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Investigation of major mutagenic substances in airborne particulate matter : biologically-driven analysis of fractions and analysis of polycyclic aromatic hydrocarbons (PAHs) nitro-PAHs and other classes of compounds

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INVESTIGATION OF MAJOR MUTAGENIC SUBSTANCES IN AIRBORNE PARTICULATE MATTER: BIOLOGICALLY-DRIVEN ANALYSIS OF FRACTIONS AND ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) NITRO-PAHS AND OTHER CLASSES OF COMPOUNDS

by

Jung-Hen Lwo

Thesis submitted to the faculty of the Graduate School of the New Jersey Institute of Technology in Partial fulfillment of the requirements for the degree of Master of Science in Environmental Science

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APPROVAL SHEET

Title of thesis: Investigation of Major Mutagenic Substances in Airborne Particulate Matter: Biologically-Driven Analysis of Fractions and Analysis of Polycyclic Aromatic Hydrocarbons (PAHs), Nitro-PAHs, and Other Classes of Compounds

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ABSTRACT

Title of thesis: Investigation of Major Mutagenic Substances in Airborne Particulate Matter: Biologically-Driven Analysis of Fractions and Analysis of Polycyclic Aromatic Hydrocarbons (PAHs), Nitro-PAHs, and Other Classes of Compounds

Jung-Hen Lwo, Master of Science in Environmental Science, 1989

Thesis directed by: Dr. Arthur Greenberg

A modified fractionation scheme involving acid-base partitioning and silica gel column chromatography has been used as the first step in the bioassay-directed search for significant levels of mutagenic compounds in extracts of inhalable (IP10) ambient air particulates. The biologically "hot" fractions were separated and analyzed chemically or subfractionated to isolate and concentrate "hot" subfractions which were then chemically analyzed by GC/MS, FTIR, and HPLC equipped with UV, Fluorescence and Photodiode Array UV detectors.

The Ames assay of mutagenicity has involved the unactivated TA98 strain of Salmonella and enzyme-activated (TA98+S9) assays. In addition, some assays have been performed in this present study using TA98NR (TA98-nitroreductase deficient) and TA98DNP (TA98- dinitropyrene reductase deficient). In essence, we are using mutagenicity as our chromatographic detector to pinpoint the most active fractions and compounds which are responsible for carcinogenicity in the air, and then monitor them as well as assess their reactivity.

The comparison of winter and summer samples indicate that the profiles are similar in these two periods. However, levels of polycyclic aromatic hydrocarbons (PAHs) are significantly greater in winter as compared to summer. In addition, nitro-PAHs are found at levels approximately an order of magnitude lower than the PAHs.

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I would like to express my deep gratitude to my advisor, Dr. Arthur Greenberg, for giving me the opportunity to study this interesting and challenging research topic. During the past two years, under his guidance, I learned not only practical experimental skills but also advanced analytical methods and techniques which will be very helpful to my future research.

I am happy to acknowledge Drs. Robert Rosen and Thomas Hartman, Center for Advanced Food Technology, Cook College, Rutgers University who helped me by using GC/MS to identify individual compounds from each fraction and Dr. Thomas Atherholt, Coriell Institute for Medical Research who ran the mutagenicity assays and helped me in interpreting these data.

Special thanks are given to Dr. David Bugay, Squibb Company who taught me how to use FTIR and some special infrared analytical techniques as well as Mr. Clint Brockway for his valuable and helpful suggestions. Additionally, I want to appreciate Dr. Edward Aig and Mr. John Van Antwerp, Waters Associates. They helped me to run the advanced HPLC with Photodiode Array Ultraviolet detector.

I would also like to thank my wife and family for their love and support, and dedicate this thesis to my mother for her encouragement in pursuing higher education.

