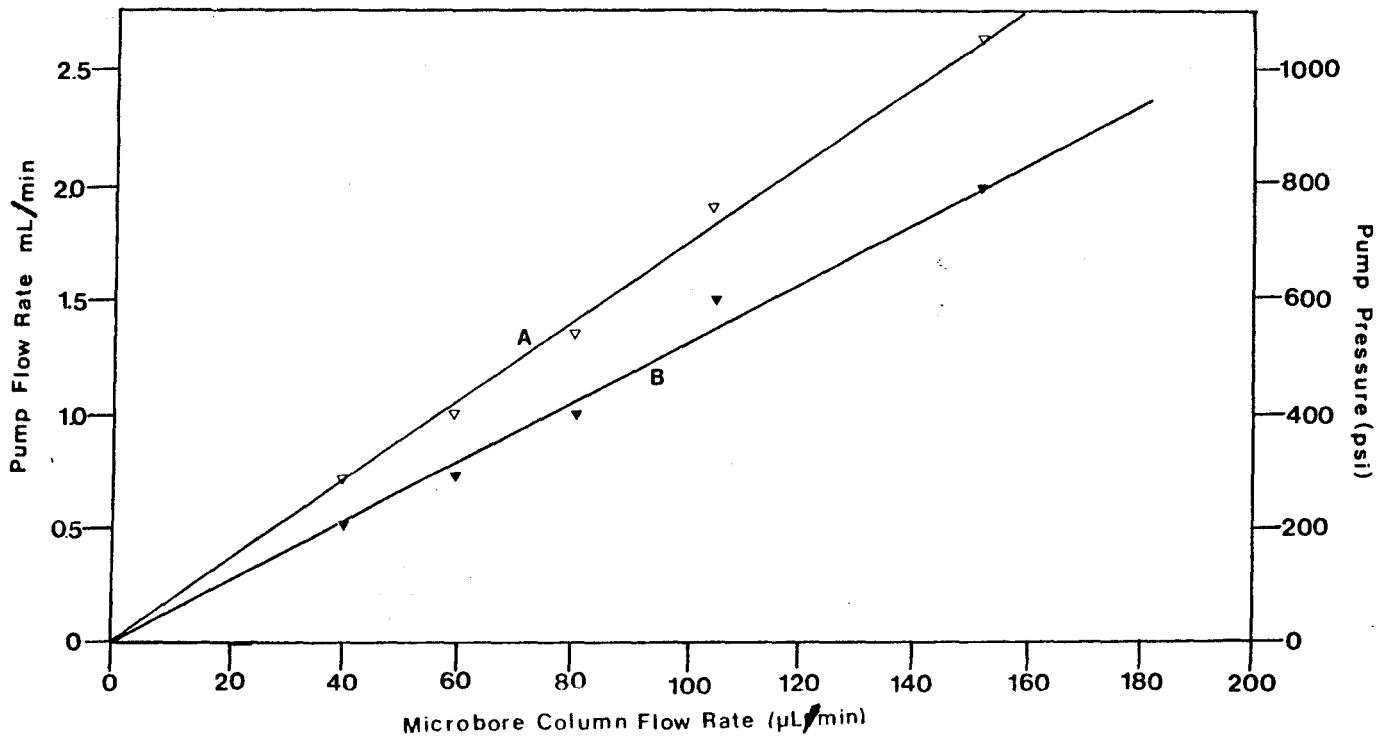


Figure 7: Relationship between flow rate through the microbore column and A) pump flow rate and B) pump pressure at 100% acetonitrile. The column used was a Vydac 201-TP reverse phase column (1 mm I.D.x 25 cm).

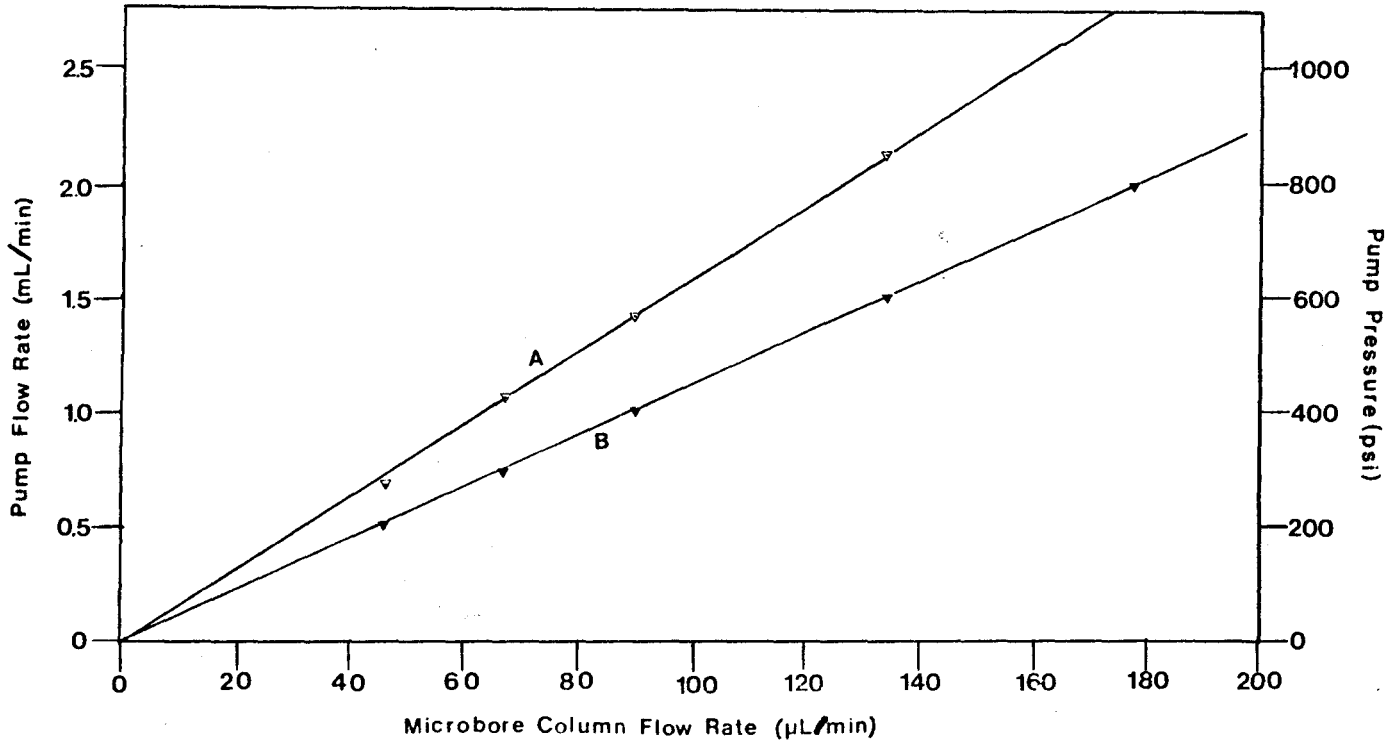


Figure 8: Relationship between flow rate through the microbore column and A) pump flow rate and B) pump pressure at 80% acetonitrile/water. The column used was a Vydac 201-TP reverse phase column (1 mm I.D. x 25 cm).

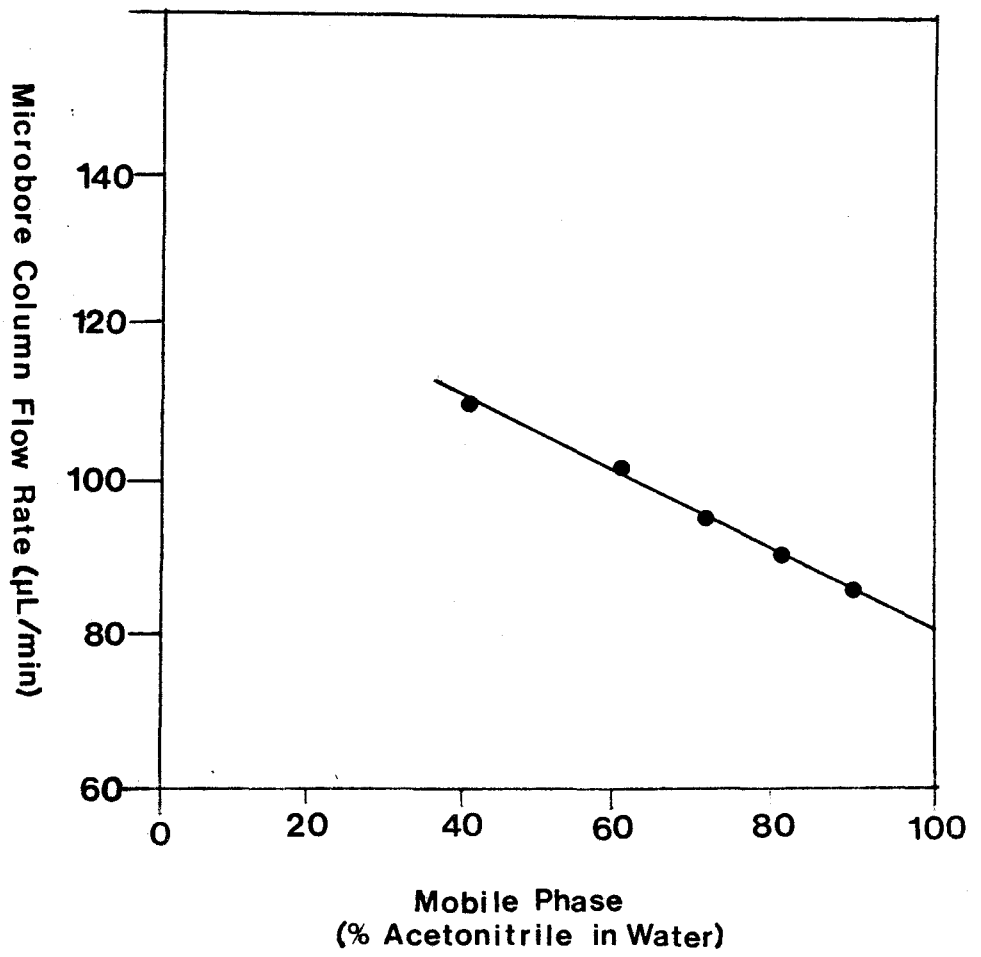


Figure 9: Relationship between mobile phase compostion and flow rate through the microbore column. Conditions: 1 mL/min of mobile phase varying from 40% to 100% acetonitrile and water using a Vydac 201-TP reverse phase column (1 mm I.D. x 25 cm), 1130 psi at start.

typical chromatogram is given in Figure 10. Excellent peak shape and sensitivity were observed. The reproducibility of retention times appeared to be quite good in the short term. However, the longer term reproducibility (over a period of a month) was poorer (a relative error of 2%). This was attributed to the gradual change in the pressure drop across the column (as discussed in the previous section).

Column efficiency values (N) calculated for biphenyl (k'=5.4) and naphthalene (k'=7.6) were 5700 and 4600 plates, respectively. In comparison to manufacturer's specifications of 6250 plates, our values were considered respectable. The effect of flow rate on N is shown in Figure 11. It was found that N maximized near a flow rate of 17 uL/min. However, operating at this flow rate was far too time consuming and we therefore chose to operate at about 30 uL/min with a reduced efficiency in order to have faster analyses.

Gradient elution operation was studied using a NBS standard containing 16 PAH. Figure 12 shows the excellent peak shape, resolution and sensitivity obtained. Short and long term reproducibility was found to be similar to the results obtained for isocratic analysis (a relative error of 3%). One disadvantage noted for gradient elution was the reduction of flow rate with the decrease of water percentage in the gradient. It would have been advantageous to have had the reverse so that the longer retained PAH would have had sharper peak shape.

The overall performance of the microbore HPLC system was deemed adequate to proceed to further studies involving LC/MS applications.

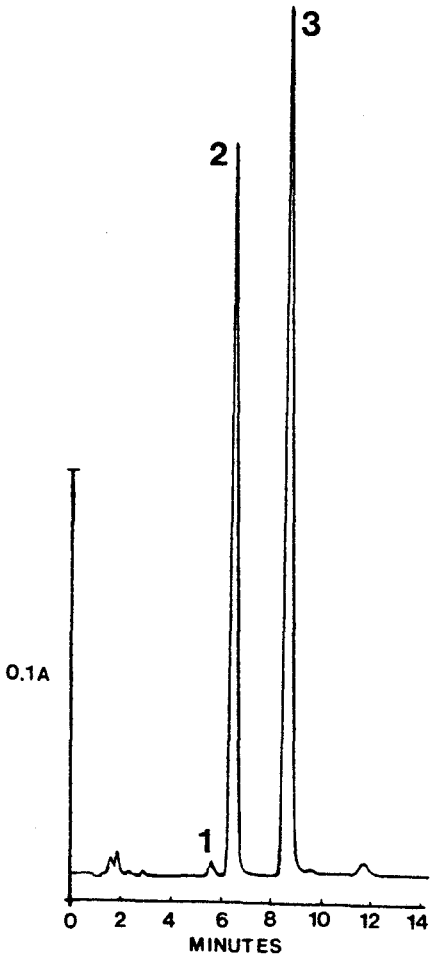


Figure 10: Microbore HPLC-UVD chromatogram of a benzene (1), biphenyl (2), and naphthalene (3) standard solution (1 uL at .25 mg/mL) on a Vydac 201-TP reverse phase column (1 mm I.D. x 25 cm). Conditions: isocratic 60% acetonitrile/water, 58 uL/min, 600 psi, .2 AUFS.

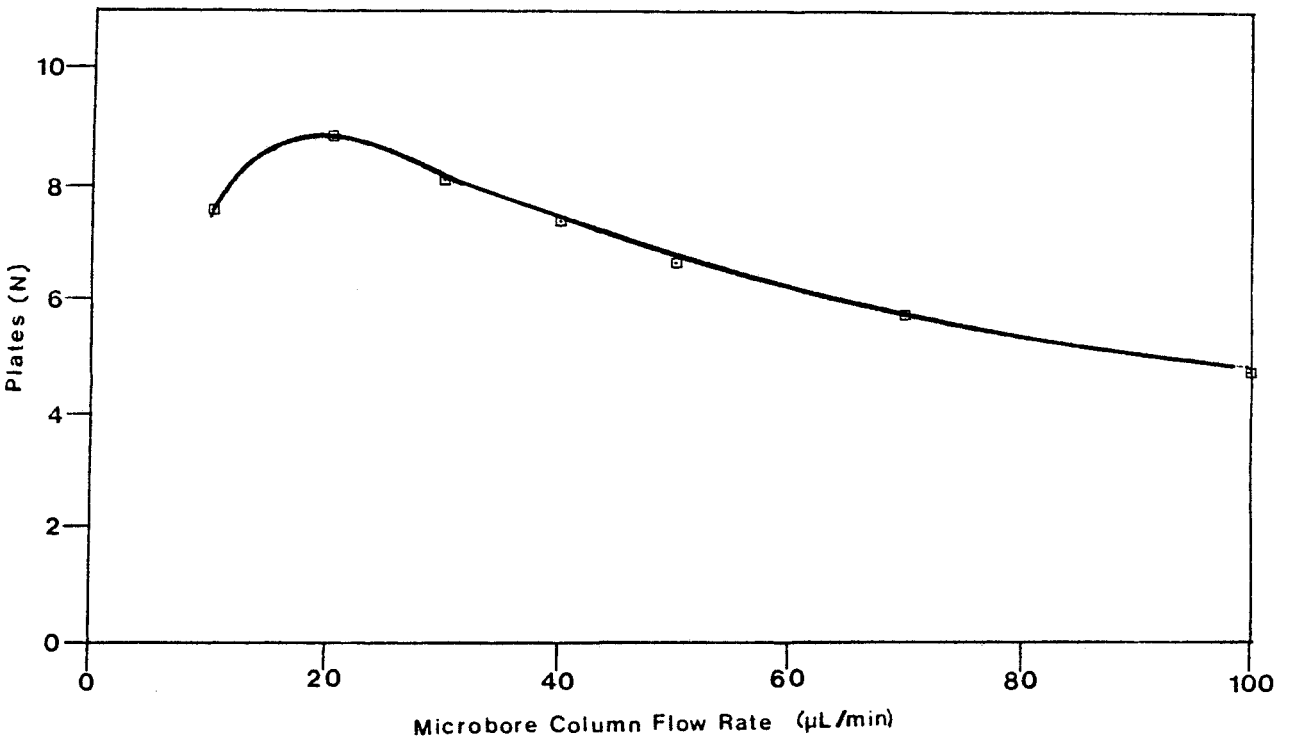


Figure 11: The effect of flow rate on column efficiency. Conditions: Vydac 201-TP reverse phase column (1 mmI.D. x 25 cm), 100% acetonitrile.

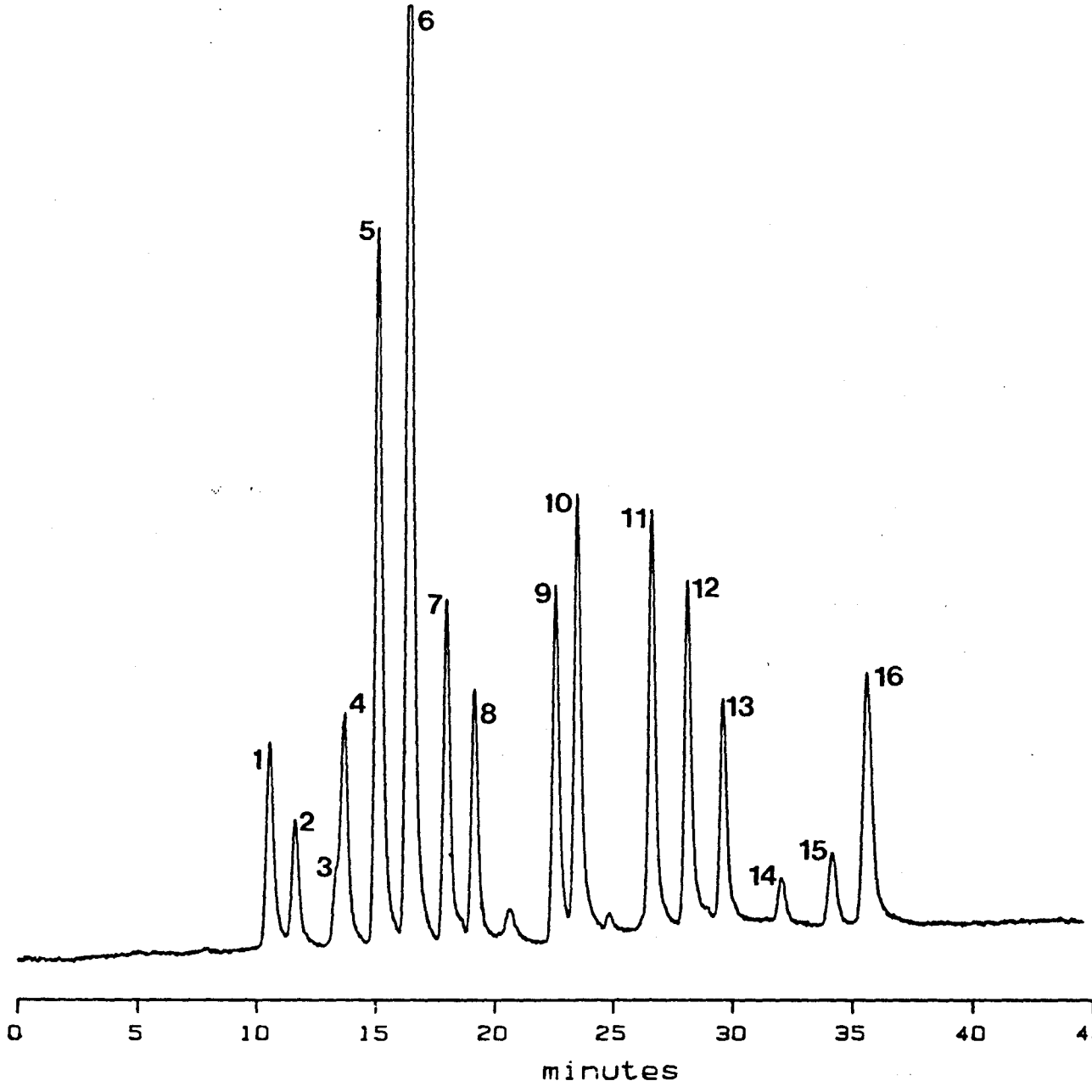


Figure 12: Microbore HPLC-UVD chromatogram of the NBS PAH standard (1uL) on a Vydac 201-TP reverse phase column (1 mm I.D. x 25 cm). Conditions: gradient of 60% to 100% acetonitrile/water in 20 min and hold, 30 uL/min, 920 psi at start, 0.25 AUFS. Refer to Table 1 for compound identities and concentrations.

3.2 MICROBORE COLUMN LC/MS

3.2.1 Conventional LC/MS Problems

The first LC/MS experiments conducted were with conventional columns (25 cm x 4.6 mm I.D.). From these studies we found three major problems when operating the LC/MS interface. Figure 13 illustrates the problems encountered. First, with flow rates of 1 mL/min, there is usually some backstreaming of the column effluent on the moving belt. This introduces band-broadening which reduces the apparent column efficiency. Another problem encountered was the formation of droplets on the belt due to the surface tension of the solvent. This is particularly troublesome with aqueous effluents. If these droplets were not all evaporated before reaching the tunnel seals, serious pressure fluctuations occurred in the mass spectrometer ion source resulting in noise due to defocussing of the instrument. In addition, the uneven distribution of the analyte on the belt yielded noisy chromatograms that were difficult to use for quantitation. Lastly, the rapid evaporation of large quantities of solvent by high infrared heater output resulted in sample loss due to codistillation and splattering of droplets. In gradient operation, it is difficult to find appropriate conditions for the changing solvent composition. Figure 14 depicts a typical conventional column LC/MS chromatogram that exhibited such problems. These problems could be minimized by drastic reduction of the flow rates into the LC/MS interface. It was found that gradient reverse phase

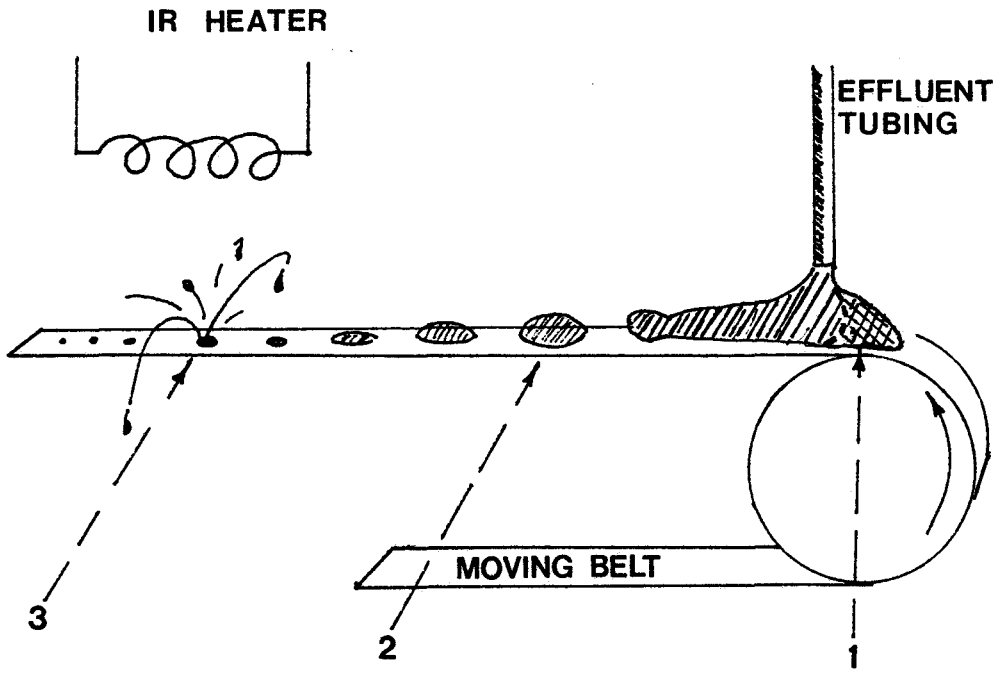


Figure 13: Coating problems encountered usaing a conventional HPLC column with a moving belt LC/MS interface: 1) backstreaming 2) droplet formation; and 3) droplet splattering.