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CHAPTER ONE

Introduction

Urban air particles contain extractable organic matter which has both mutagenic (1) and carcinogenic (2,3) activities. The polycyclic aromatic hydrocarbons (PAHs) have been the classic family of airborne carcinogens analyzed and are considered to be the surrogate for airborne carcinogenicity. (4-12) In fact, one member of this class, benzo(a)pyrene has been used to represent the PAH class itself. The most recent U.S.E.P.A. report on the subject attributes the major carcinogenic impact of air pollution to Products of Incomplete Combustion (PICs) (13) for which benzo(a)pyrene (BaP), a representative of the class of compounds termed polycyclic aromatic hydrocarbons (PAHs), is also considered as the surrogate.

Over the past decade, the realization occurred that nitro- derivatives of polycyclic aromatic hydrocarbons (14) (nitro-PAHs) may make a very important contribution to the carcinogenicity and mutagenicity of airborne particulates. Therefore, some interesting trends in research have taken place. Previously, research in the area had been "chemically-driven". That is to say, one would have advanced knowledge of carcinogens known to be produced and found in the air and monitor them as well as assess their reactivity. However, detection of the specific compounds responsible for this mutagenic and carcinogenic activities is limited by the complexity of these extracts of ambient air particles. Consequently, these extracts must be separated

into substantially less complex fractions to faciliate detection of the mutagenic compounds. Thus, in this study a fractionation scheme involving acid-base partition and silica gel column chromatography has been used as the first separation step in the bioassay-directed search for mutagenic compounds in extracts of ambient air particulates.

At the same time, bioassay directed fractionation and characterization has been proposed as the most cost-effective and time-effective approach for identifying mutagenic compounds in ambient air particulate extracts.(15) Thus, a new approach rests upon the fractionation of extracts of air samples and the use of biologically "hot" fractions which are separated and analyzed chemically or subfractionated to isolated "hot" subfractions which are then chemically analyzed. Additionally, GC/MS and other techniques such as FTIR, UV and semi-preparative HPLC as well as HPLC equipped with a photodiode array UV detector have been used to explore the presence of other known or unknown compounds and classes of carcinogens and mutagens in the air. This is the approach we have adopted in this research program.

Samples have been collected from the Newark ATEOS site, (16) which is about thirty feet above ground, on the roof of the Boy's Club in the Ironbound section of Newark. This site is bounded primarily by industry to the south as well as inner-city housing to the north. Samples were sequentially soxhlet extracted with two different solvents, dichloromethane (DCM) and acetone (ACE). Each of these two extracts was fractionated by liquid-liquid partition and then subfractionated

using silica gel open column chromatography. The Ames mutagenicity assay has been employed by Dr. T. Atherholt, Coriell Institute for Medical Research, to identify "hot" fractions. They have employed the TA98 *Salmonella strain* ($\pm$S9) on the basis of the ATEOS experience. (17) In addition, nitroreductase-deficient (TA98NR) and dinitropyrene reductase-deficient (TA98DNP) strains have also been employed.

The fractionation procedure employed here is basically the same employed by Nishioka et al (18,19) which is based upon the scheme developed by Peterson. (20) We have made one significant modification in the Nishioka et al scheme. Detailed descriptions of this modified fractionation procedure and the Ames assay results are presented in Chapter Two. The chemical methods employed are, in part, based upon HPLC. Analysis of PAH is done using our earlier HPLC technique as well as a more advanced HPLC system equipped with a photodiode array UV detector. The results are presented and discussed in Chapter Three. The results achieved by Fourier Transform Infrared (FTIR) Spectroscopy and High Performance Liquid Chromatography equipped with Photodiode Array Ultraviolet detector are presented and discussed in Chapter Four. In Chapter Five we show the Gas chromatography/Mass Spectrometry (GC/MS) data. A limited group of fractions were analyzed by Drs. Robert Rosen and Tom Hartman of the Center for Advanced Food Technology of Cook College, Rutgers University. Chapter Six summarizes the conclusions of our study.

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CHAPTER TWO

Fractionation and Ames Assay Results

2.1 Sample Collection

The air particulate samples were collected using four samplers for 10 days in Winter and 15 days in Summer on the roof of the Newark Ironbound Boys Club building on Clifford Street in Newark. Samplers used are all IP10 high volume type which includes two stage fractionators and one hi-vol blower. Suspended particles in the air are sampled for 24 hours in each day at 40 SCFM (Standard Cubic Feet per Minute). At this flow rate, particles greater than 10 microns will cut-point impact onto the impaction surface and the thoracic particles smaller than 10 microns are carried vertically upward by the air flow and then down multiple vent tubes to the 8 in. x 10 in. hi-vol filter where they are collected. The IP10 sampler was designed to collect particles less than 10 microns because this size of air particles can be inhaled by human and might damage the human organs.

In addition, the collection periods were 1/6-1/20/88 for Winter samples and 7/27-8/19/88 for Summer samples. Since a typical 24- hour air volume is ca 1,700 m^3, an equivalent of nearly 70,000 m^3 of Winter air particulates was present in the 10 ml DCM and ACE extract composites as well as 100,000 m^3 of Summer air particulates was present in the 25 ml DCM and ACE extract composites. Before

the samples were collected, pre-fired high-volume quartz filters supplied by NJDEP (New Jersey Department of Environmental Protection) were dried in a desiccator for 24 hours and then weighed. The particulate samples collected from Newark were stored overnight in aluminum foil and dried again in desiccator for another 24 hours prior to soxhlet extraction.

After weighing in order to determine the net total masses of airborne particulates collected, each filter was first soxhlet extracted with 200 ml of dichloromethane (DCM), Photrex grade (J.T. Baker) and GC^2 grade (Burdick & Jackson), then 200 ml of acetone (ACE), same grade and suppliers as DCM. Each 200 ml extract was concentrated to 10 ml using a Kuderna-Danish apparatus, and ultimately all extracts were concentrated to one extract composite for DCM and another extract composite for ACE. A 150-ul aliquot of each extract was used for a residue mass measurement, and a 1 or 2-ml aliquot of each extract was also removed and prepared for bioassay. Moreover, all glassware was cleaned by a special procedure: (1) cleaned with strong base detergent, (2) washed with 5% HNO_3 solution, (3) rinsed with distilled water, (4) dried at a oven, (5) rinsed with methanol three times.

2.2 Separation/Fractionation Procedure

The modification fractionation scheme employed here is based on the scheme of Peterson et al, (1) which involved acid-base partitioning to separate the extract

initially into organic acid, organic base, and neutral component fractions. We feel that this scheme is more useful than a similar one used by the Rome research group. (2) However, one significant modification was made in this scheme because we are interested in attempting to separate classes of acids and bases at the fractionation (extraction) level. Our separation scheme for the DCM extract composite [This work discusses only the DCM extract; the same technique was used by Ms. Wenhui Wu for the ACE extract except that first this solvent was replaced by hexane so that an aqueous extraction could be done. (3)] is depicted in Figure 2-1.

Since 2 ml of Winter composite and 1 ml of Summer composite were sent for Ames assay and an additional (0.15 ml=150 microliter) was employed for determination of extractable organic matter (EOM), only 7.85 ml of Winter DCM extract composite and 23.85 ml of Summer DCM extract composite were used in this fractionation.

The initial extract composite was first extracted with pH 7.0 water, reasoning that strong acids (e.g. carboxylic acids, RCOOH), strong bases (e.g. alkylamines, RNH_2), and possible highly polar neutrals and inorganic salts, if any, would be removed at this point. After this the aqueous phase was split into two parts, one part was used for the separation of strong acids which were back extracted into methylene chloride after adjusting the pH to 1 with 6M H_2SO_4, and another was for strong bases which were back extracted into methylene chloride after adjusting pH to 13 with 40% KOH. Subsequent extraction of acids and bases in the manner of Nishioka et

al (4,5) should presumably remove weaker acids (e.g. phenols, Ar-OH) and weaker bases (e.g. anilines, $Ar - NH_2$ and azaaromatics) from the remaining extract. At the same time, an aliquot of 150 ul was removed for the EOM (Extractable Organic Matter) determination and these fractions also submitted for Ames bioassay. If the fraction give a significantly positive Ames response, chemical analysis was then carried out.