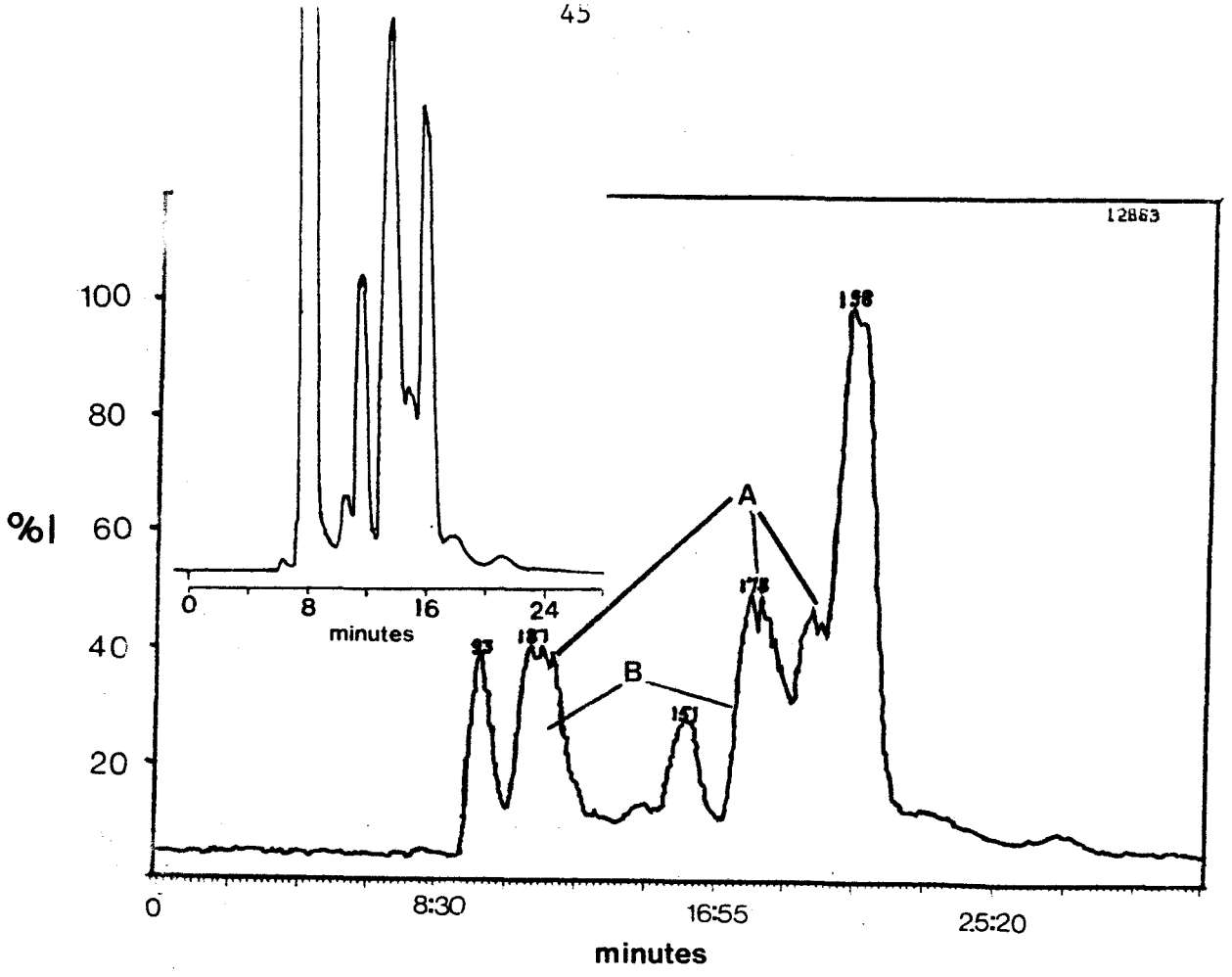


Figure 14: Conventional column moving belt LC/MS and HPLC-UVD traces of benzo[a]fluorene nitration products with a normal phase amino-cyano column (4.6 mm I.D. x 25 cm). Conditions: 1ml/min of isopropanol/hexane (85/15): A) noise due to droplet formation and splattering; and B) loss of resolution due to backstreaming.

operation was easiest when less than 100 uL/min was fed into the interface. Since such low flow rates are not suitable for conventional column operation, an effluent splitter is necessary. We have found that it is very difficult to design a splitter that does not introduce excessive dead volume and subsequent band broadening. In addition, a large portion of the sample is wasted in such an approach. This is certainly of importance in trace analysis where the amount of sample is usually limited.

3.2.2 Spray Deposition

Using a design similar to those of Vouros (18) and Smith (19) we constructed a spray deposition system (see Figure 15). In this method, a venturi with heated gas (helium) sprays the mobile phase onto the belt surface as a fine mist. In this way, the problems of backstreaming, droplet formation and splattering were avoided. One mL/min of 80% acetonitrile/water was easily accomodated. The system proved quite reliable once working, but was found to be time-consuming to set up. In addition, if the solvent composition changes drastically, as is the case during gradient elution, the spray changed from a fine mist to heavy droplets. Since completion of our work, commercial spray systems have become available that are easier to use and that can be programmed for gradient elution. However, these units are only designed to handle a maximum of 200 uL/min of polar solvent. This still requires an effluent splitter with conventional columns or the use of medium bore (2 mm I.D.) columns.

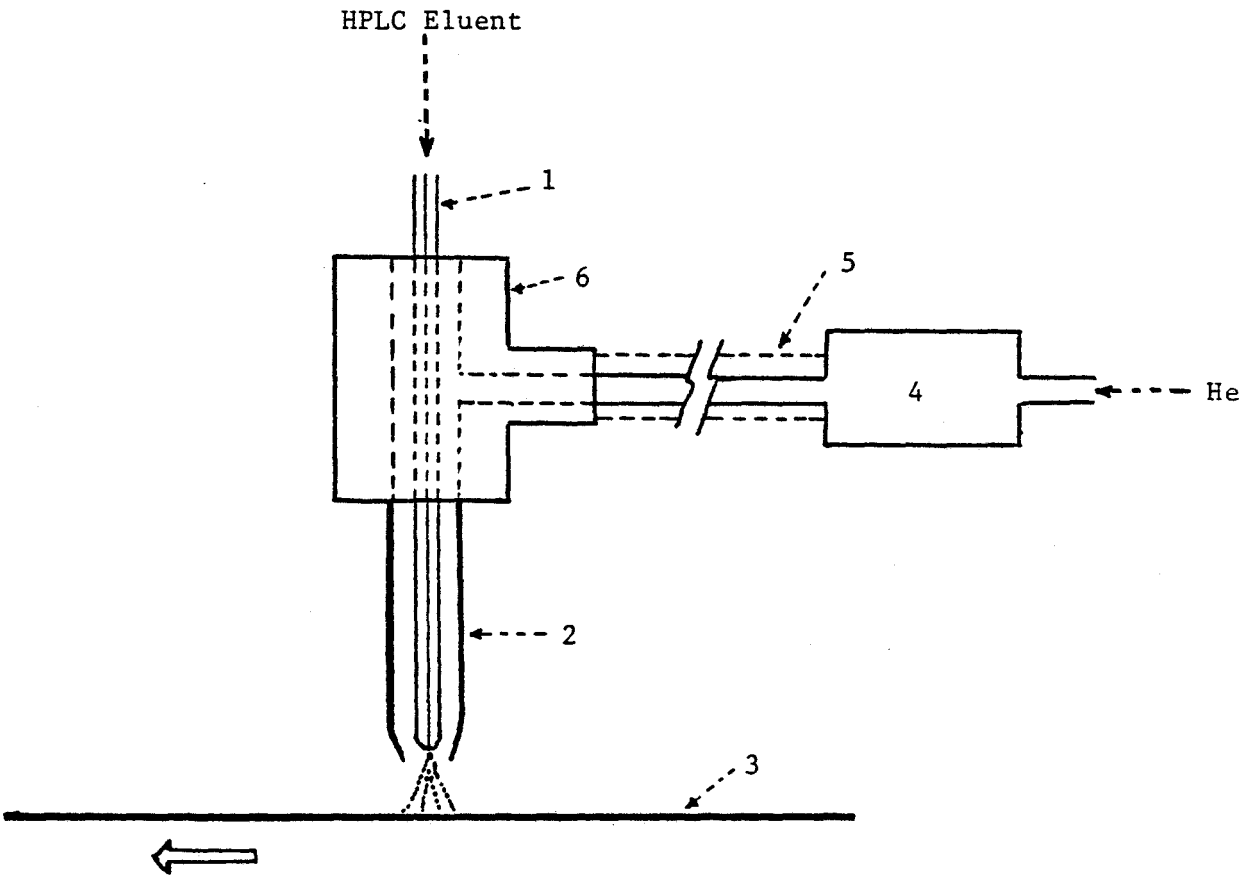


Figure 15: Spray deposition system used for HPLC-MS with conventional HPLC column.

1: Microbore capillary tubing from column outlet
2: Glass tubing
3: HPLC-MS belt
4: Gas heater system
5: Heated gas transfer line
6: Swagelok union-tee

3.3 MICROBORE LC/MS

Figure 16 shows representative microbore LC/MS results for an injection of 1 uL of a PAH standard mixture. A comparison with Figure 12, the microbore HPLC-UVD trace of the PAH standard, shows that there was no apparent degradation of resolution incurred when changing detection systems. This is best demonstrated by comparing the separation of compounds 3 and 4 in each figure. In both cases, the two compounds are eqully well resolved if not slightly better in LC/MS. Other parameters, such as peak shape and sensitivity also show no apparent change. A big advantage of the microbore system over the conventional column plus spray deposition system is the ease of setup and optimization. A range of flow rates (30 to 100 uL/min) caused no problems with uneven spreading of the eluent. Background noise, typically a problem with moving belt interfaces, was found to be manageable. A representative background spectrum is shown in Figure 17. The most common source of background was found to be the polyimide belt, with major ions at m/z 141 and 170, and silicone grease in the seals, with ions at m/z 207 and 281. Attempts to reduce background by washing the belt with various solvents and acids proved unsuccessful. It was found that the only way to reduce background was by continuous operation of the interface. As previously stated, in most cases the background did not pose any great problems since it could usually be subtracted from the raw data using various subtraction routines avaliable in the VG 2035 data system. However, for compounds at very low

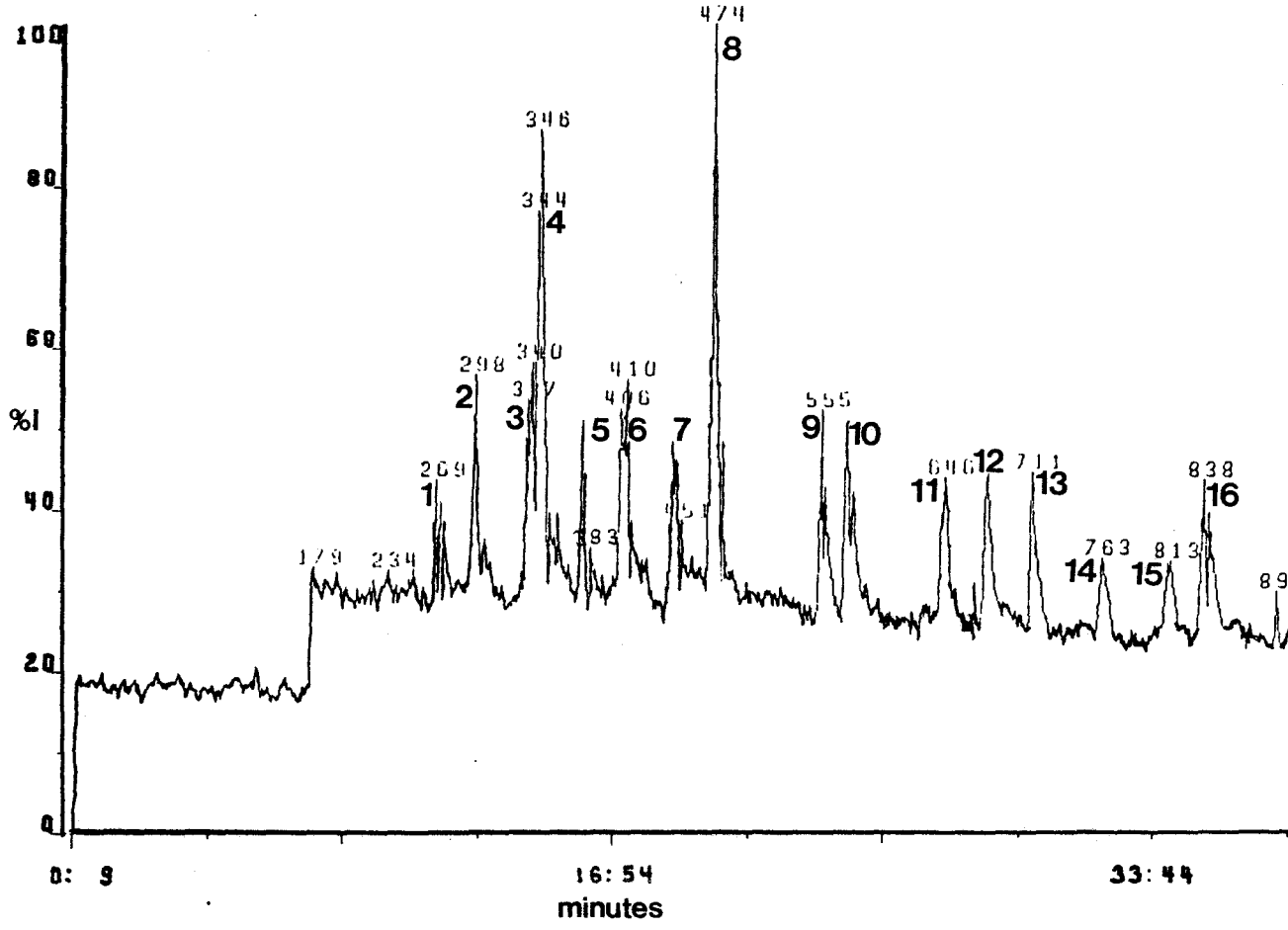


Figure 16: Microbore column LC/MS chromatogram of NBS PAH standard solution (1:1, 1 uL) using a Vydac 201-TP reverse phase column (1 mm I.D. x 25 cm). Conditions: gradient of 60% to 100% acetonitrile and water in 20 min and hold for 50 min, 30 uL/min 900 psi at start. See Table 1 for compound identities and concentrations.

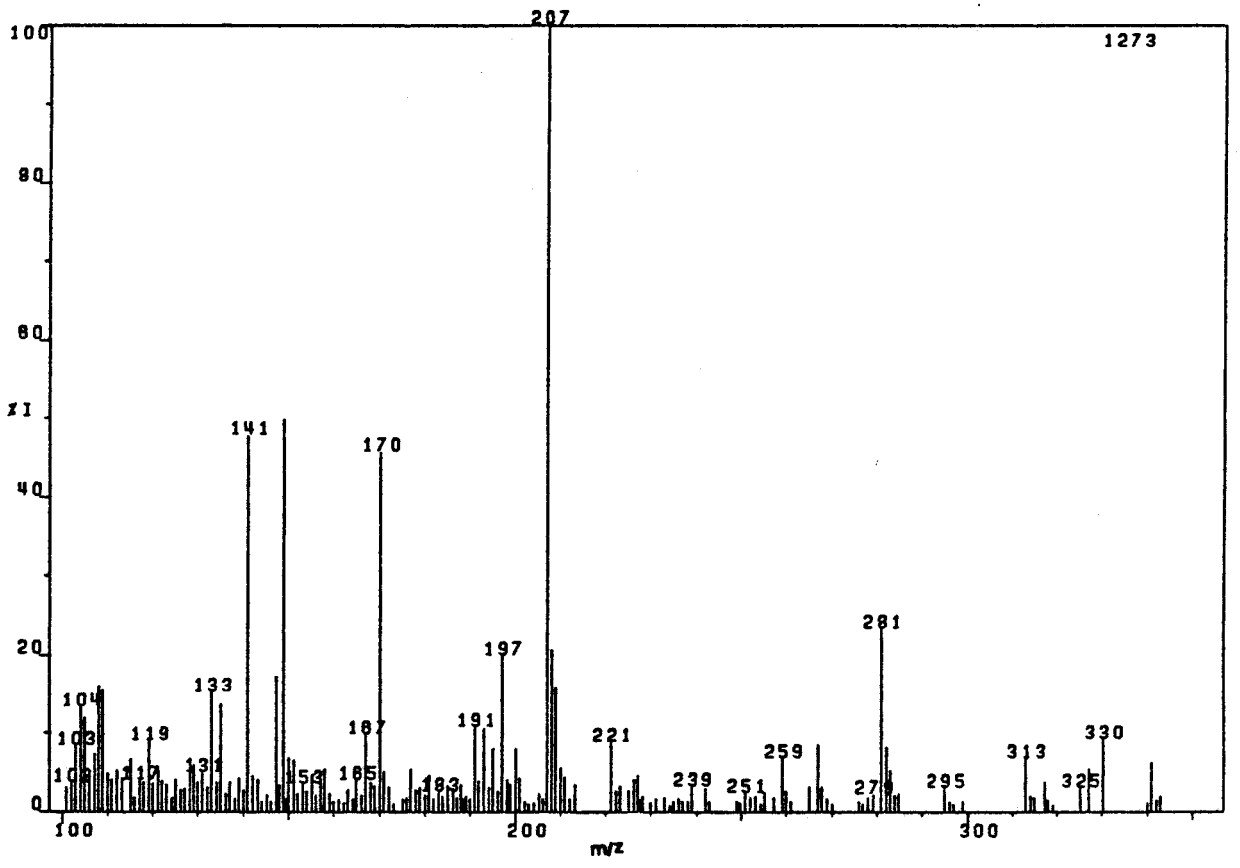


Figure 17: Representative electron ionization mass spectrum of the background obtained from a LC/MS experiment.

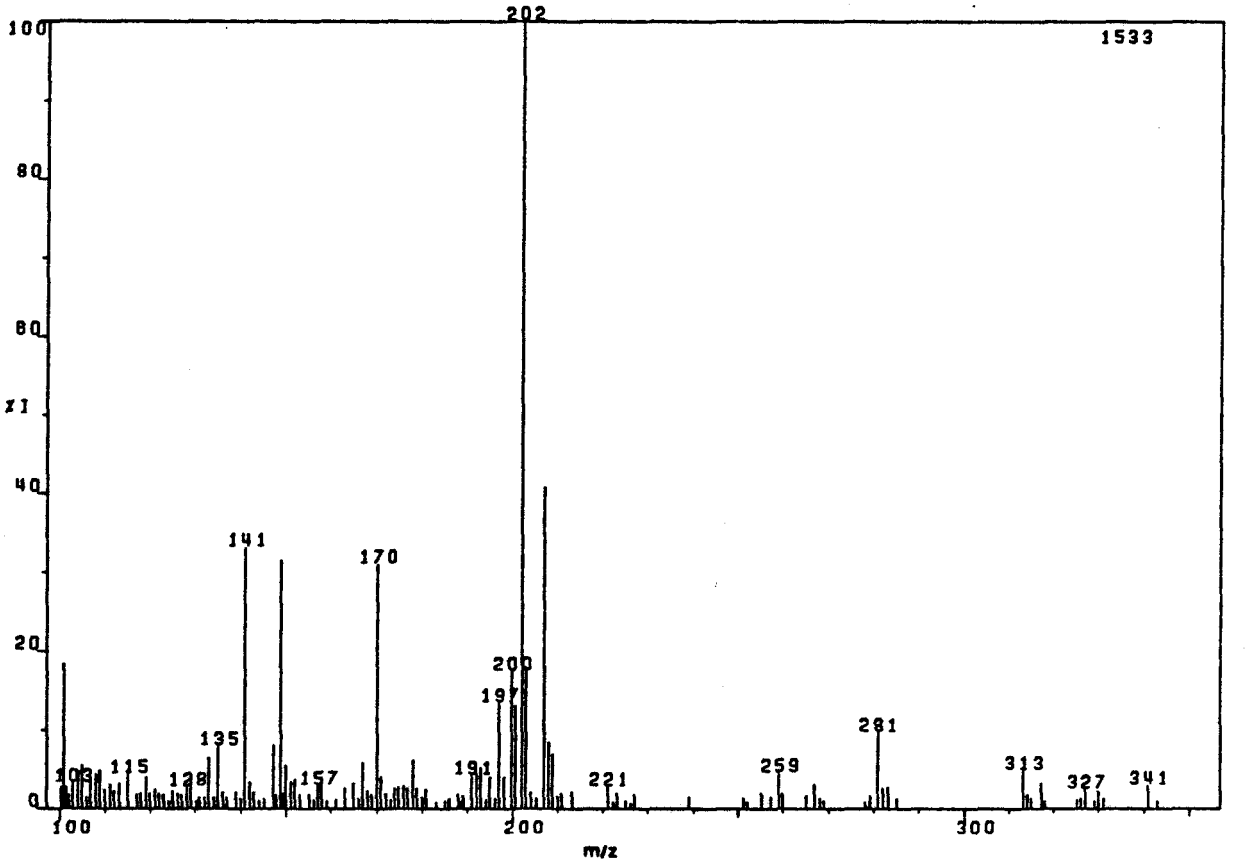


Figure 18: Electron ionization mass spectra of pyrene from a microbore LC/MS analysis of PAH standard (background not subtracted), 1 uL injected.

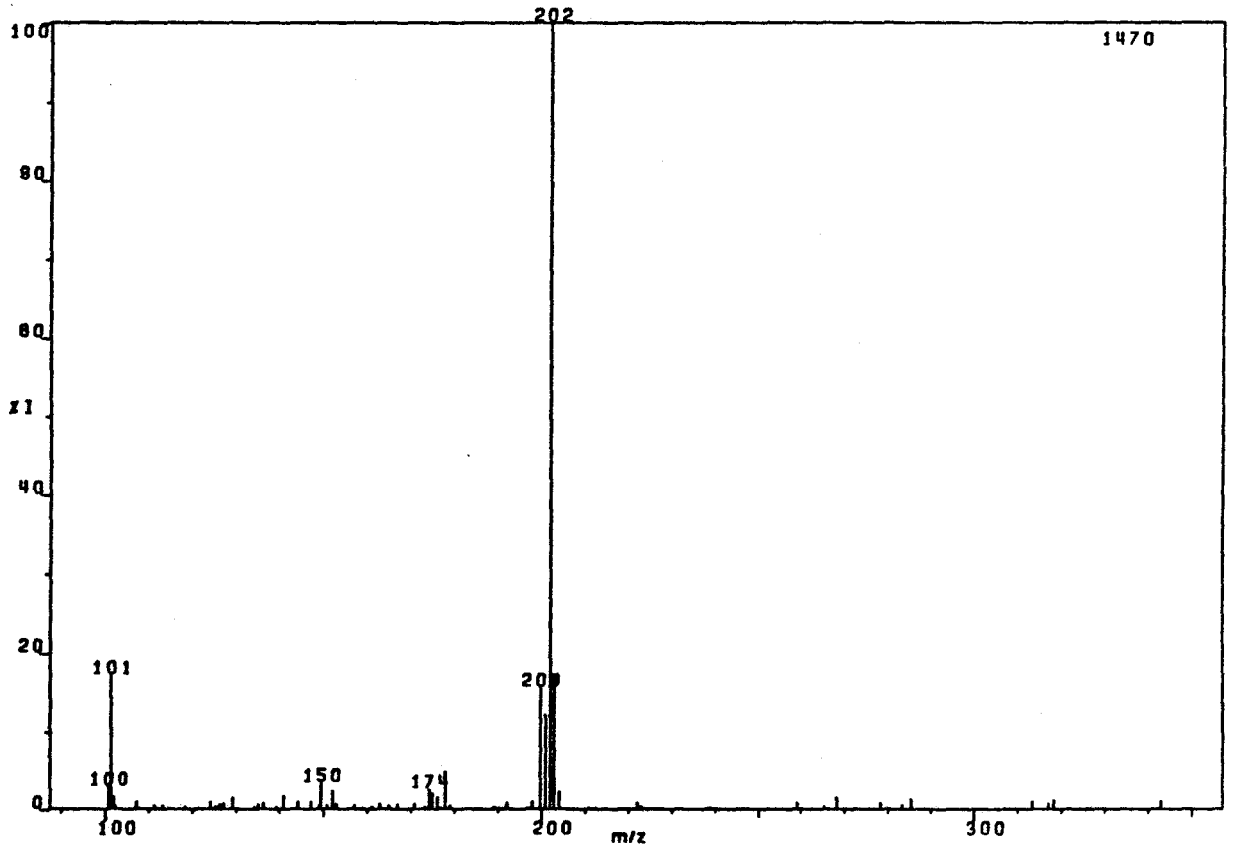


Figure 19: Electron ionization mass spectra of pyrene from a microbore LC/MS analysis of a PAH standard (background subtracted). Refer to Figure 18.

concentrations the background did cause conciderable problems, even when subtraction routines were used. To overcome this problem, sample concentration was necessary, since simply increasing the amplifier gain would proportionatly increase the background along with the sample signal. Figures 18 and 19, a raw spectrum and a background subtracted spectrum respectively, demonstrate that background can be effectivly removed without the loss of pertinent information. It was found that the various parameters of the moving belt interface (i.e., housing pressure, belt speed and sample evaporator temperature) play an important role in optimizing the analysis. These parameters and their effects are listed in Table 2.

3.4 QUANTITATION BY SELECTED ION MONITORING

Ultimately, in any analytical scheme, quantitation of the detected compounds is required. Selected ion monitoring (SIM) is an accepted quantitative method since it offers excellent sensitivity and selectivity. One of our objectives was the use of microbore LC/MS for the quantitative determ

Table 2: Common problems and system optimization techniques encountered with the moving belt interface.