As noted earlier by Nishioka (4) and in our work (see later discussion), the neutral component is the most active fraction, so it is further separated by column chromatography using 5% H_2O-deactivated silica gel (70-150 mesh, Woehlm Pharma) eluted with solvents of increasing polarity. The solvents used, hexane, hexane/benzene (1:1 v/v), methylene chloride, and methanol correspond to the fractions collected. These fractions correspond to the general compound classes of aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAH) and nitro- PAH, moderately polar, and high polar neutral compounds. The column size remained constant (25 mm i.d. x 450 mm length), and the quantities of silica gel as well as eluent solvents used were also kept constant.

The method of Nishioka et al (6) involves four levels of chromatography which were applied to the most active (polar neutral) fraction: 1) Extraction into Fractions, 2) Silica column chromatography into subfractions, 3) HPLC of the most active subfraction to provide "subsubfractions" which we termed second- order subfractions, 4) HPLC of the "subsubfraction" to produce a third-order subfraction.

It was at this fourth stage that Nishioka et al (6) identified hydroxynitropyrenes as important mutagenic components of airborne particulate matter. However, we adopted the first three stages of Nishioka et al procedure for the most active fraction. We completed Level 1 fractionation and fractioned the neutral fractions of the DCM composite using silica gel open column chromatography to form subfractions (Level 2), and then the major mutagenic subfraction was further separated to subsubfractions (Level 3) on a semi-prep silica column (Du Pont Zorbax silica, 9.4 mm id x 25 cm) by using normal-phase HPLC. Table 2-1 identifies the extracts separated and tested for mutagenicity. In Figure 2-1, Table 2-1 and subsequently we have used an abbreviation technique illustrated for a particulate collected in Winter, 1988, extracted by DCM, collected in the neutral (number 6) fraction and obtained from DCM elution (third fraction) from the silica column: *W88- DCM-N6-S3.*

Tables 2-2 and 2-3 show the mass balance results of the first two levels of separation for Winter and Summer DCM composite extracts. The recoveries of organic masses through the acid-base partitioning are 75% and 60% for Winter and Summer composites, respectively. The majority of sample loss which occurred during this level of fractionation might be due to discarding of the aqueous phase (DCM-P5 fraction) containing high-polar neutral, strong acid and strong base compounds. However, mass recoveries are 75% and 80% for Winter and Summer neutral fraction by silica gel column chromatography with only four elution solvents- hexane, hexane/benzene, methylene chloride and methanol. Because we did not use the acidic

methanol to pull out the last neutral fraction, some dark-colored organic material remained at the top of the silica gel column at the end of the fractionation procedure. This indicated that the extract contained extremely polar compounds which did not migrate through the silica gel bed. According to Nishioka study (4), presumably this unrecovered material would appropriately be called extremely polar neutral compounds and might have accounted for as much as 20% of the original extract mass. In addition, the Nishioka group also used acidic methanol [2% 2N HCl in Methanol (v/v)] to pull out the extremely polar neutral compounds from the silica gel column. However, the mutagenic activity both with and without activation of the acidic methanol fraction was quite low and less than any other mutagenic fraction. Therefore, this subfraction was ignored.