Problem	Parameter adjustment
1) ghosting	- reduce belt speed - increase sample evaporator
2) source pressure fluctuations	- reduce interface housing pressure - increase infrared solvent evaporator output - decrease belt speed
3) droplets formation	- reduce belt speed - increase housing pressure by purging with argon - move column eluent tube closer to belt
4) droplet splattering	- increase housing pressure - decrease infrared solvent evaporator output
5) high background	- increase belt speed - decrease sample evaporator temperature - bake belt for several hours (by increasing sample evaporater temperature and source temperature)

second, which allowed about 20 to 30 data points across each peak. Figure 20 shows the results obtained from a SIM run performed on the NBS standard. Excellent peak shape with very little noise was observed, which greatly facilitated the quantitation. There was also no apparent loss of chromatographic efficiency, resulting in well resolved components.

Figure 21 represents some of the results of experiments designed to test the sensitivity and response linearity of the LC/MS-SIM system. In these experiments, isocratic conditions were used to reduce the required analysis time. Three dilutions of the NBS PAH standard solution (1:1,1:5 and 1:10) were injected in random order. Both peak heights and areas were measured using the VG 2035 data system interactive software. Plots of peak heights or area versus amount injected and a least squares linear fit revealed that there was a linear response with a near zero intercept for most compounds. There were exceptions, however; acenaphthalene, for example, exhibited an appreciable positive y-intercept of the linear regression fit (see Figure 21, top box). This effect was probably due to signal saturation above 10 ng as suggested by the dotted line in Figure 21. Indeno[1,2,3-cd]pyrene on the other hand showed an intercept on the x-axis (see Figure 18, bottom box). This may be due to incomplete evaporation of this low volatility compound from the moving belt into the source. A detection limit, defined by a signal to noise ratio of 2, of approximately 50 pg (injected) for the higher molecular weight PAH was estimated. The response factors are presented in Table 4(see section 3.6).

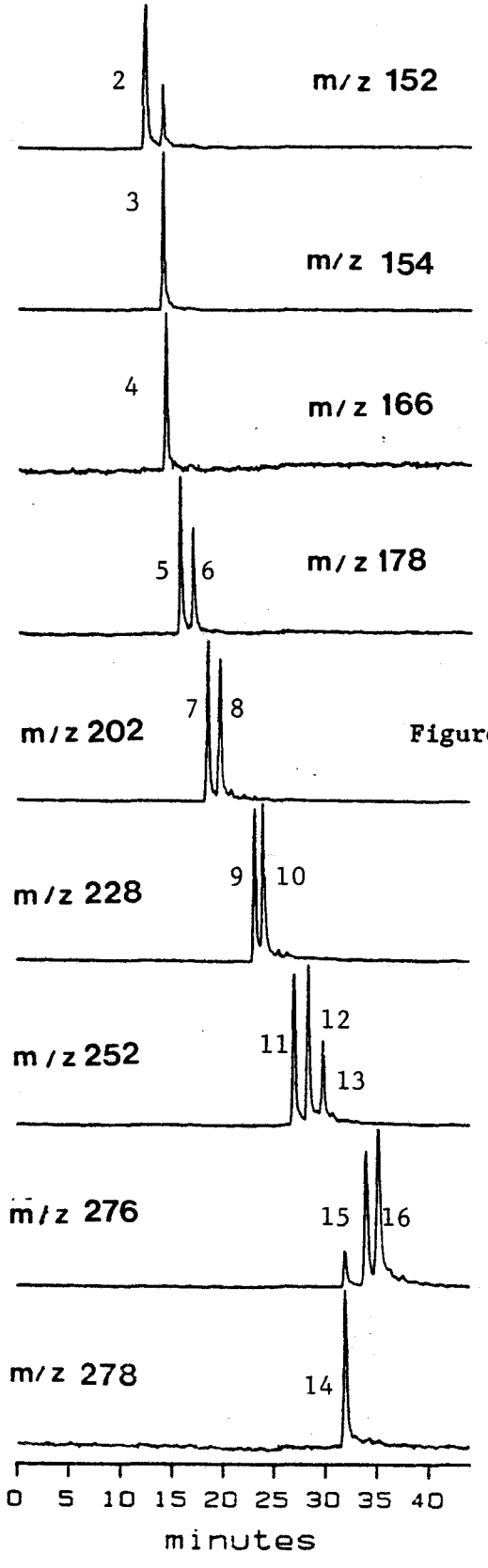
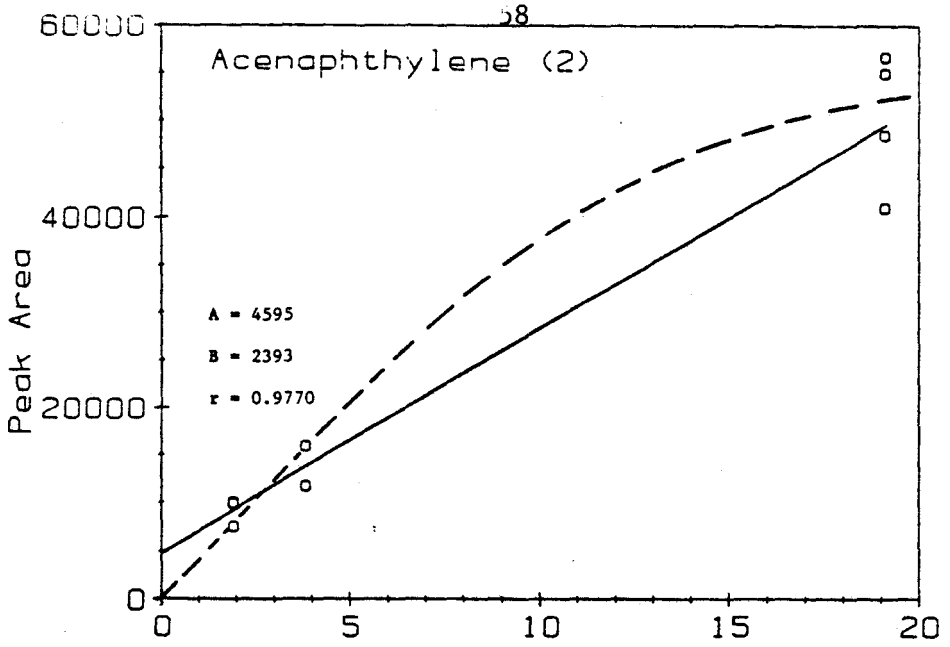
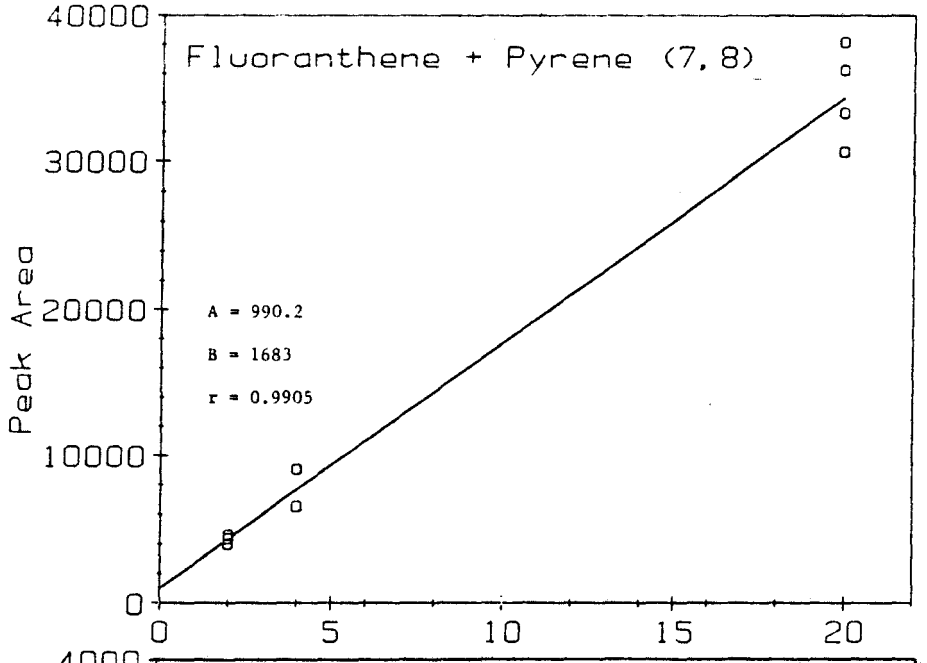
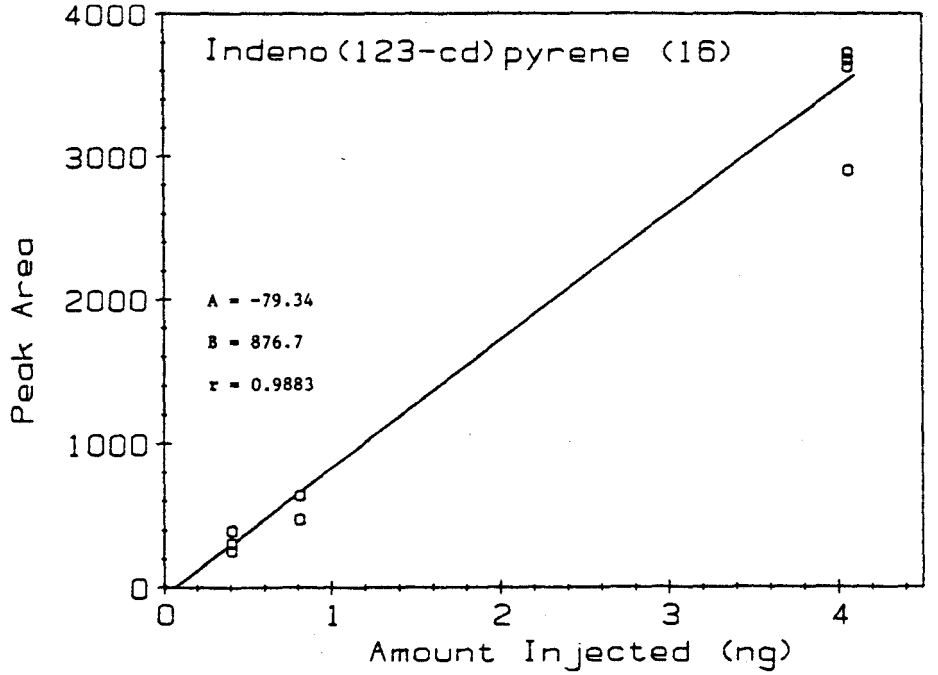


Figure 20: Representative selected ion chromatograms from a microbore column HPLC/MS-SIM analysis of the NBS PAH standard (1:2, 1 uL). Conditions: 100 msec dwell time per ion, gradient of 60% to 100% acetonitrile/water in 20 min and hold, uL/min, 900 psi at start. See Table 1 for compound identities. Note the bottom trace is a HPLC-UVD chromatogram performed under the same conditions.

Figure 21: Data from HPLC/MS-SIM experiments on acenaphthylene, pyrene, fluoranthene, and indeno[1,2,3-cd]pyrene standards. Conditions outlined in Figure 20.


Acenaphthylene (2)
A = 4595
B = 2393
r = 0.9770
Peak Area
60000
40000
20000
0
0
5
10
15
20


Fluoranthene + Pyrene (7, 8)
A = 990.2
B = 1683
r = 0.9905
Peak Area
40000
30000
20000
10000
0
0
5
10
15
20


Indeno(123-cd)pyrene (16)
A = -79.34
B = 876.7
r = 0.9883
Peak Area
4000
3000
2000
1000
0
0
1
2
3
4
Amount Injected (ng)

3.5 FAST LC/MS-SIM

To help facilitate the quantitation of a large number of samples, it is desirable to reduce the turn around time for each sample. In our situation this meant speeding up the chromatography by increasing the flow rate and using isocratic conditions (thereby eliminating column reconditioning necessary when using gradients). Usually, this creates problems with respect to poorer resolution. However, by taking advantage of the inherent selectivity of the mass spectrometer, we were able to enhance the chromatographic separation by deconvoluting coeluting compounds on the basis of their mass. Figure 22 shows the results obtained from a fast LC/MS-SIM experiment. Resolution of single components, and therefore the quantitative results, was not compromised. However, the analysis time was reduced by a third. To achieve the same results with conventional columns a flow rate of approximately 3 mL/min would be required. The increased flow would, if the effluent splitter was left unchanged, overload the LC/MS interface. Therefore an increased split is required, resulting in reduced sensitivity due to increased sample loss. To circumvent this, it would be possible to increase the amount of sample injected, however, this would lead to increased sample consumption, which is especially undesirable when dealing with limited samples. Therefore, by taking advantage of the low flow rates associated with microbore HPLC we were able to effectively facilitate reduced analyses without encountering any of the problems associated with performing the same experiment using

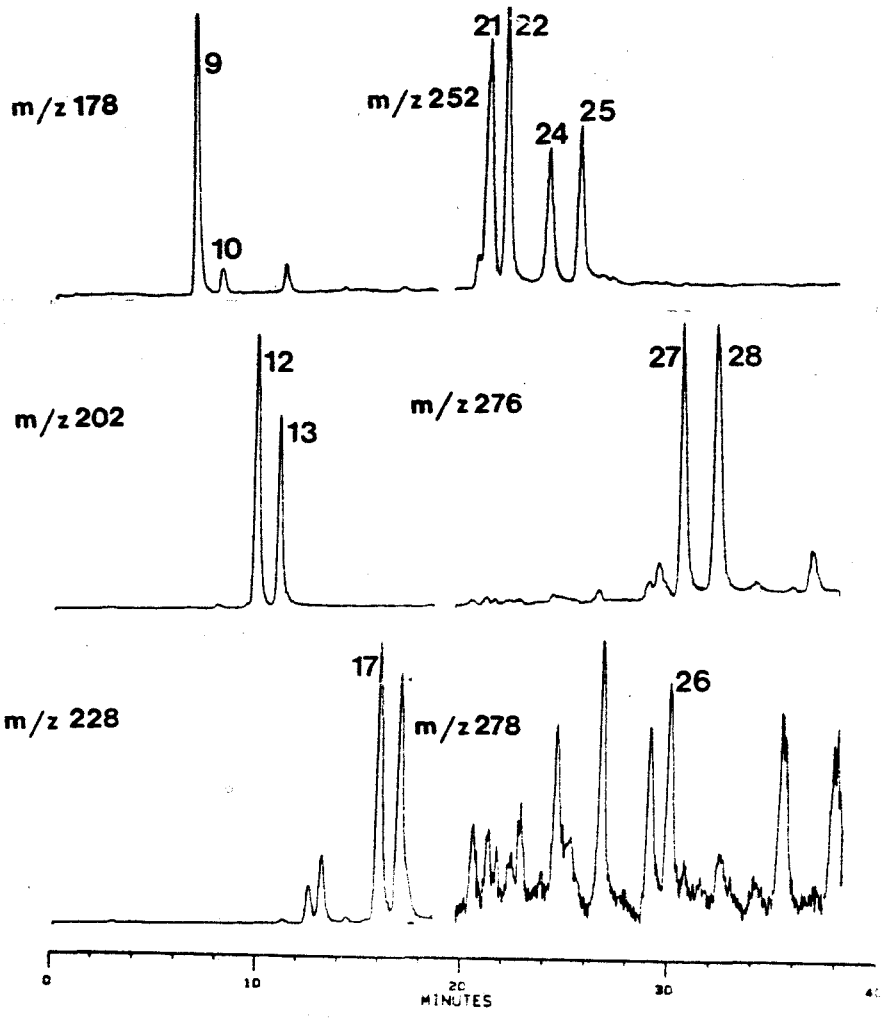


Figure 22: Representative selected ion chromatograms from a fast microbore column HPLC/MS-SIM analysis of the marine sediment. Conditions outlined in Figure 20, except flow rate is 80 uL/min.

conventional column LC/MS.

3.6 APPLICATION TO SEDIMENT SAMPLE

In order to complete the performance evaluation of microbore column LC/MS, an analysis of PAH in a marine sediment extract was undertaken. The extract had been obtained from an analytical reference sediment sample (code CASS-794), provided by the National Research Council of Canada (Atlantic Regional Laboratory, Halifax). The sample was taken through the extraction and cleanup scheme in Figure 3 (experimental details are outlined in Section 2.1.3). Microbore LC/MS (scanning mode) results are shown in Figures 23 and 24 and Appendix A. The microbore HPLC/UVD results in Figure 25 show fairly good resolution and good sensitivity. Since the pressure drop across the column was only 900 psi under these conditions, it is possible to either increase the resolution by going to a series of concatenated columns, or to decrease the analysis time by going to higher flow rates. Comparisons between the UVD and MS results indicate that there is no apparent loss of resolution in the microbore LC/MS results. The mass spectra (see Appendix A) are also of good quality as demonstrated by their strong ion intensities with respect to the background ions.

Table 3 presents the tentative and confirmed identities of peaks observed in the extract. The compounds identified in this study, listed in the above Table 3, have all been previously seen. This, however, does not exclude the possibility that that further examination of these

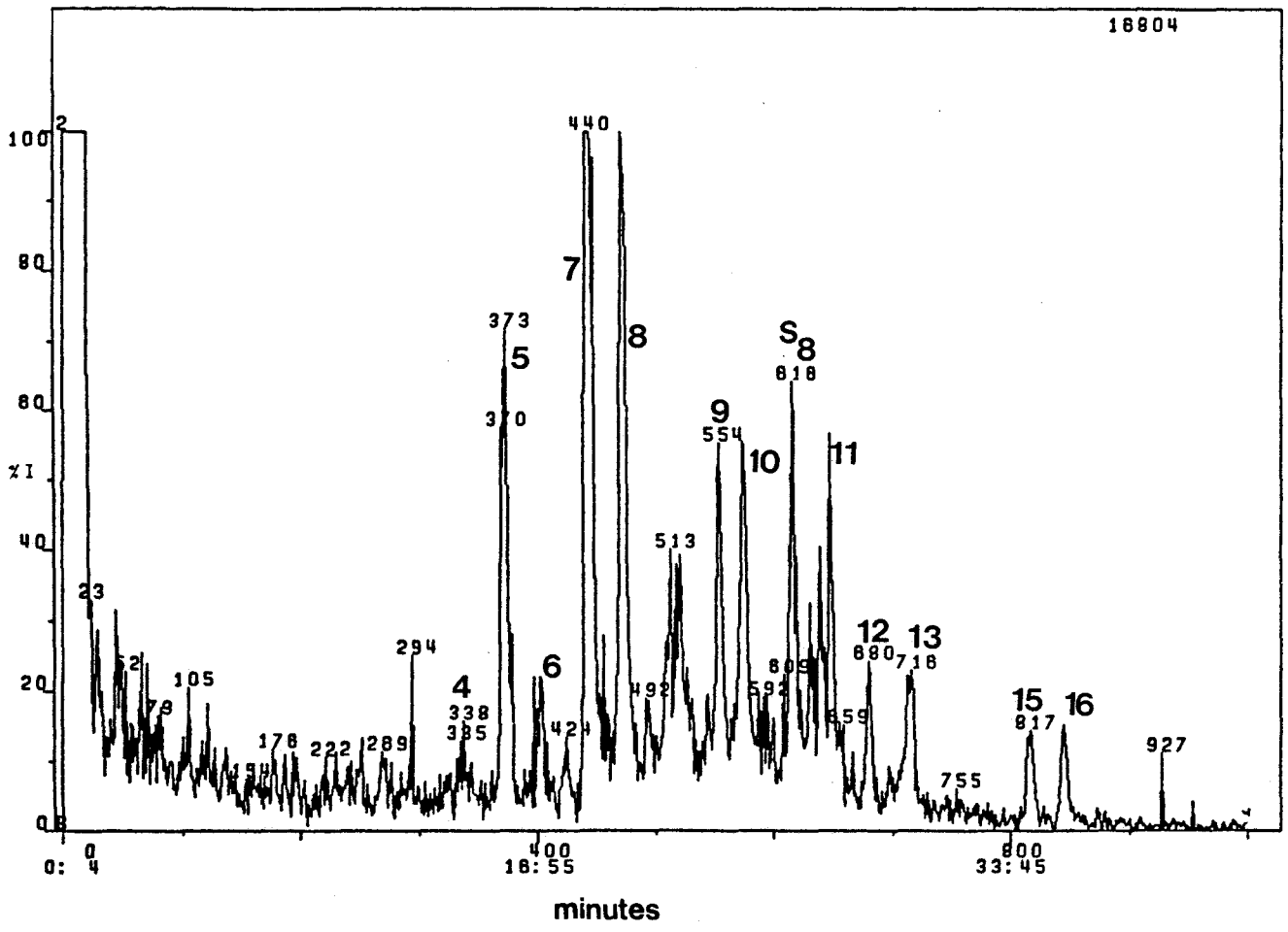


Figure 23: Representative mass chromatogram from the microbore column HPLC/MS analysis on the marine sediment extract. Conditions: gradient of 60% to 100% acetonitrile and water in 20 min and hold, Vydac 201-TP reverse phase column (1 mm I.D. x 25 cm), 30 uL/min, 1 uL injection of sample in 10 mL.

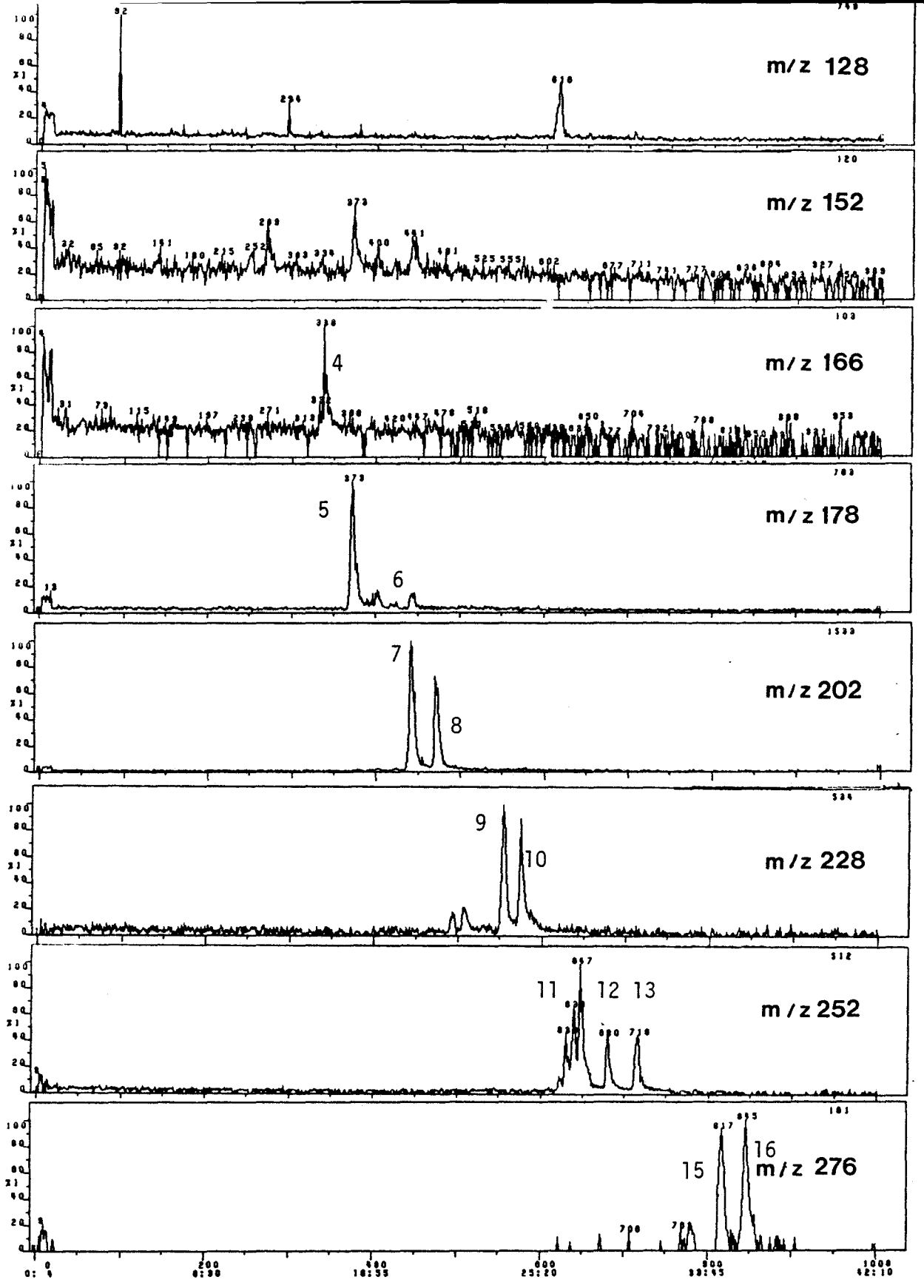


Figure 24: Representative mass chromatograms from the microbore column HPLC/MS analysis of the marine sediment extract. Conditions as in Figure 20.

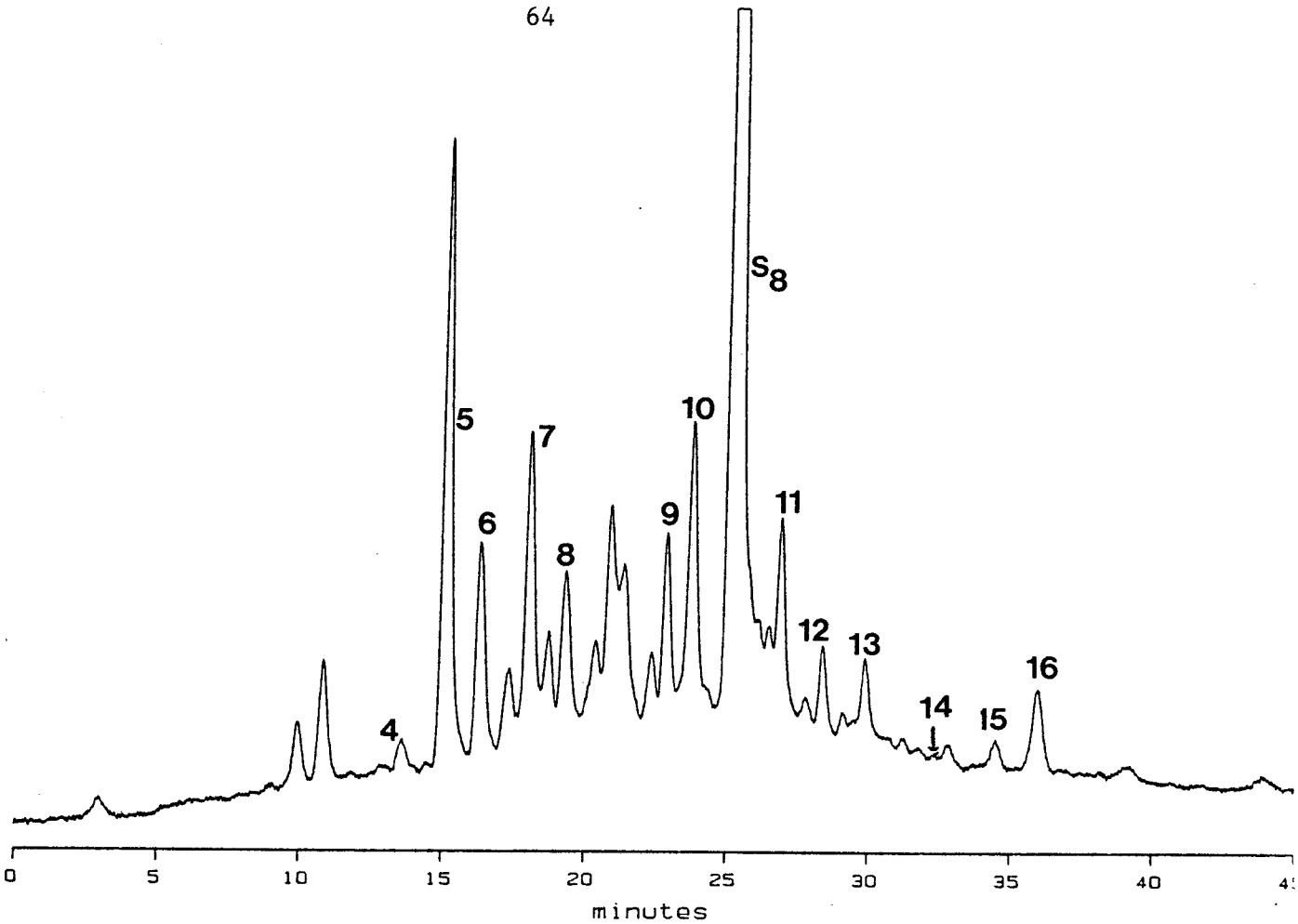


Figure 25: Microbore column HPLC-UVD chromatogram of the marine sediment extract (1:1, 1 uL) on a Vydac 201-TP reverse phase column. Conditions: gradient of 60% to 100% acetonitrile and water in 20 min and hold, 30 uL/min, 920 psi at start, 0.025 AUFS, 254 nm. See Table 1 for compound identities.

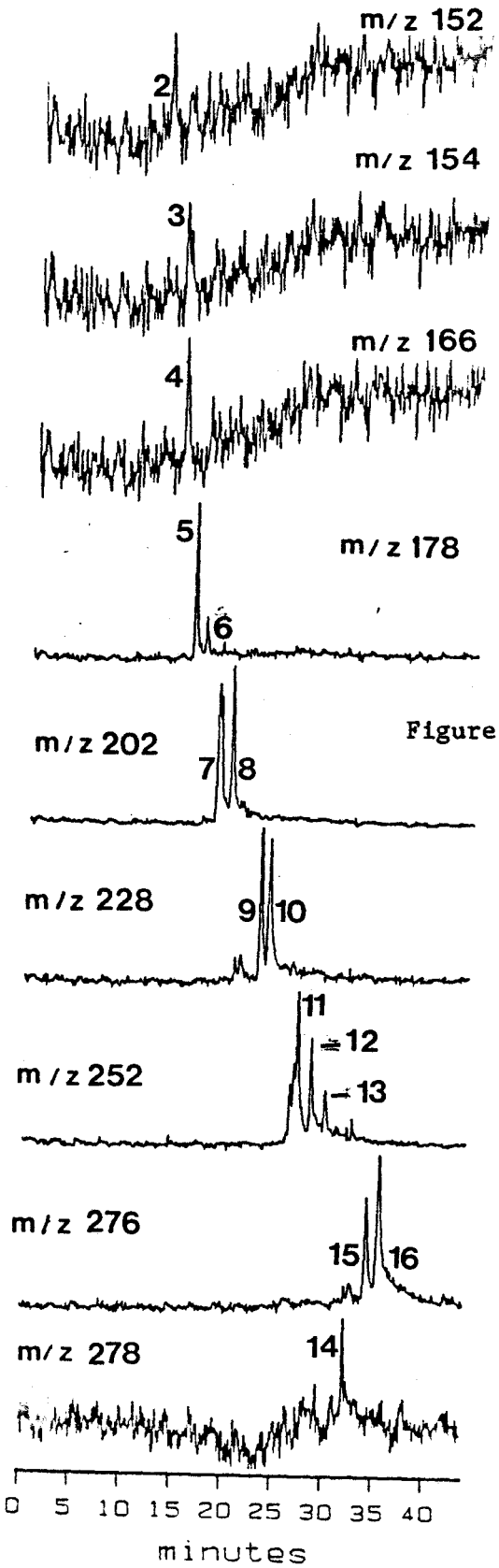


Figure 26: Representative selected ion chromatograms from a microbore column HPLC/MS-SIM analysis of the marine sediment extract (1:2, 1 uL). Conditions: 100 msec dwell time per ion, gradient of 60% to 100% acetonitrile/ water in 20 min and hold, 30 uL/min, 920 psi at start. See Table 1 for compond identities.

Table 3: Compounds identified in marine sediment extract by microbore column LC/MS analysis (see Figure 25 for chromatograms)

Compound Number	Scan No.	Mol. Wt.	Compound Identity
1	261	128	Naphthalene
2	290	152	Acenaphthylene
3	334	154	Acenaphthene
4	338	166	Fluorene
5	373	178	Phenanthrene
6	404	178	Anthracene
7	441	202	Fluoranthene
8	470	202	Pyrene
9	554	228	Benz[a]anthracene
10	574	228	Chrysene
11	647	252	Benzo[b]fluoranthene
12	680	252	Benzo[k]fluoranthene
13	716	252	Benzo[a]pyrene
14	769	278	Dibenz[ah]anthrcene
15	817	276	Benzo[ghi]perylene
16	845	276	Indeno[1,2,3-cd]anthracene
17	228	167	Carbazole
18	249	180	9-Fluorenone
19	269	208	Anthraquinone
20	320	168	Dibenzofuran
21	412	190	4H-Cyclopenta[def]phenanthrene
22	521	216	Benzo[a]fluorene
23	549	216	Benzo[b]fluorene
24	616	256	Sulphur
25	623	252	Benzo[j]fluoranthene
26	630	252	Benzo[e]pyrene
27	656	252	Perylene
28	747	278	Benzo[b]chrysene
29	906	278	Picene
30	382	182	C2-alkyl-biphenyl [a]
31	448	196	C3-alkyl-biphenyl [a]
32	460	196	"
33	470	218	Benzoxanthene [a]
34	544	226	Cyclopenta[cd]pyrene [a]
35	574	242	Methyl Benz[a]pyrene [a]
36	576	240	Methyl Cyclopenta[cd]pyrene [a]
37	598	242	Methyl Benz[a]anthrcaene [a]
38	628	242	"
39	634	242	"
40	663	242	"

a - identified only by mass spectra

Table 4: Summary of target compound concentrations in marine sediment sample as determined by microbore column LC/MS-SIM experiments using peak areas.

Compound No.	RWR[a]	S.D.[b]	Conc'n[c] (ppm)	S.D.[b] (ppm)	C.I.[d] (ppm)
2	3600	1100	0.2	0.1	0.3
3	1280	250	0.5	0.3	0.8
4	2150	640	0.6	0.1	0.3
5	2460	460	4.7	1.8	4.5
6	2460	460	0.7	0.2	0.4
7	1920	280	10	3	6
8	1920	280	7	3	6
9	1420	190	3.2	0.5	1.3
10	1420	190	3.0	0.7	1.8
11	1200	200	3.2	0.5	1.3
12	1330	240	1.5	0.2	0.4
13	360	80	1.5	0.4	0.9
14	840	130	0.4	0.2	0.5
15	380	120	1.6	0.3	0.7
16	800	140	1.7	0.3	0.7

a - RWR = relative weight response (unit area/ng)
b - S.D. = standard deviation (n=3)
c - Concentration in ug of compound per g of dry sediment
d - C.I. = 95% confidence interval: = $ts/\sqrt{N}$
where; N = number of repetitions,
s = standard deviation,
t = confidence interval factor for n=3.

samples will not reveal any new compounds. Confirmation was performed by a variety of techniques (i.e., GC/FID, GC/MS, and LC/MS).

Table 4 summarizes the quantitative results obtained from SIM experiments (Figure 26) performed on the marine sediment extract. The external calibration method was used and the results obtained are outlined in section 3.4. A precision of about 20% relative error on average was observed, although there were values as high as 100% for some components at low concentrations. After taking into consideration the standard deviations of both standards and samples and the propagation of error in calculations, the 95% uncertainty levels turned out to be rather high. The major sources of error appeared to be injection volume control and fluctuations of mass spectrometer conditions and sensitivity. A solution to this problem, that has been investigated by Dr. Quilliam, is the use of fully deuterated PAH as internal standards. This improvement would circumvent any injection volume fluctuations, along with problems such as errors due to changes in ion source conditions, solution volume errors, and poor compound transmission in the interface.

Along with the HPLC-UVD and LC/MS experiments performed on PAH standards and samples, GC/FID and GC/MS-SIM experiments were also performed. A capillary GC/FID chromatogram is given in Figure 27. It clearly shows the high resolution obtainable with capillary GC. This high degree of resolution greatly simplifies quantitation in comparison to HPLC-UVD. In addition, with the aid of retention indices, compound

identification can be made more easily. These points can be clearly seen if one compares Figure 27, the capillary GC/FID trace, and Figure 25 the microbore HPLC-UVD chromatrogram of the marine sediment extract. As previously mentioned, HPLC has a number of advantages over GC, however, particularly the ability to accomodate thermally unstable and involatile compounds. In addition, we found through comparing LC/MS and GC/MS results that in some cases LC was better at separating various PAH isomers. For example, when the SIM traces for mass m/z 252 (Figure 28) of a LC and GC separation are compared, LC showed superior selectivity of separation. That is, LC was better able to resolve the various isomers while on the other hand, complete resolution of the same isomers was not achieved by GC despite its higher column efficiency. Of course, this is not true for all cases - examples of better separation have been observed in GC also. Taking this into consideration, it can be concluded that LC and GC are complementary techniques. Table 5 presents the GC-FID, GC/MS and LC/MS results obtained from interlab analysis of the marine extract. A comparison of the LC/MS results obtained in our lab demonstrates that the values remained fairly consistent in the two experiments; however, the precision achieved for the internal standard calibration was far better, as would be expected for this inherently more precise method, than that observed with external calibration. A similar comparison of the GC/MS results does not yield the same consistent quantitative results as did LC/MS. The concentrations obtained from the GC analysis tend to vary significantly between experiments. This may be due to sloppy sample preparation and handleing

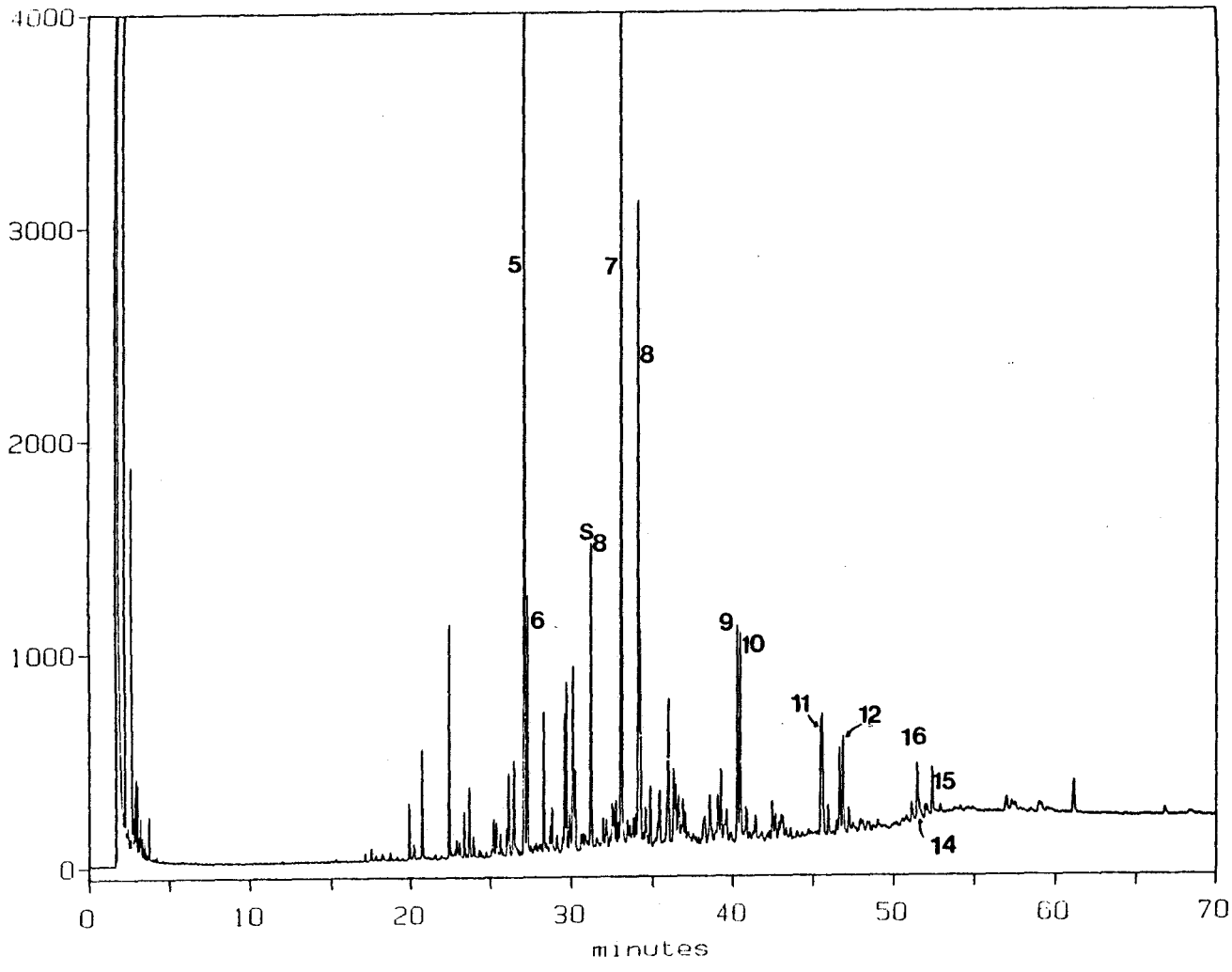


Figure 27: Capillary GC-FID chromatogram of the marine sediment extract. Conditions: cold on-column injection, programmed column temperature from 60°C to 320°C at 5°C/min, 30 m, 0.32 mm I.D., DB-5 fused silica column with a carrier gas (He) linear velocity of 30 cm/min.

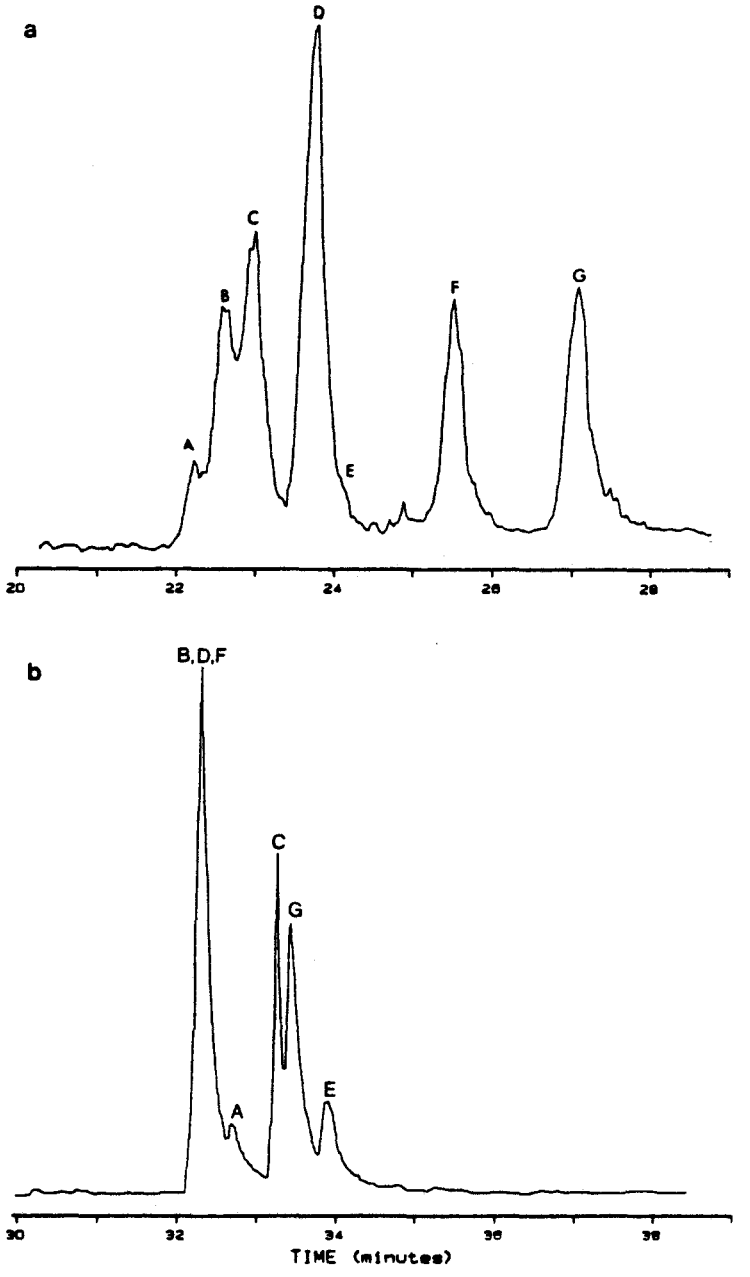


Figure 28: Comparison of a HPLC/MS-SIM trace (a) and a GC/MS-SIM trace (b) of m/z 252 from the marine sediment extract. Conditions are listed in the experimental section. Compound identities are: A) unknown; B) Benzo[j]fluoranthene; C) Benzo[e]pyrene; D) Benzo[b]fluoranthene; E) Perylene; F) Benzo[k]fluoranthene; and G) Benzo[a]pyrene.

and/or injection technique. However, similar to the LC/MS results, the internal standard calibration GC/MS experiments yielded better results than the external calibration experiments. Another interesting point to note is that benzo[b]fluoranthene and benzo[k]fluoranthene could not be quantitated due to their poor separation. This same reason, quantitation of poorly resolved peaks, may be responsible for some of the other inconsistencies found. One important point these results indicate is that LC/MS and GC/MS are equally well suited for PAH quantitation. The pooling of information from both GC/MS and LC/MS can provide higher confidence in both qualitative and quantitative determination of PAH in complex mixtures. In addition, these results clearly demonstrate that internal calibration, using deuterium labelled standards, is far superior to the external calibration method. Compensation for injection errors, sample miss handleing and solvent evaporation, and varying instrument sensitivity are clearly refected in the results, given in Table 5.

TABLE 5: The GC/MS and LC/MS quantitative results for the marine sediment extract

Cmpd. #	Compound Name	GC/MS(a) external standards	GC/MS(a) internal standards	LC/MS(b) external standards	LC/MS(b) internal standards
1.	Naphthalene	0.08±0.03	0.155±0.008	NA	0.25±0.04
2.	Acenaphthylene	0.05±0.02	NA	0.02±0.1	NA
3.	Acenaphthene	NA	0.016±0.009	0.5 ±0.3	0.11±0.006
4.	Fluorene	0.38±0.08	0.234±0.019	0.6 ±0.1	0.38±0.03
5.	Phenanthrene	5.2 ±0.8	3.99 ±0.05	4.7 ±1.8	5.47±0.13
6.	Anthracene	0.48±0.16	0.198±0.012	0.7 ±0.2	0.49±0.05
7.	Fluoranthene	8.9 ±1.2	6.91 ±0.13	10 ±3	13.5 ±0.5
8.	Pyrene	4.5 ±0.7	3.94 ±0.03	7 ±3	5.9 ±0.2
9.	Benz[a]anthracene	5.1 ±1.6	1.68 ±0.02	3.2 ±0.5	2.8 ±0.3
10.	Chrysene	2.1 ±0.5	2.15 ±0.03	3.0 ±0.7	2.4 ±0.15
11.	Benzo[b]fluoranthene	NA	1.62 ±0.10	3.2 ±0.2	2.5 ±0.14
12.	Benzo[k]fluoranthene	NA	1.75 ±0.21	1.5 ±0.2	1.24±0.01
13.	Benzo[a]pyrene	1.8 ±0.5	1.07 ±0.02	1.5 ±0.4	1.47±0.09
14.	Dibenz[ah]anthrocene	NA	0.300±0.006	0.4 ±0.2	0.18±0.03
15.	Benzo[ghi]perylene	0.30±0.07	1.56 ±0.06	1.6 ±0.3	1.38±0.03
16.	Indeno[1,2,3-cd]pyrene	2.9 ±0.6	0.52 ±0.02	1.7 ±0.3	1.47±0.09

All values quoted are of ppm (dry sediment) ± standard deviation

(a) performed by NRC Atlantic Regional Laboratory

(b) performed by our laboratory

4. CONCLUSION

An inexpensive method has been developed for the conversion of a conventional HPLC system to accommodate microbore columns. This system has demonstrated satisfactory chromatographic performance under both isocratic and gradient conditions. However, due to design limitations, some advantages associated with microbore HPLC have not been realized, namely: solvent economy and higher sensitivity with concentration dependent detectors.

The combination of microbore HPLC and mass spectrometry proved to be successful. Many problems commonly associated with conventional column LC/MS, such as backstreaming, droplet formation and splattering, have been for the most part eliminated. This resulted in far better chromatographic and mass spectrometric performance for both qualitative and quantitiative studies. In addition, the ability to perform fast analysis LC/MS, without compromising the operating requirements of the LC/MS moving belt interface and the mass spectrometer, was shown to be possible with microbore LC/MS. These points and others, summarizing the advantages and disadvantages of microbore and conventional column LC/MS, are presented in Table 6.

Quantitative studies performed on a marine sediment extract using microbore LC/MS-SIM also proved successful. Sixteen PAH were quantitated and a detection limit of 50 pg was determined. However, due to the combination of poor injection volume control and the use of external calibration, poor precision was obtained. Since the completion of this

Table 6: The advantages and disadvantages of conventional column LC/MS with spray deposition and microbore LC/MS.

Conventional column LC/MS plus spray deposition system

Advantages:	Disadvantages:
-High sample capacity (20 uL sample)	-Limited to narrow gradients (e.g. 80% to 100% ACN) unless effluent splitter used
-Direct transfer of conditions from conventional HPLC analyses	-Sample wastage if effluent splitting used
-Greater range of stationary phases available in commercially packed columns	-More complex set-up and more things to go wrong
	-Pressure in source high and fluctuations cause noise.

Microbore Column LC/MS

Advantages:	Disadvantages:
-Ease of setup and reliability.	-Smaller injection volumes (requires more concentrated sample solutions, although this is common for GC and GC/MS)
-Allows wider ranging gradients with high water content	
-No sample wastage in comparison with effluent splitting	-Special equipment or modification of existing equipment is required
-Good source pressures	-Microbore columns with a wide variety of packing materials are not readily available
-Use of expensive or exotic stationary and mobile phases (e.g., deuterated solvents)	
-Ability to perform fast LC/MS analysis	

study, Dr. Quilliam has found that precision can be greatly improved if an internal standard method using deuterated PAH is used. Overall, microbore LC/MS has proven itself to be a promising technique, complementary to the well established technique of GC/MS, and on the basis of the results obtained, the purchase of a dedicated microbore LC system would be highly recomended.