2.3 Ames Assays and Results

The primary interest of the present study is the biological effect (carcinogenicity) of airborne particulates on humans. In recent years the Ames mutagenicity assay (7) has been employed as a screen in testing environmental samples. Since it is accepted that cancer can be initiated by an alteration in DNA, mutagenicity appears to be a reasonable first-order surrogate. Furthermore, 83% of the known animal and human carcinogens have been detected as mutagens using the Ames assay. (8)

In the present study, Dr. T. Atherholt of the Coriell Institute for Medical

Research has employed the TA-98 Salmonella strain for the assay since this has been shown to be highly sensitive to airborne mutagens. (9) The assay has involved unactivated (TA98- S9) and enzyme-activated (TA98+S9) assays. The significance of the former is that some substances are known to be direct mutagens: capable of reacting with DNA without metabolism ("activation"). Other compounds, notably the PAH, must first be metabolized ("activated") before attacking DNA. In addition, some assays have been performed in this present study using TA98NR(TA98- nitroductase deficient) and TA98DNP(TA98- dinitropyrene reductase deficient). If there are significant reductions in mutagenicity using these microorganisms, then the active compounds are presumed to be mononitrated or dinitrated respectively.

The purpose of the Ames assays of extracts and fractions of extracts is to pinpoint the most mutagenic fractions of the extracts. In essence, we are using mutagenicity as our chromatographic detector and, thus, employing biology to drive our chemical strategy. This approach has been used to deduce that most of the mutagenic activity of airborne particulates is associated with polar nitrated compounds. (6,10)

As noted earlier, samples were extracted and fractionated by using a modification of the Nishioka-Peterson scheme and silica gel column chromatography. Then, the whole extracts, each fraction and subfraction for winter and summer samples were bioassayed using the TA98 strain with and without enzyme metabolic activation (S9), as well as by some other assays. The calculated distributions of mutagenic

activities coupled with EOM (extractable organic matter) results for the first two levels of fractions in Winter and Summer are shown in Tables 2-4 to 2-7, and the relative distributions of mutagenic activities between fractions and subfractions are also listed in Tables 2-8 to 2-11. Table 2-12 and Table 2-13 display the corrected mutagenicity of each fraction and subfraction which is based upon the 100% recovery of TA98-S9 strain, and the activated mutagenicity (TA98+S9) is calculated from the corrected TA98-S9 multiplied by TA98+S9 over TA98-S9. This correcting method is used to keep the original trend betwwen TA98-S9 and TA98+S9.

After comparing these data, we found that the neutral fractions, in fact, are the most active fractions in the first level of separation for both winter and summer samples, which are responsible for 39% and 53% recovered TA98-S9 as well as 55% and 61% recovered TA98+S9 activities. At the same time, subfraction 2 (W88-DCM-N6-S2, S88-DCM-N6-S2) and subfraction 3 (W88-DCM-N6-S3, S88-DCM-N6-S3) also show the most significant mutagenicity in the second level of fractionation. Furthermore, the poor recoveries of mutagenic activities at the liquid-liquid partitioning step (14.05% - 47.74%) are also shown in Tables 2- 4 to 2-7, but the mutagenic recoveries of subfractions in silica gel column chromatography level (40.44% - 183.07%) are better than the former.

The amount of EOM mass in the summer DCM extract is comparable to that of the winter DCM extract. This is interesting and very surprising to us because our early study indicated that even though the EOM mass of DCM fraction stayed

remarkably constant through year, it did, however, exhibit one very high reading during January which is the same time as our winter sample collection period. Moreover, it is clear that the total mutagenicities of the winter extract are considerably greater than for the summer extract, and total mutagenicity, in the winter, was fairly evenly divided between the weak base, weak acid, strong base and strong acid fractions. For the summer, the two acidic fractions appear to have more than double the mutagenicity of the two basic fractions. This point is consistent with the results of Nishioka et al. (4,5)

Figures 2-2 to 2-5, include graphs which compare mass data and mutagenicity results between fractions and between subfractions. The relative percentage distribution charts are shown in Figures 2-6 to 2-9. From these charts, we can more easily understand that the ordering of mutagenic activities for unactivated TA98 strains are slightly different between winter and summer samples. Specifically, the orderings are Winter: neutrals > weak acids > weak bases > strong acids > strong bases and Summer: neutrals > weak acids > strong acids > weak bases > strong bases, respectively. Additionally, the orderings for activated TA98 strains are also different in Winter and Summer but the weak acid fractions still are more important than other fractions, except the neutrals.