4.1 FUTURE WORK

A number of interesting studies could be done in the future resulting, in part, from work performed in this thesis. The combination of diode-array UV-VIS detector with microbore HPLC would be of considerable interest. An on-line method of obtaining individual UV spectra of peaks would provide important information for the identification of isomers, something that has proven difficult with mass spectrometry. Polar PAH, some of which may be mutagens, have been found in a variety of samples. These compounds are not easily analyzed by GC/MS because of their involatile and thermally sensitive nature. The identification and determination of these compounds in various samples by microbore LC/MS could form the basis for other investigations. It would also be interesting to study the effects of coupling several microbore columns together for the purposes of achieving higher separation efficiencies.

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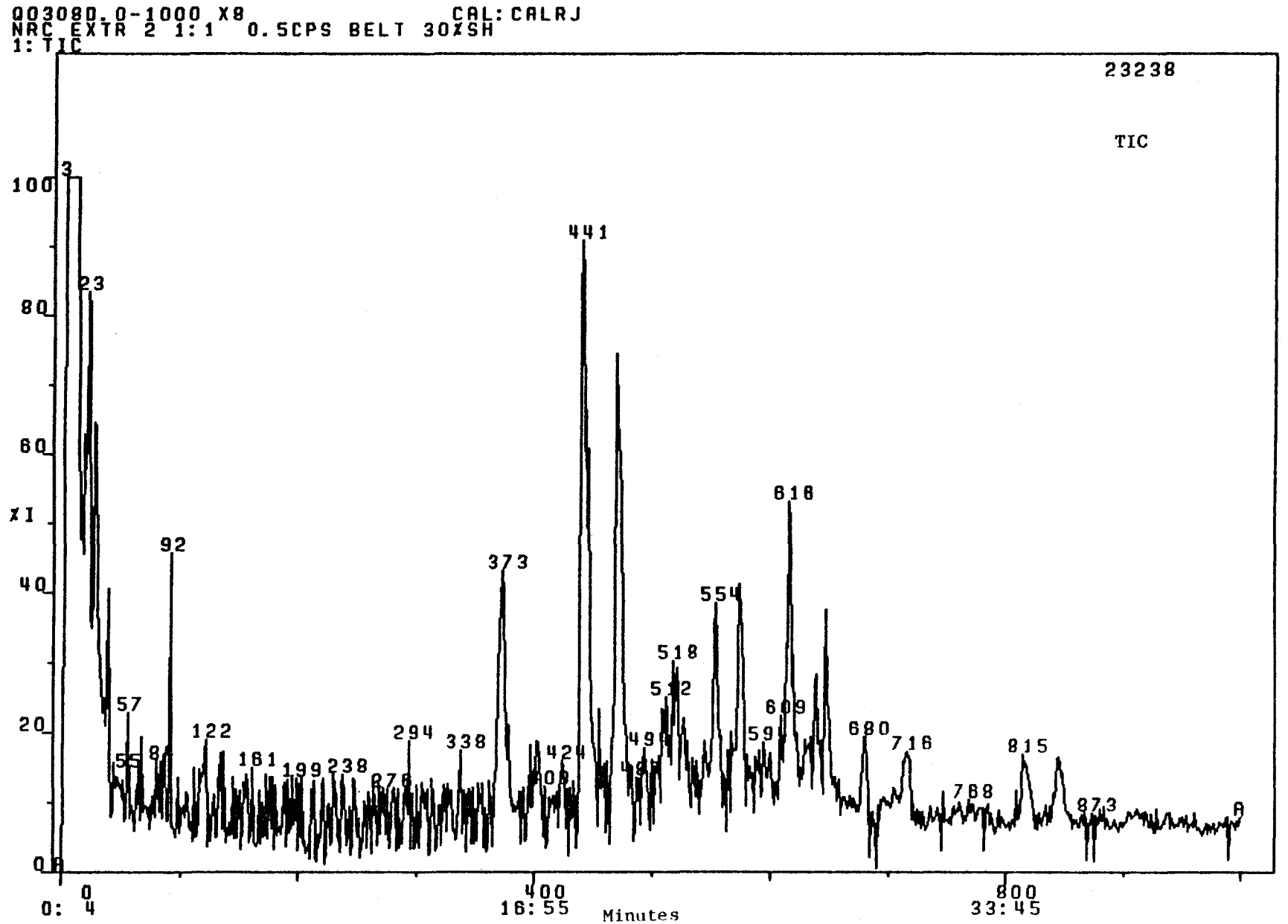
6. APPENDIX

Appendix A

Microbore column HPLC/MS mass spectra for the marine sediment extract. Conditions: 1 uL of sample in 10.00 mL, 30 uL/min of 60% to 100% acetonitrile and water in 20 min using a Vydac TP-201 reverse phase column (1 mm I.D. * 25 cm), 920 psi.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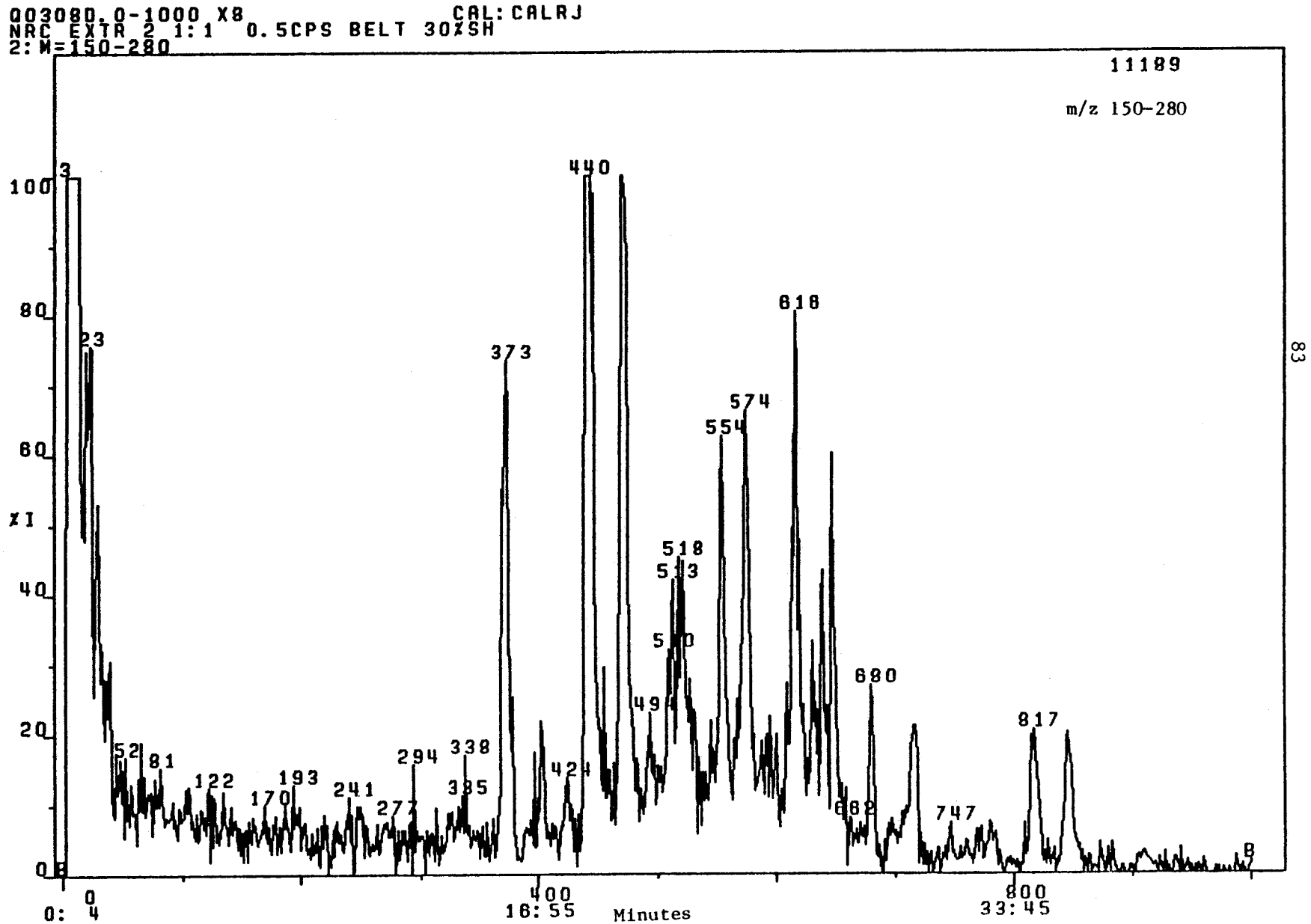
CAL:CALRJ
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
1:TIC
23238
TIC
XI
100
80
60
40
20
0
3
23
57
92
122
161
199
238
294
338
373
424
441
490
518
554
609
618
680
716
788
815
873
0
0:
4
400
16:55
800
33:45
Minutes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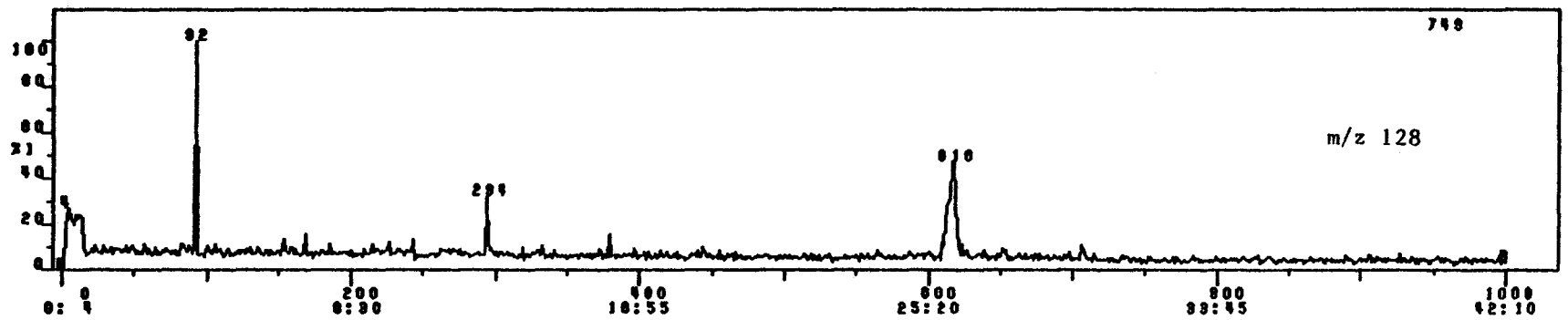
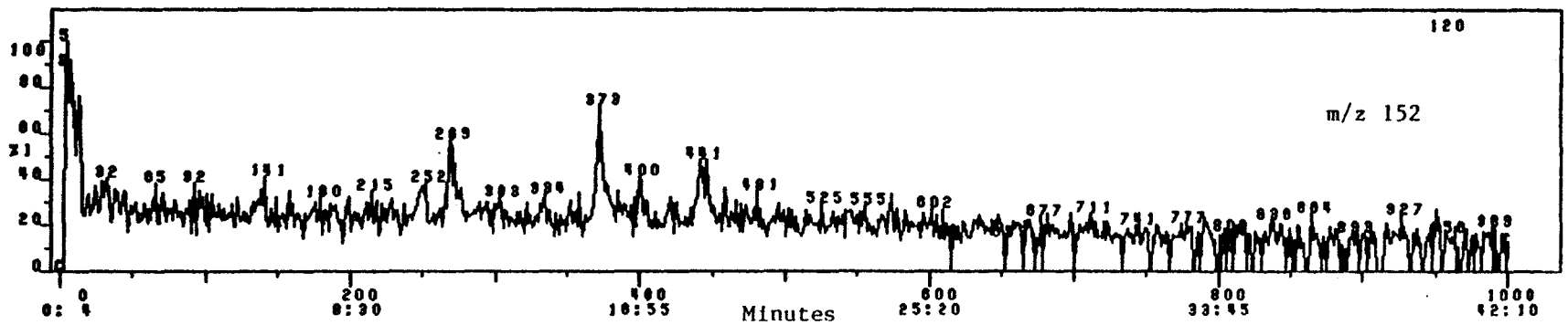
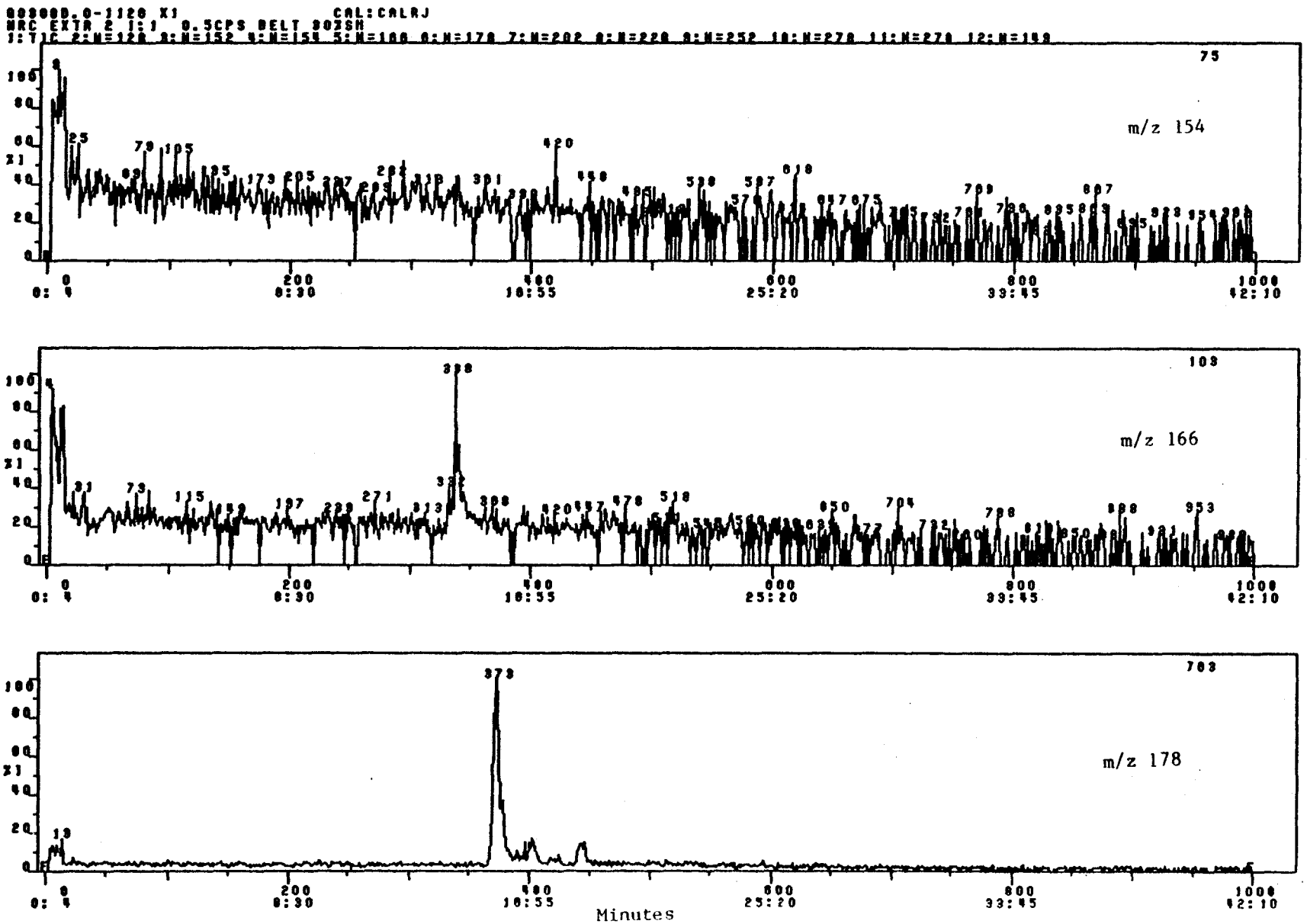
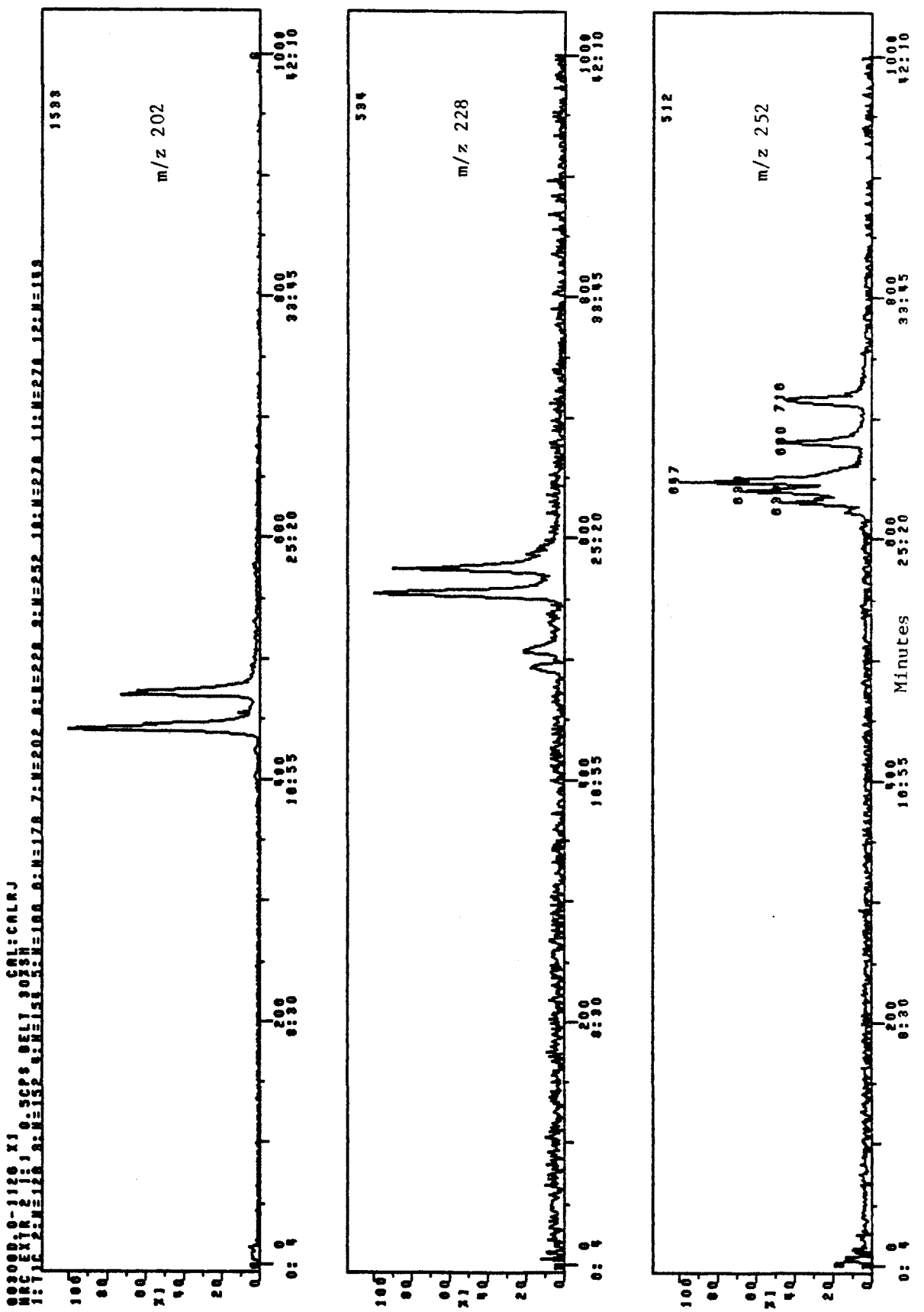
CAL:CALRJ
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
2:M=150-280
11189
m/z 150-280
100
80
60
40
20
0
%I
3
23
52
81
122
170
193
241
272
294
338
335
373
424
440
490
513
518
554
574
618
680
682
747
817
0
400
800
0: 4
16:55
33:45
Minutes
83


m/z 128
748
92
294
610
0
200
400
600
800
1000
0:00
8:30
16:55
25:20
33:45
42:10


m/z 152
120
379
289
551
Minutes
0
200
400
600
800
1000
0:00
8:30
16:55
25:20
33:45
42:10


CAL:CALRJ
m/z 154
m/z 166
m/z 178
Minutes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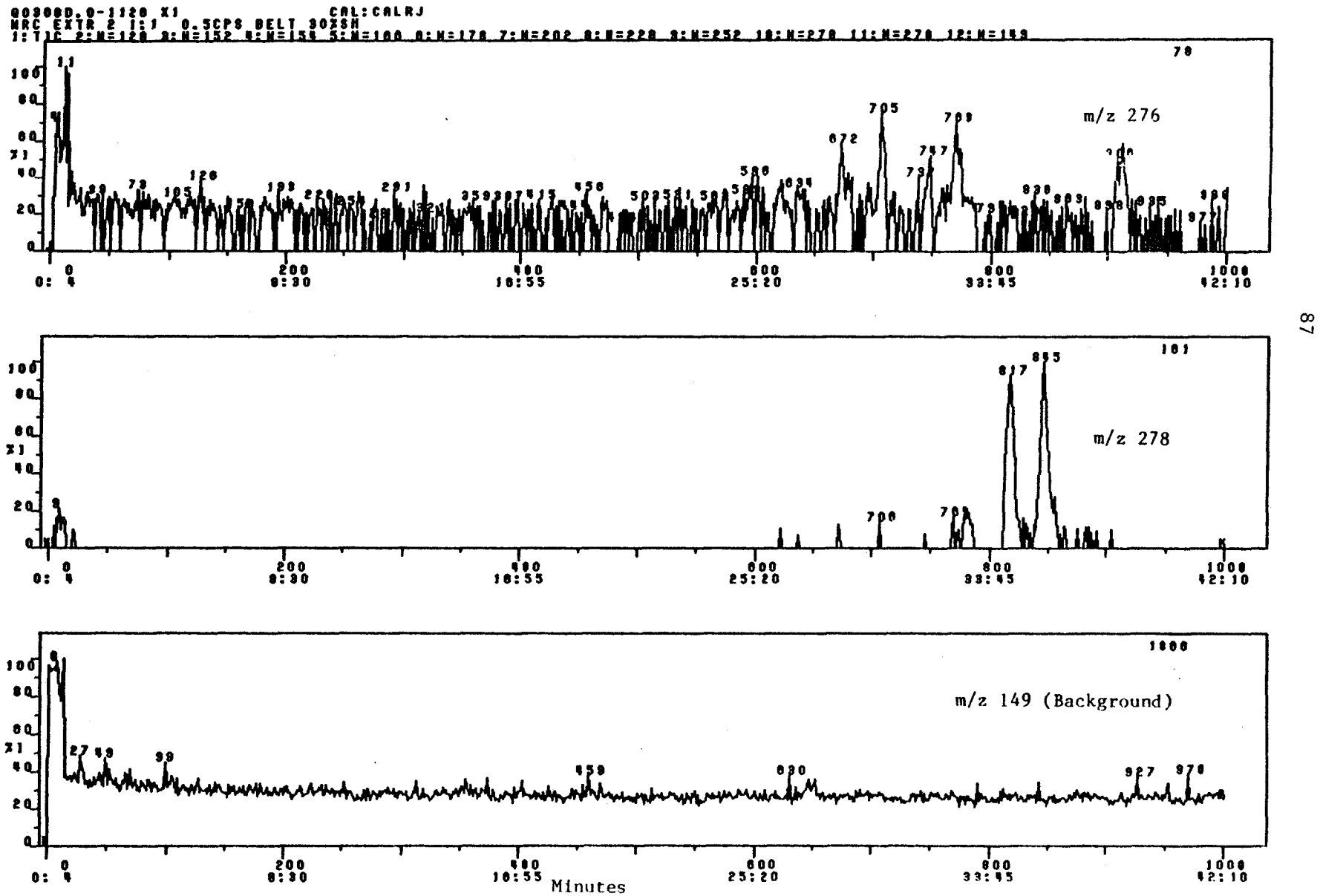
m/z 228
m/z 252
Minutes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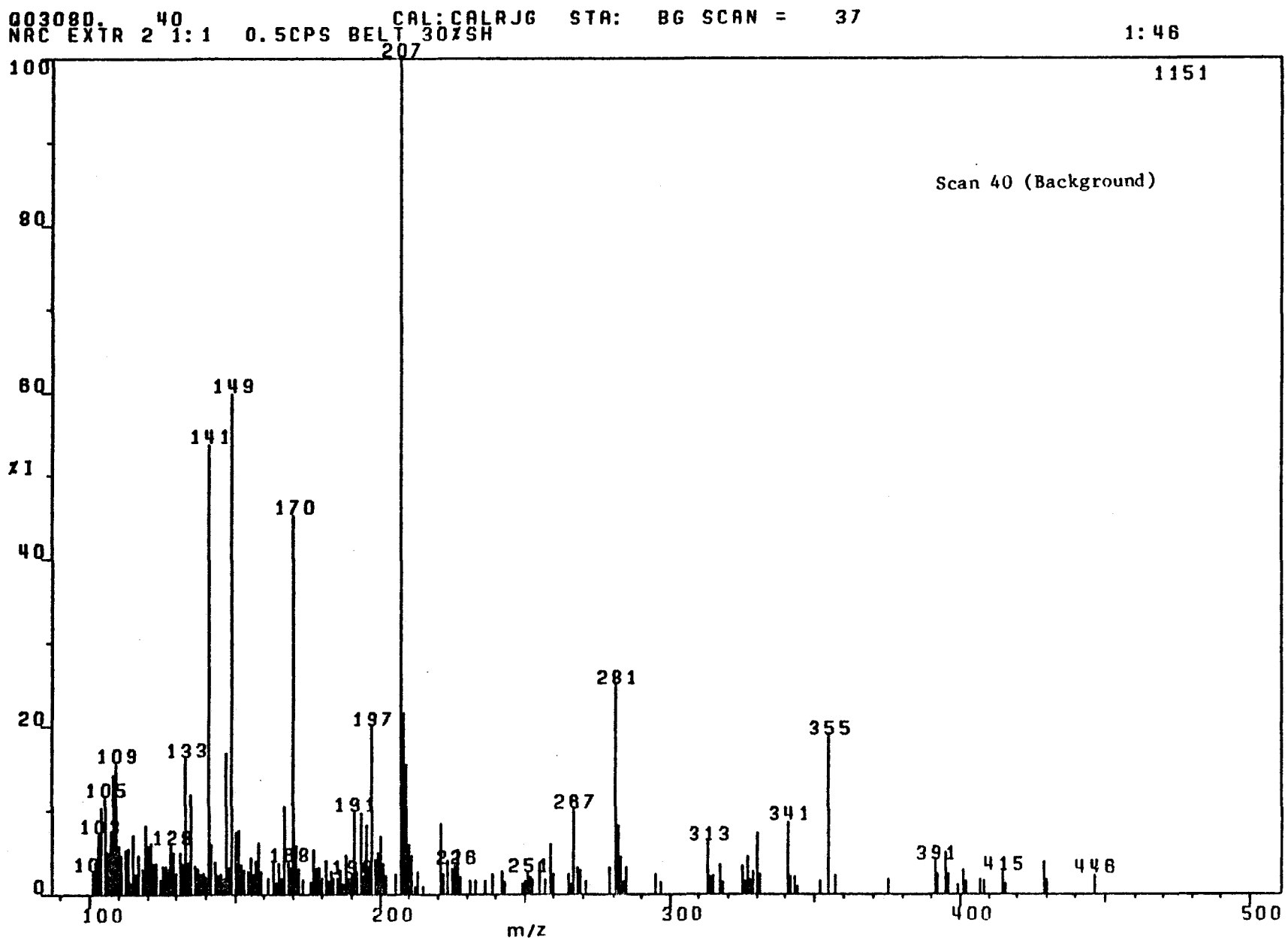
m/z 278
m/z 149 (Background)
Minutes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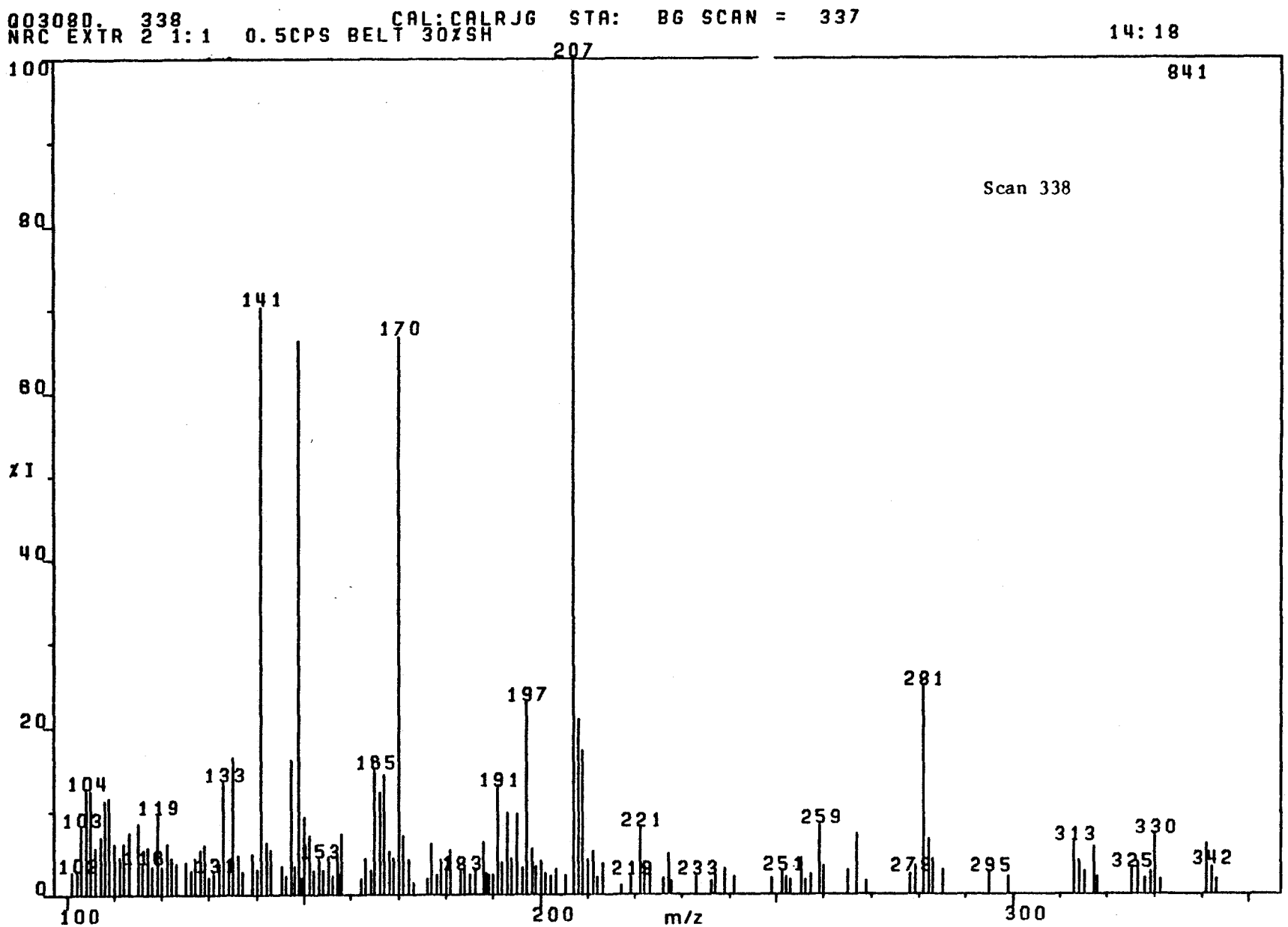
NRC EXTR 2 1:1 0.5CPS BELT 30%SH 1:46
1151
Scan 40 (Background)
207
149
141
170
197
281
355
109
133
105
287
341
313
391
415
446
128
226
251
100
200
300
400
500
m/z
%I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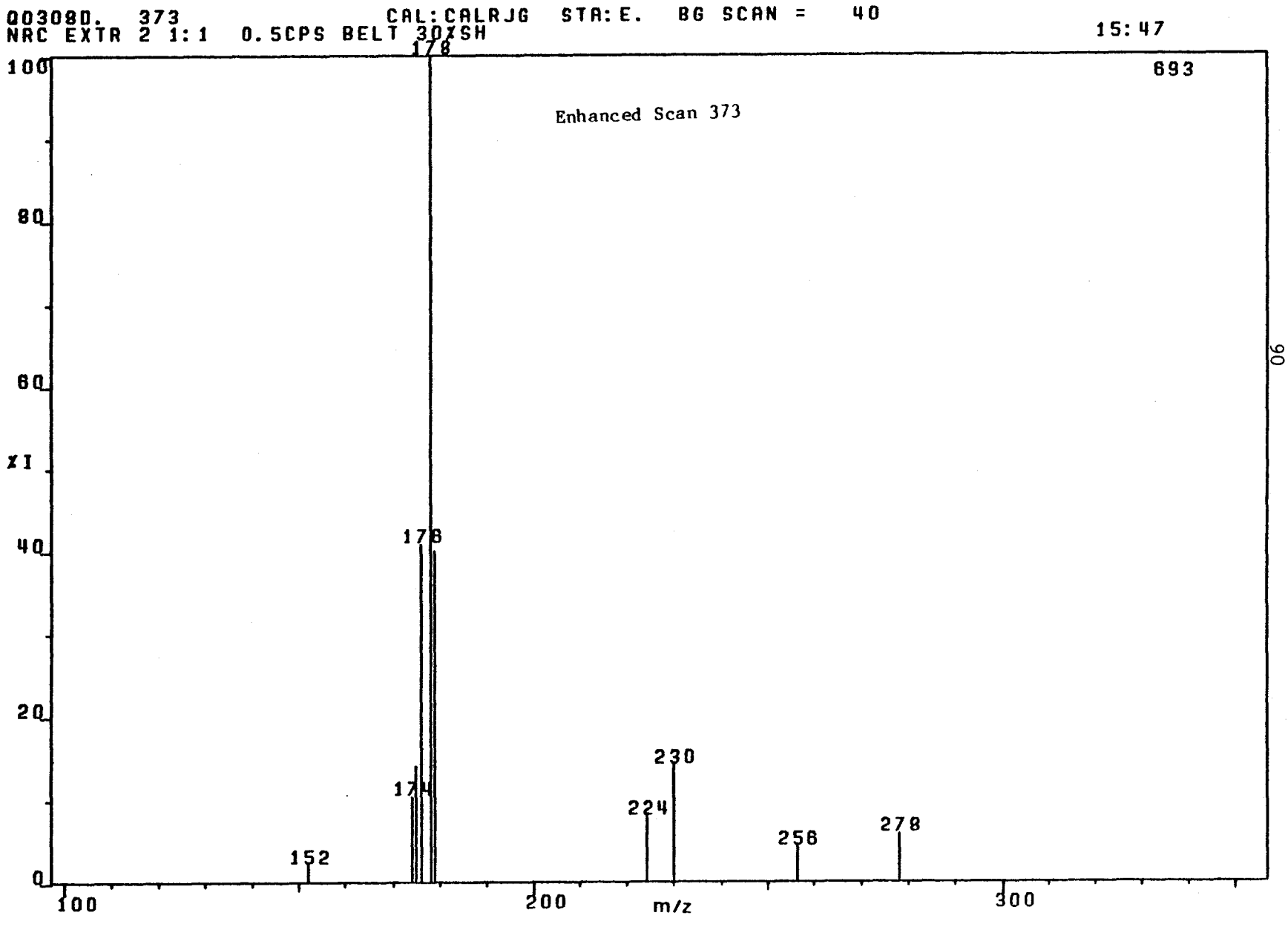
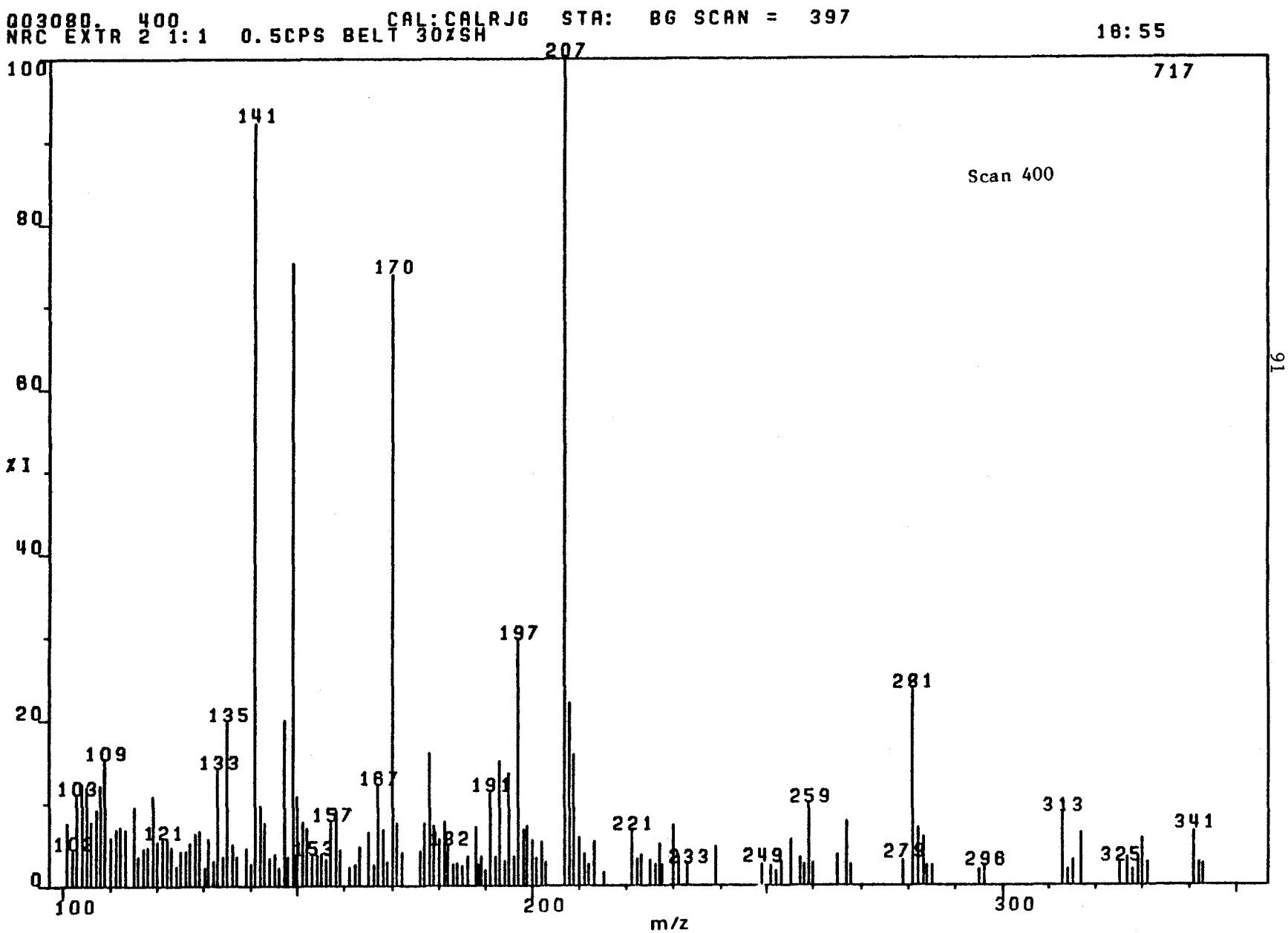
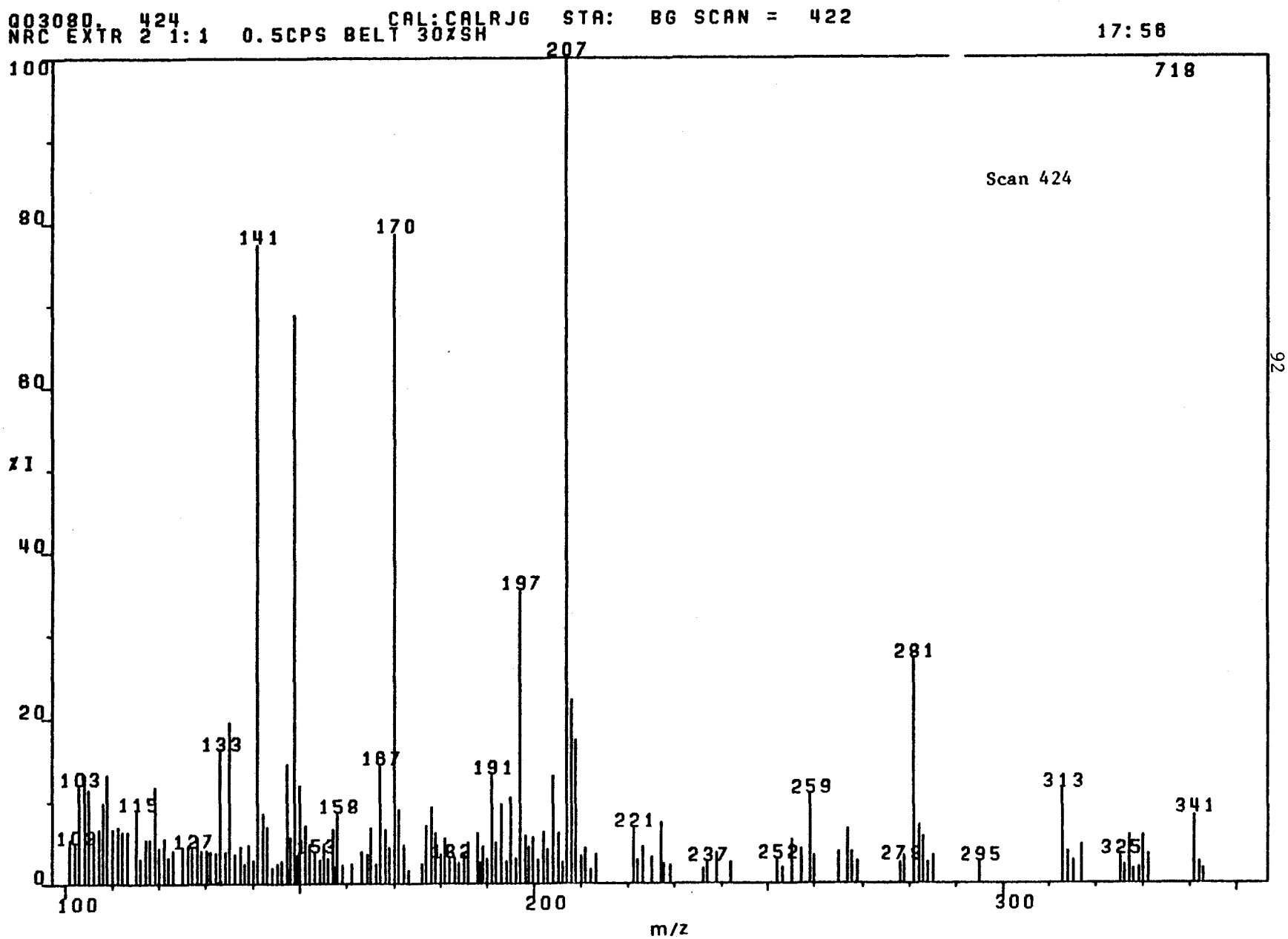
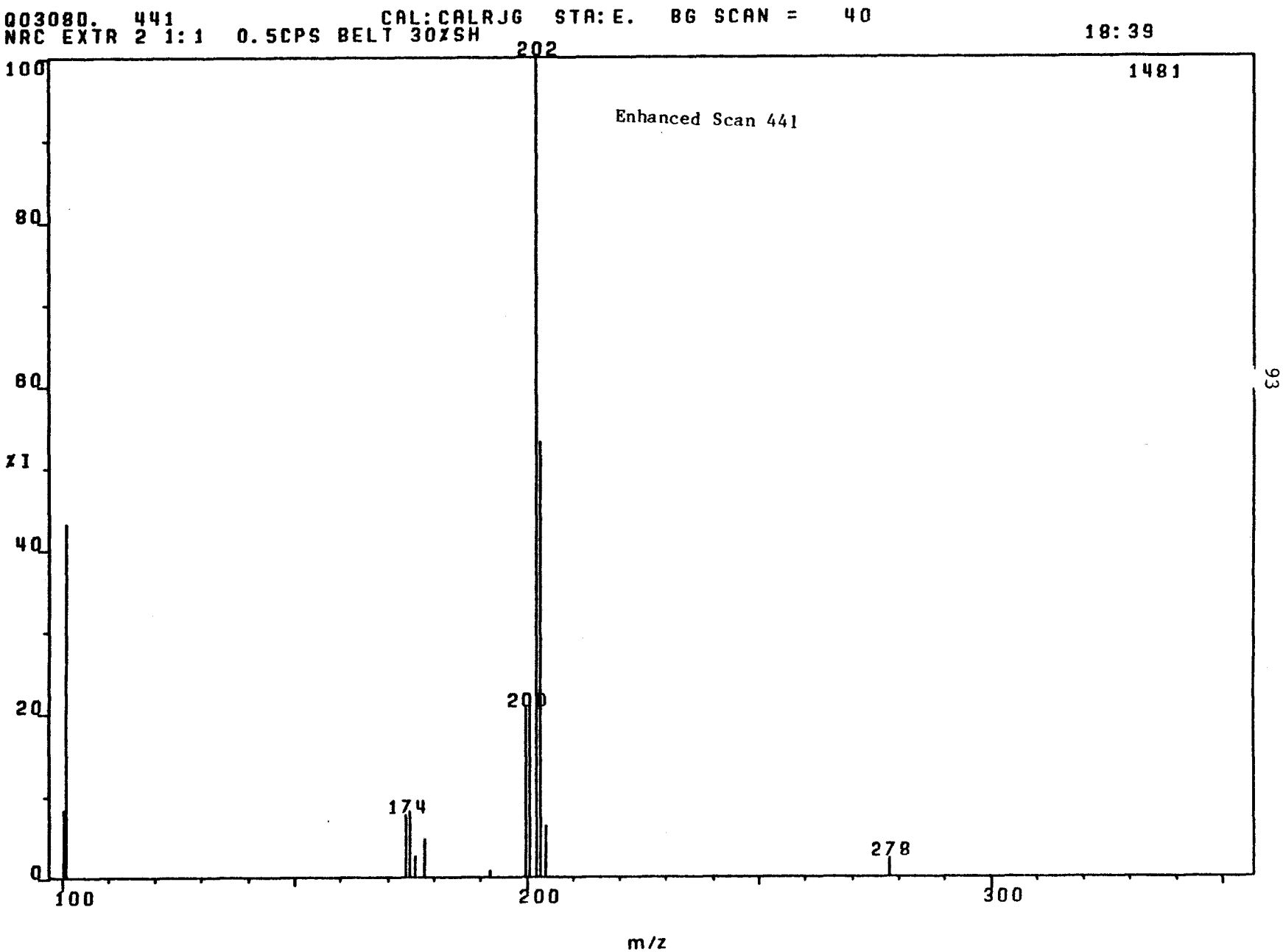
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
14:18
841
Scan 338
207
141
170
197
281
185
133
191
104
119
103
221
259
313
330
325
342
295
278
251
233
218
183
153
116
102
100
80
60
40
20
0
%I
100
200
300
m/z


Q03080. 373 CAL:CALRJG STA:E. BG SCAN = 40
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
15:47
693
Enhanced Scan 373
178
100
80
60
40
20
0
XI
178
174
152
230
224
256
278
100
200
300
m/z
90


003080. 400 CAL:CALRJG STA: BG SCAN = 397
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
18:55
717
Scan 400
91
207
141
170
197
281
135
109
133
167
191
259
313
341
221
121
157
233
249
279
296
325
100
80
60
40
20
0
%I
100
200
300
m/z


Q03080. 424 CAL:CALRJG STA: BG SCAN = 422
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
17:58
718
Scan 424
207
141
170
197
281
133
187
191
259
313
341
103
115
158
221
237
252
278
295
325
127
100
80
60
40
20
0
%I
100
200
300
m/z
92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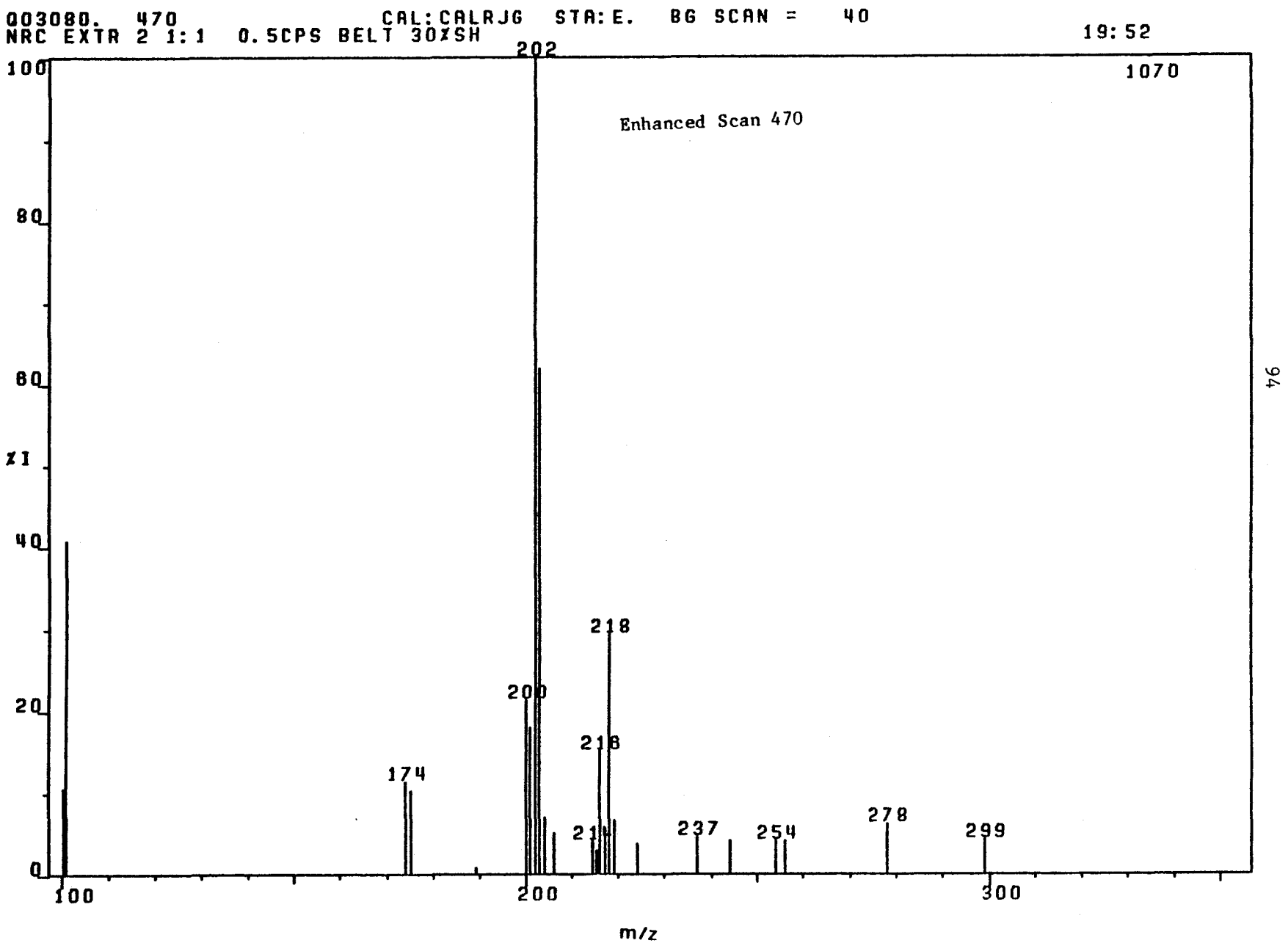
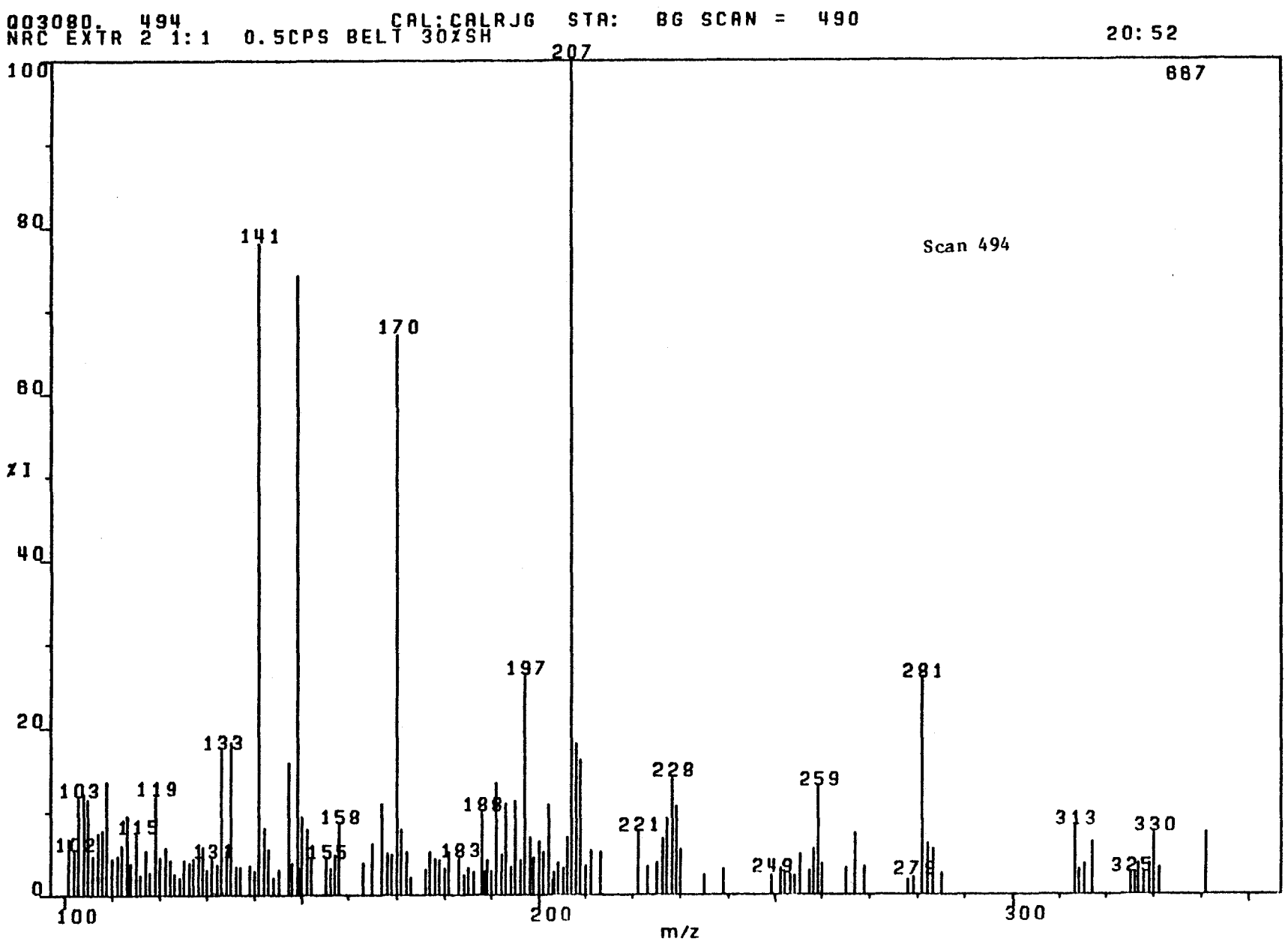
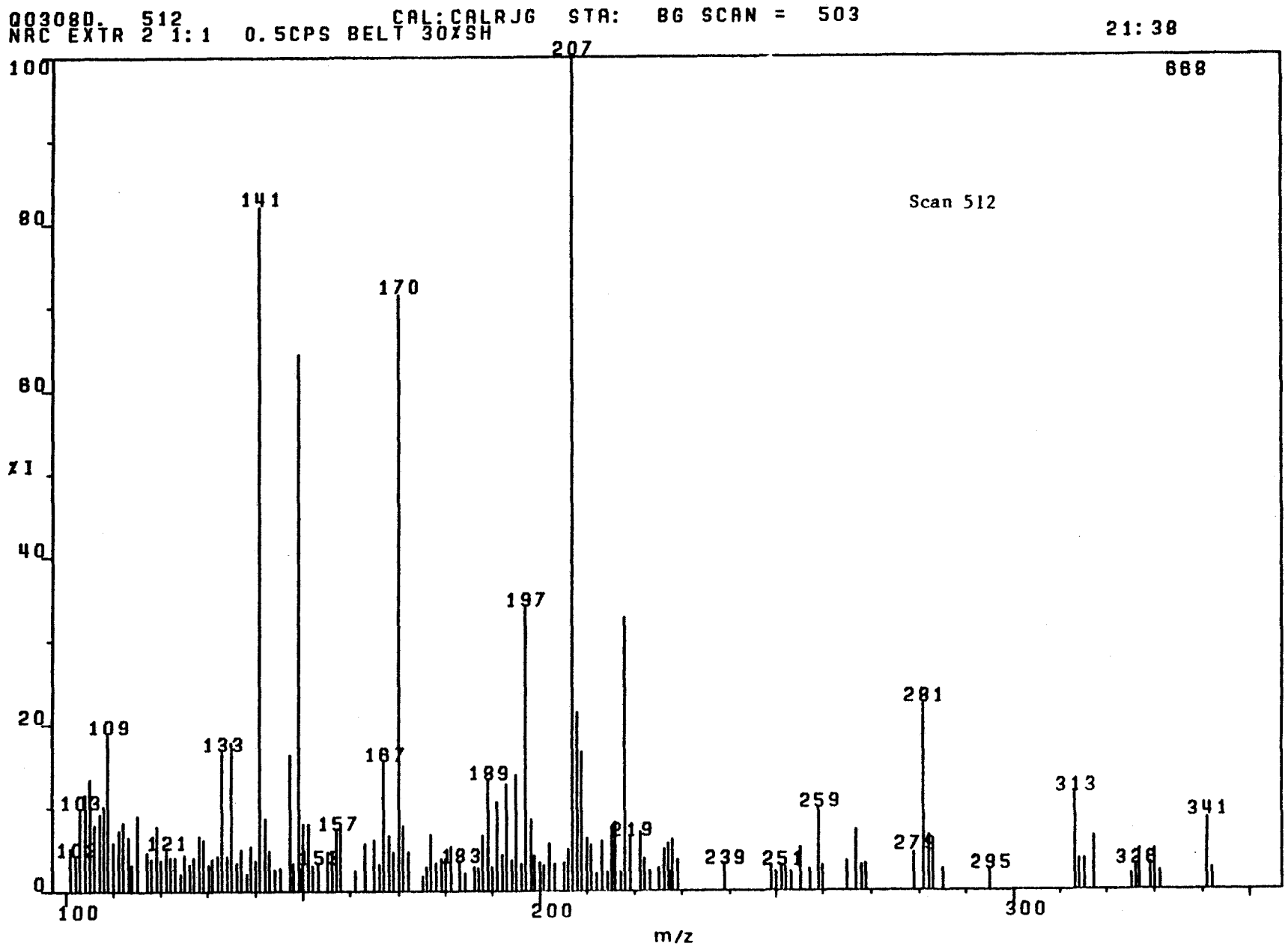
CAL:CALRJG STA:E. BG SCAN = 40
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
18:39
1481
Enhanced Scan 441
202
100
80
60
%I
40
20
0
200
174
278
100
200
300
m/z


003080. 470
CAL:CALRJG STA:E. BG SCAN = 40
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
19:52
1070
Enhanced Scan 470
202
218
200
218
174
237
254
278
299
100
80
60
%I
40
20
0
100
200
300
m/z


003080. 494
CAL:CALRJG STA: BG SCAN = 490
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
20:52
887
Scan 494
207
141
170
197
281
133
228
259
103
119
158
188
221
313
330
115
102
131
155
183
249
278
325
100
80
60
40
20
0
%I
100
200
300
m/z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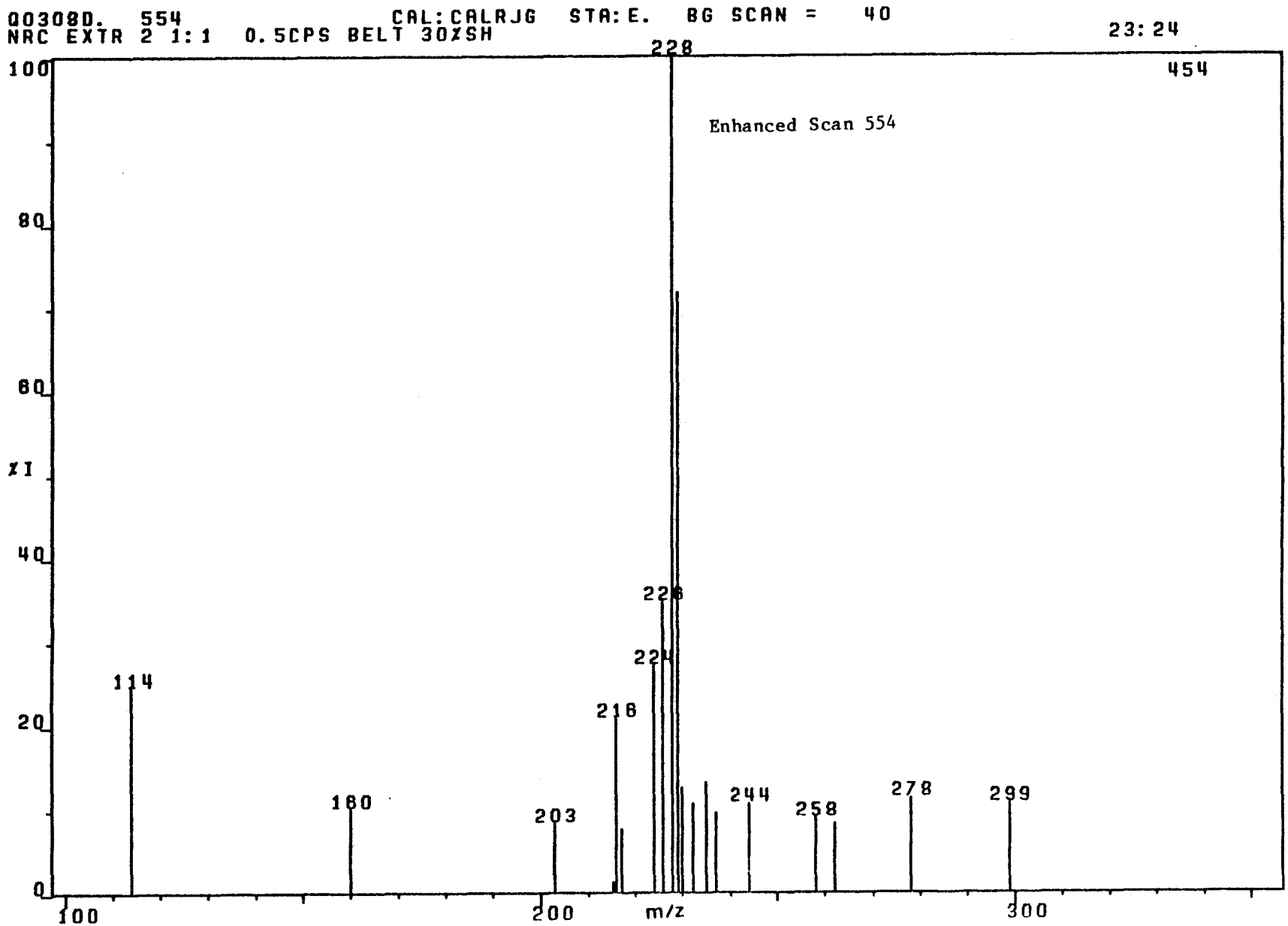
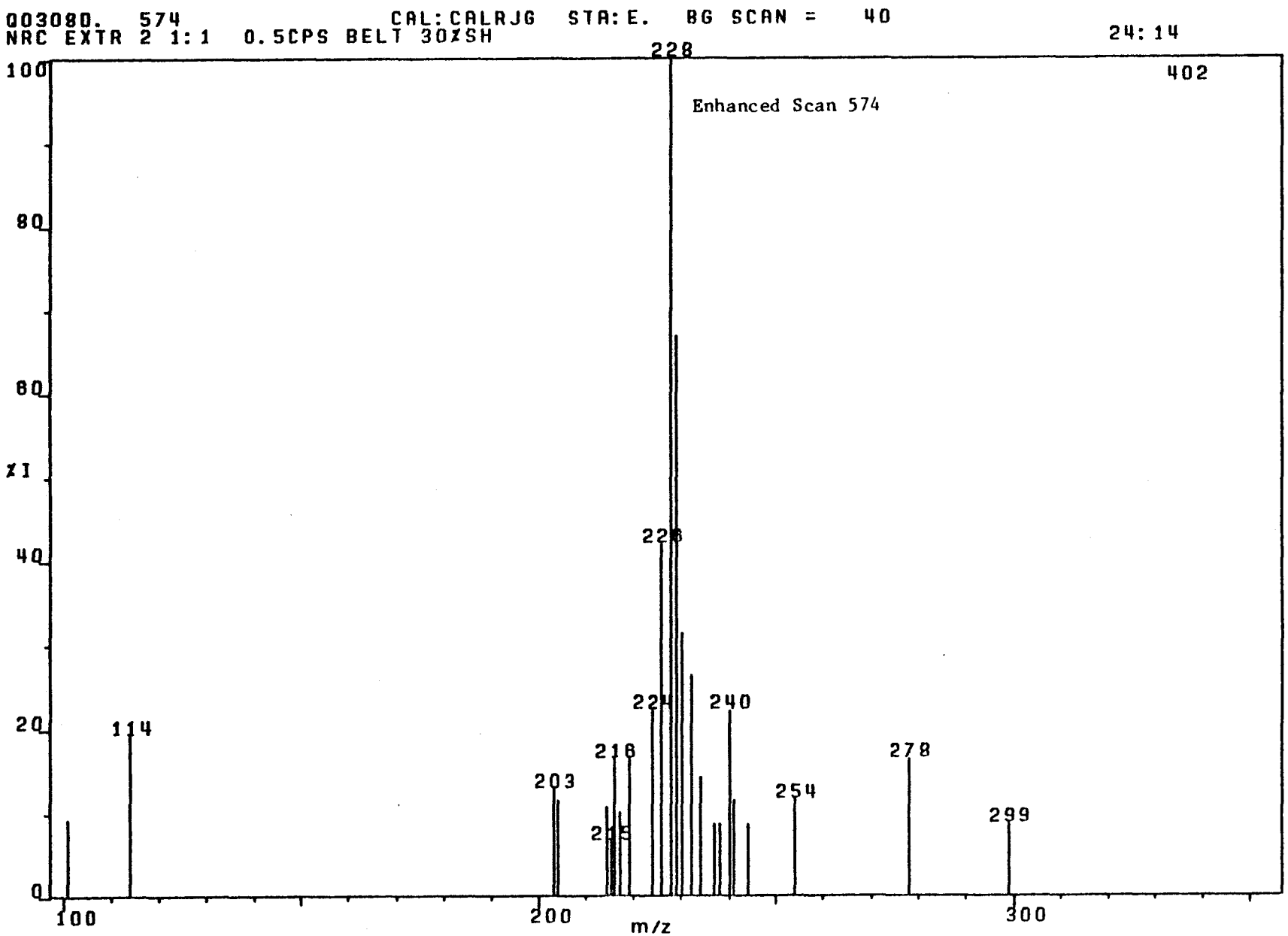
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
21:38
888
Scan 512
207
141
170
197
281
109
133
167
189
259
313
341
157
219
239
251
295
183
121
103
100
200
300
m/z
XI
100
80
60
40
20
0


Q03080. 554 CAL:CALRJG STA:E. BG SCAN = 40
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
23:24
228
454
Enhanced Scan 554
100
80
60
%I
40
20
0
114
160
203
216
224
244
258
278
299
100
200
m/z
300


003080. 574
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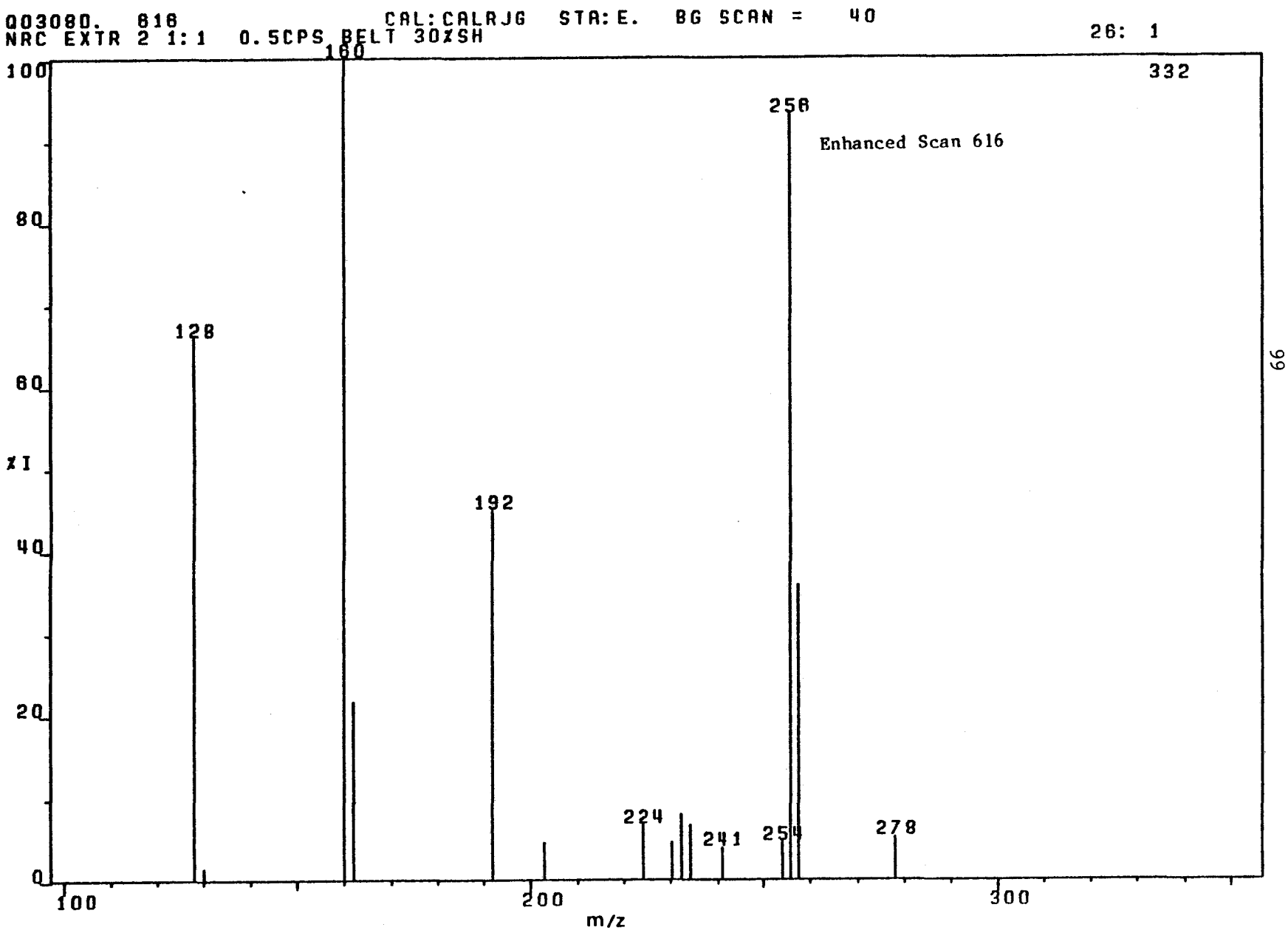
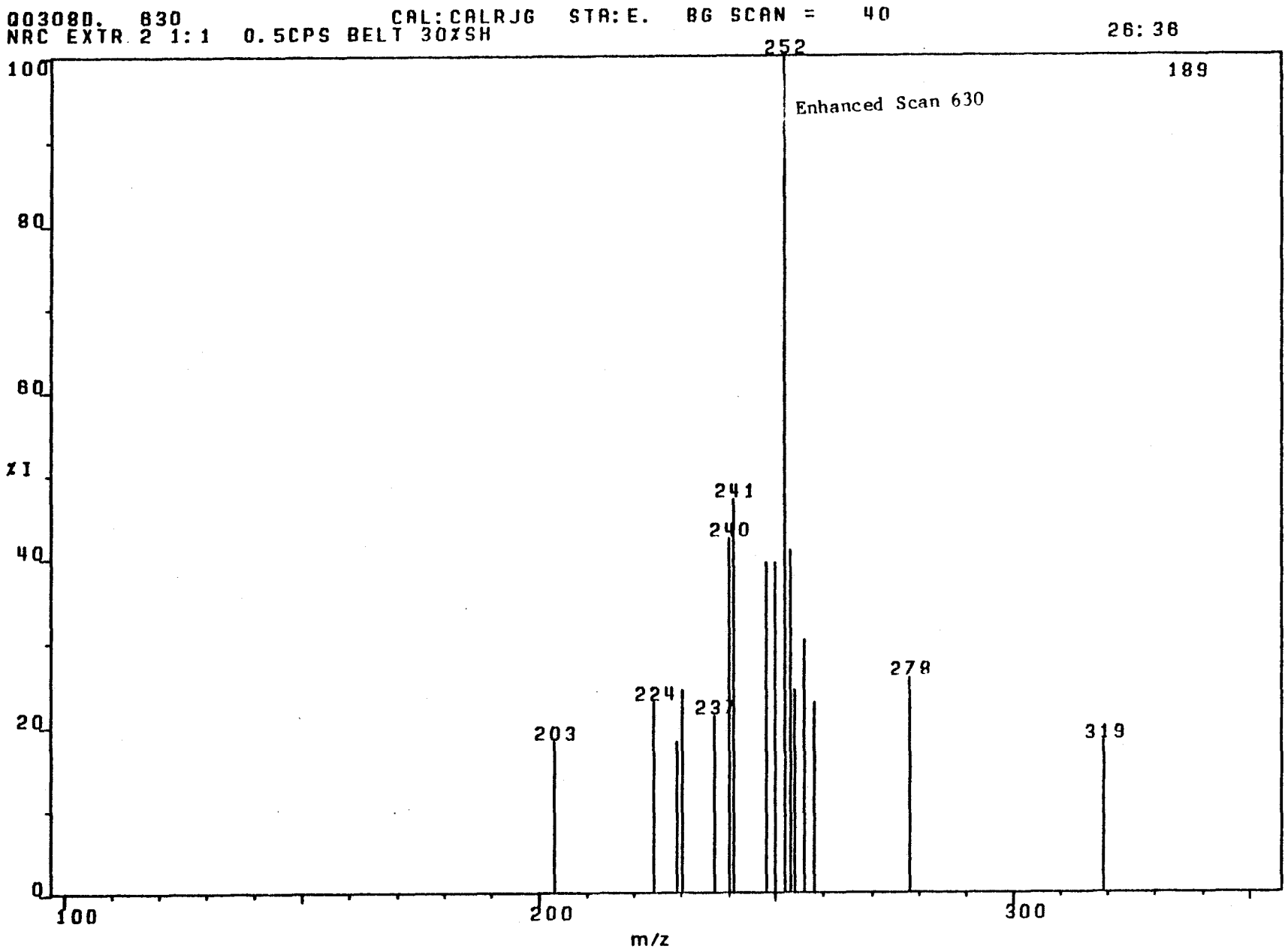
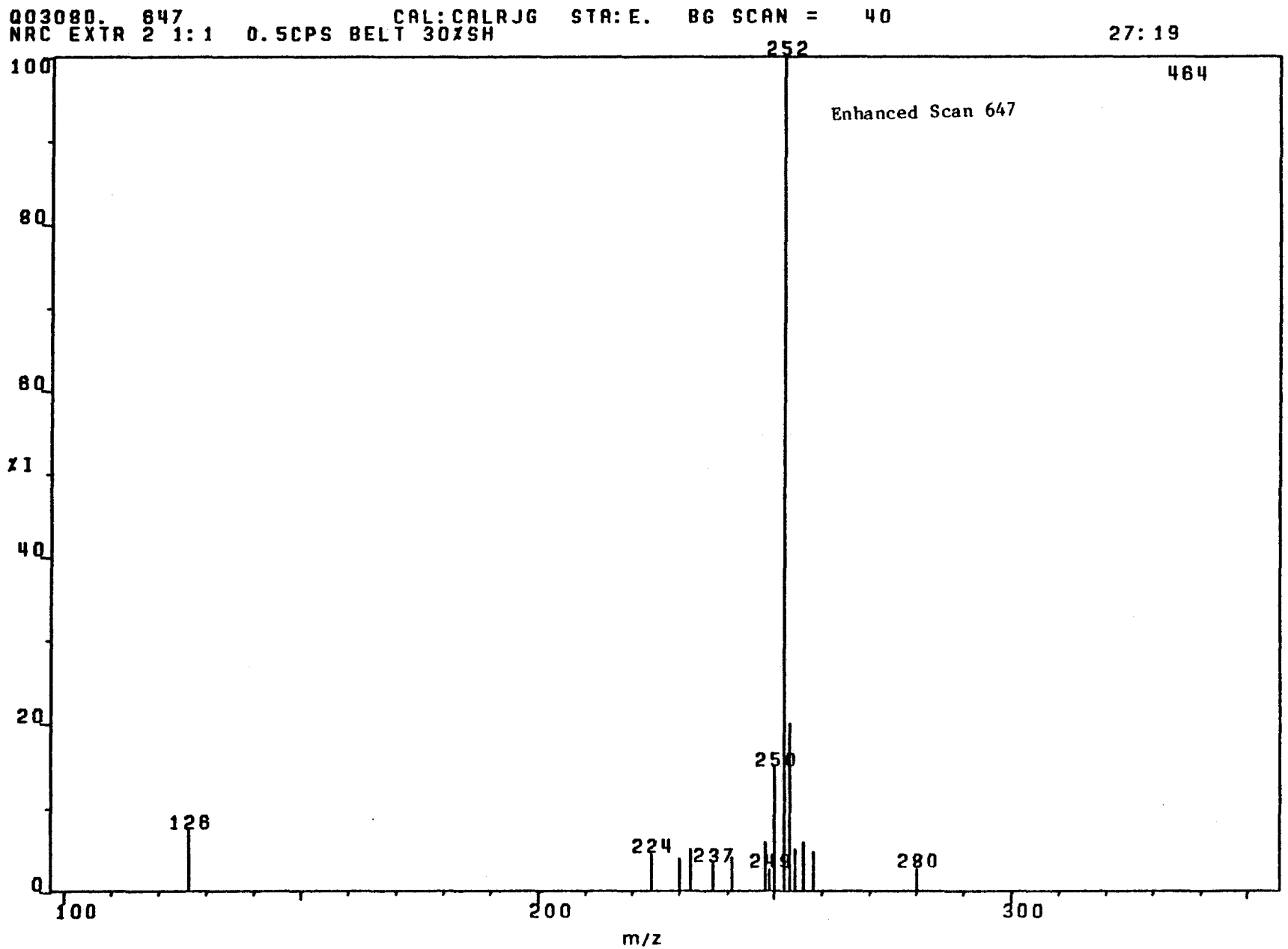
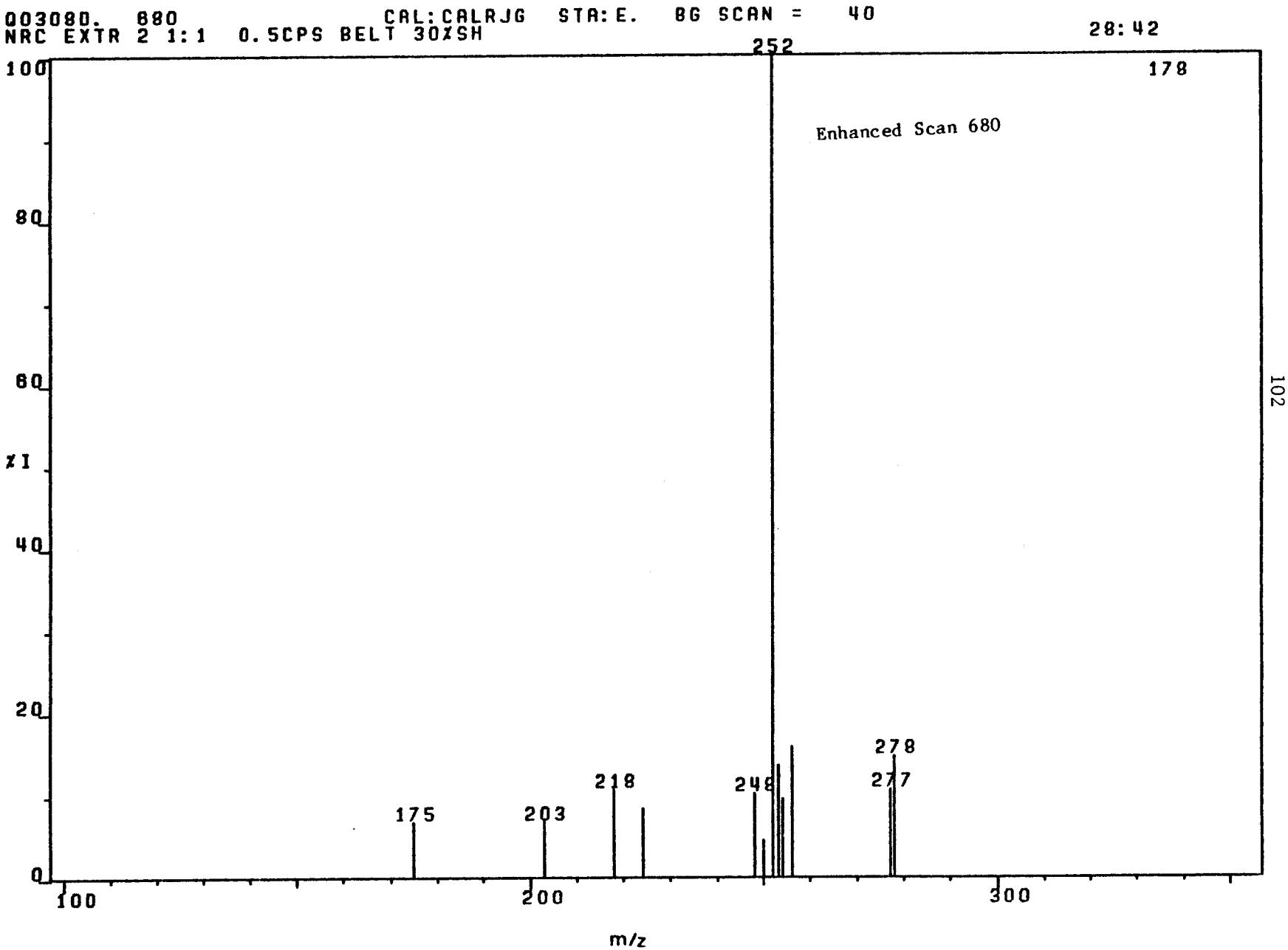
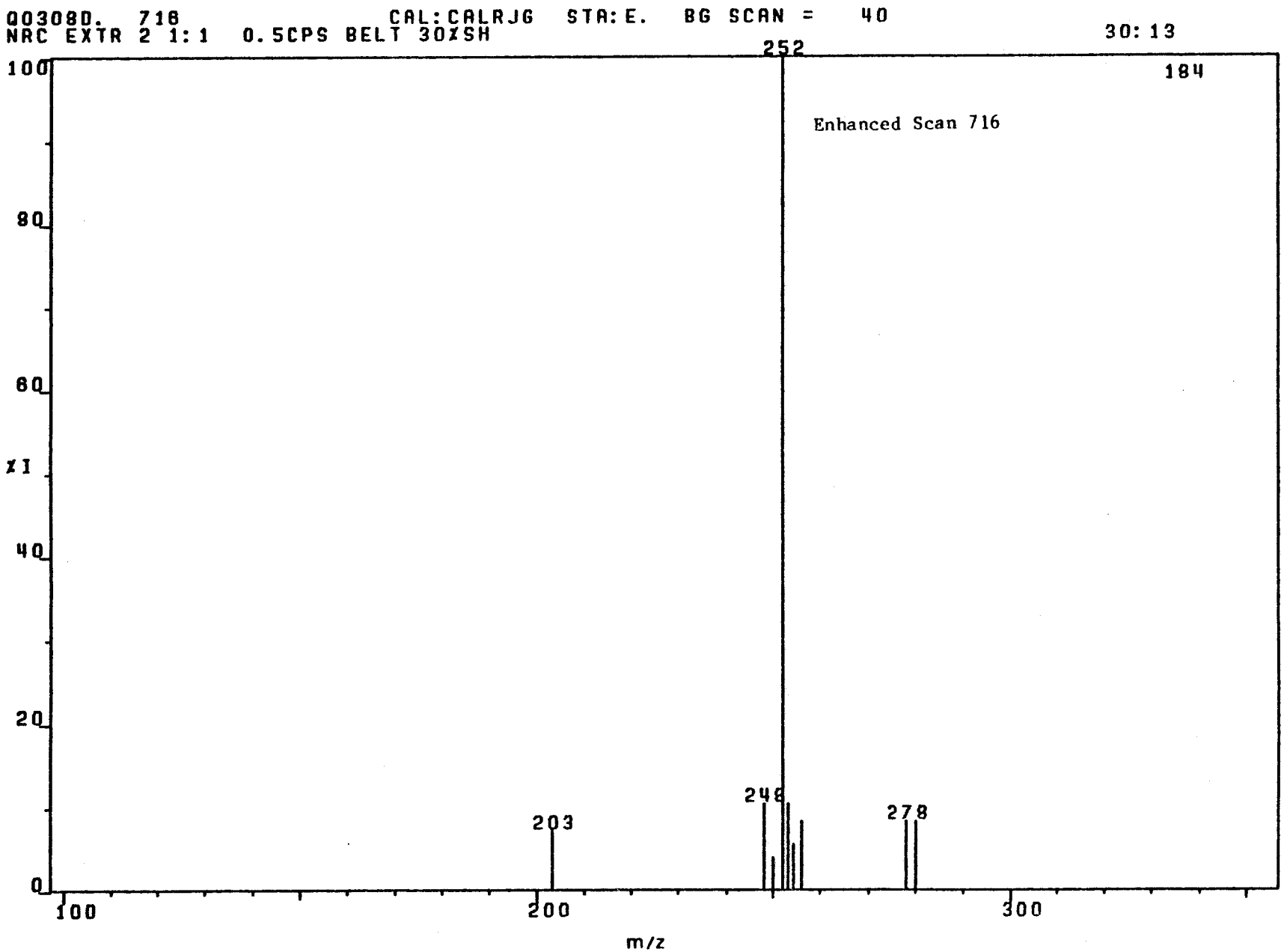
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
24:14
228
402
Enhanced Scan 574
100
80
60
%I
40
20
0
114
203
216
224
240
254
278
299
100
200
300
m/z

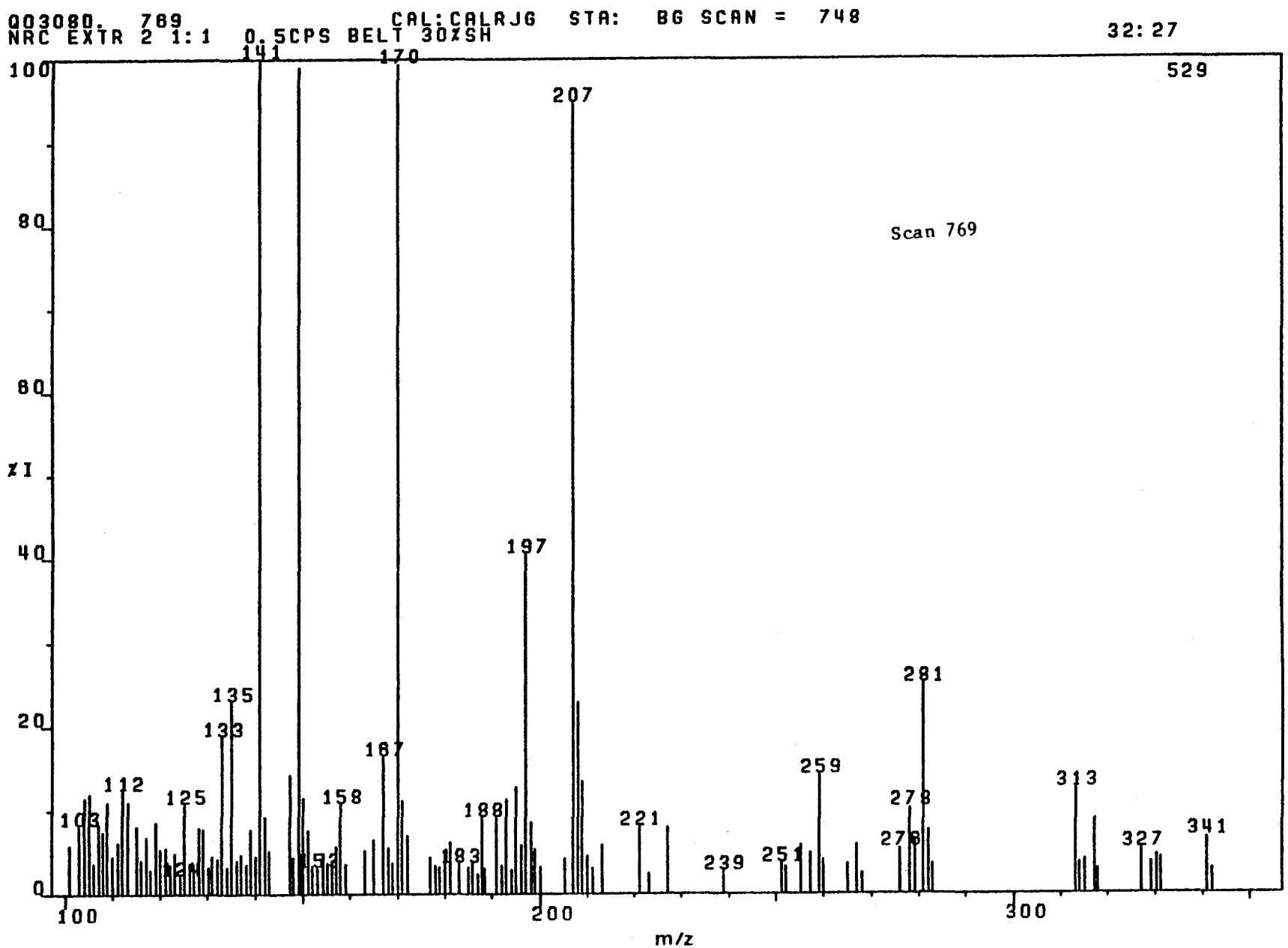

Q03080. 816
CAL:CALRJG STA:E. BG SCAN = 40
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
26: 1
160
332
256
Enhanced Scan 616
128
192
224
241
254
278
100
80
60
%I
40
20
0
100
200
300
m/z
99

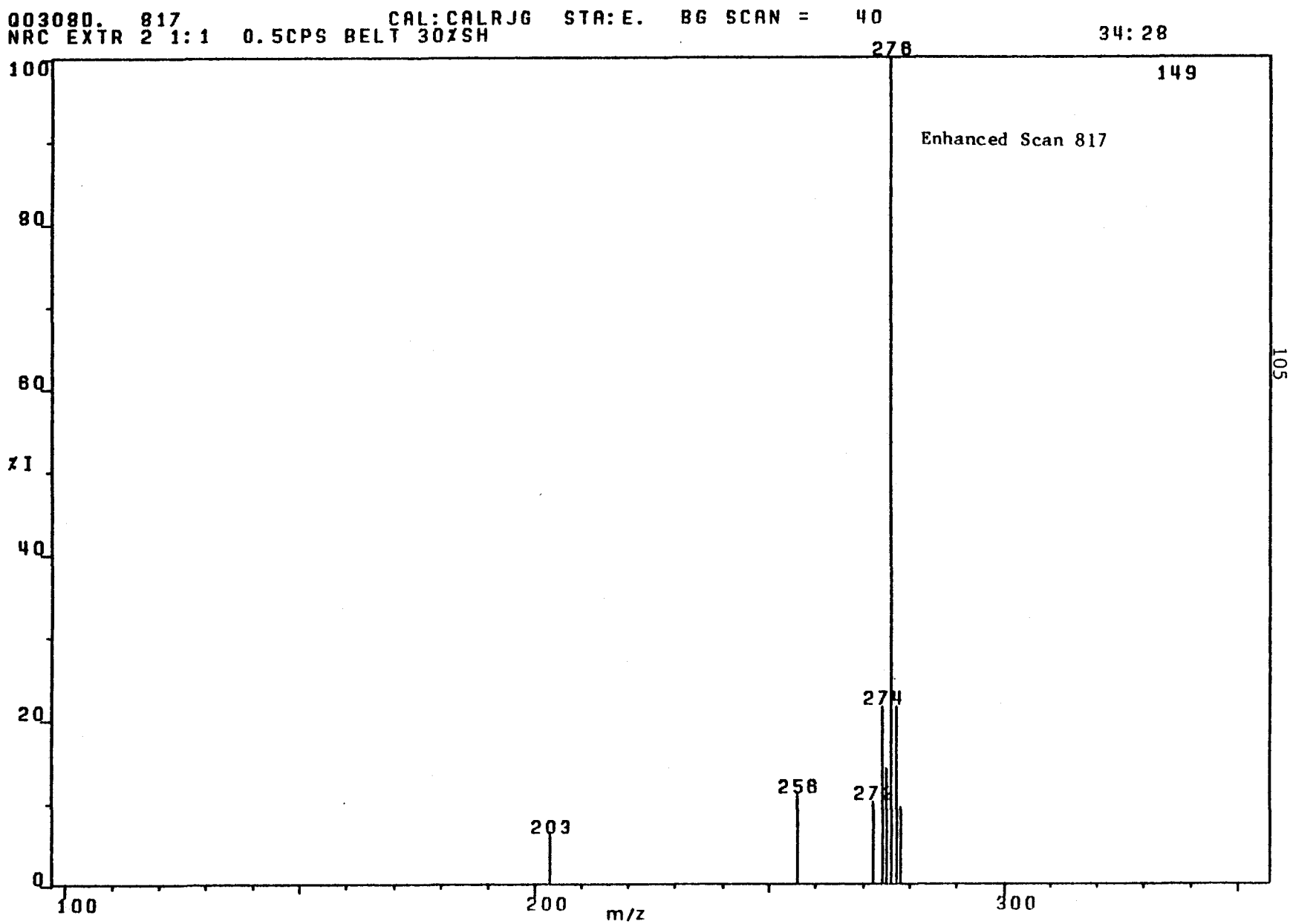

003080. 830
CAL:CALRJG STA:E. BG SCAN = 40
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
26:38
252
189
Enhanced Scan 630
100
80
60
%I
40
20
0
241
240
224
237
203
278
319
100
200
300
m/z

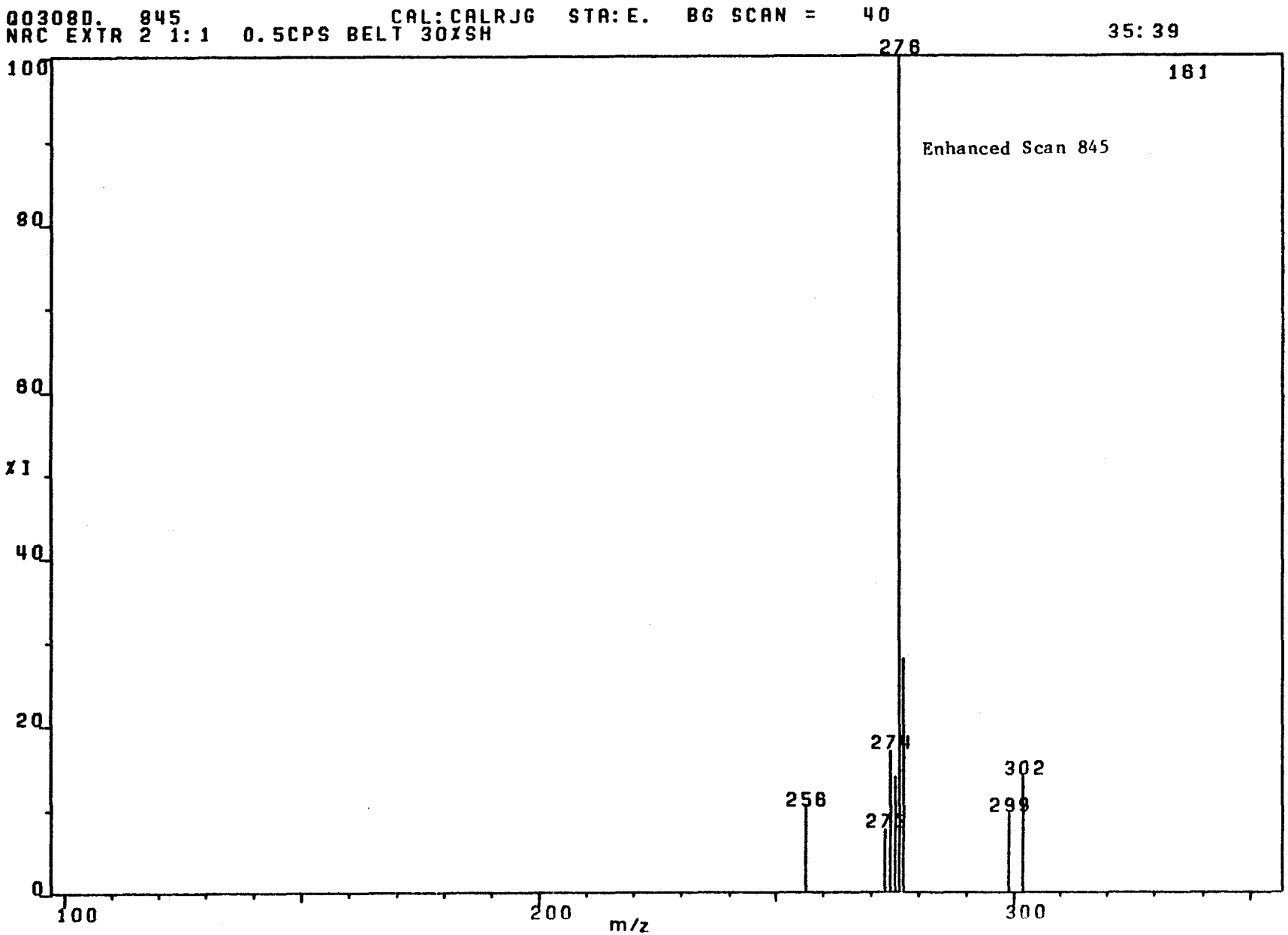

Q03080. 647 CAL:CALRJG STA:E. BG SCAN = 40
NRC EXTR 2 1:1 0.5CPS BELT 30%SH 27:19
252
464
Enhanced Scan 647
100
80
60
%I
40
20
0
126
224
237
250
280
100
200
300
m/z


003080. 680 CAL:CALRJG STA:E. BG SCAN = 40
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
28:42
252
178
Enhanced Scan 680
100
80
60
%I
40
20
0
175
203
218
248
277
278
100
200
300
m/z


Q0308D. 718
CAL:CALRJG STA:E. BG SCAN = 40
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
30:13
252
184
Enhanced Scan 716
100
80
60
%I
40
20
0
203
248
278
100
200
300
m/z


003080. 769
CAL:CALRJG STA: BG SCAN = 748
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
32:27
529
Scan 769
%I
m/z
100
80
60
40
20
0
100
200
300
141
170
207
197
135
133
167
112
125
158
188
221
259
281
313
278
327
341
239
251
183


003080. 817
CAL:CALRJG STA:E. BG SCAN = 40
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
34:28
276
149
Enhanced Scan 817
105
100
80
60
%I
40
20
0
274
258
272
203
100
200
300
m/z


Q03080. 845 CAL:CALRJG STA:E. BG SCAN = 40
NRC EXTR 2 1:1 0.5CPS BELT 30%SH
35:39
276
161
Enhanced Scan 845
100
80
60
%I
40
20
0
256
274
275
299
302
100
200
300
m/z