For the subfractions of DCM extracts, the DCM-N6-S1 seems to be elicit no mutagenic response. The remaining three subfractions have significantly increased mutagenic activities upon activation, and the largest effect is seen for DCM-N6-S2

which contains the PAH. Most of the mutagenic activity is found in the most polar DCM-N6-S3 and DCM-N6-S4 fractions, and these two subfractions show significant contributions from nitro-PAH. In contrast, subfraction, DCM-N6-S2, shows virtually no contributions by nitro-PAH as expected. Therefore, the chemical analysis or further fractionation of these three subfractions using HPLC will be discussed in the next chapter.

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Table 2-1 List of Winter & Summer Fractions and Subfractions

Name	Identify	
W88-DCM-B1	*Winter 1988 DCM Extract:*	*weak bases in DCM*
W88-DCM-A2		*weak acids in DCM*
W88-DCM-B3		*strong bases in DCM*
W88-DCM-A4		*strong acids in DCM*
W88-DCM-N6		*nonpolar-moderate polar neutral in DCM*
W88-DCM-N6-S1	*W88-DCM-N6 Subfractions:*	*hexane eluant*
W88-DCM-N6-S2		*1:1 hexane-benzene eluant*
W88-DCM-N6-S3		*dichloromethane eluant*
W88-DCM-N6-S4		*methanol eluant*
S88-DCM-B1	*Summer 1988 DCM Extract:*	*weak bases in DCM*
S88-DCM-A2		*weak acids in DCM*
S88-DCM-B3		*strong bases in DCM*
S88-DCM-A4		*strong acids in DCM*
S88-DCM-N6		*nonpolar-moderate polar neutral in DCM*
S88-DCM-N6-S1	*S88-DCM-N6 Subfractions:*	*hexane eluant*
S88-DCM-N6-S2		*1:1 hexane/benzene eluant*
S88-DCM-N6-S3		*dichloromethane eluant*
S88-DCM-N6-S4		*methanol eluant*

Table 2-2 Mass Balance Results of Winter and Summer Fractions

	Total Masses (mg)*		Percentage (%)	
Fraction Name	*W88-DCM*	*S88-DCM*	*W88-DCM*	*S88-DCM*
#B1 Weak Base	*90.97*	*10.02*	*20.55*	*3.14*
#A2 Weak Acid	*47.12*	*36.67*	*10.64*	*11.51*
#B3 Str. Base	*35.22*	*10.40*	*7.95*	*3.26*
#A4 Str. Acid	*41.70*	*34.37*	*9.42*	*10.78*
#N6 Neutral	*227.77*	*227.24*	*51.44*	*71.30*
Sum of Masses	*442.78*	*318.70*	*100*	*99.99*
Original Mass	*593.78*	*531.98*	-	-
Recovery	*74.57%*	*59.91%*	-	-

*Total masses of each fraction were placed on original volume scale for as 25 ml for Summer.

Table 2-3 Mass Balance Results of Winter and Summer Subfractions

	Total Masses (mg)*		Percentage (%)	
Fraction Name	*W88-DCM*	*S88-DCM*	*W88-DCM*	*S88-DCM*
#S1 Hexane	*37.88*	*49.90*	*28.10*	*28.47*
#S2 Hex/Ben	*9.38*	*5.62*	*6.96*	*3.21*
#S3 D.C.M.	*30.44*	*18.94*	*22.58*	*10.81*
#S4 Methane	*57.11*	*100.82*	*42.36*	*57.52*
Sum of Masses	*134.81*	*175.28*	*100.00*	*100.01*
Original Mass	*178.80*	*216.79*	-	-
Recovery	*75.40%*	*80.85%*	-	-

*Total masses of each subfraction were placed on original volume scale for as well as 15 ml for Summer.

Table 2-4a

Mass and Ames Assay Results for W88-DCM and Fractions

		TA98-S9		TA98+S9	
Fraction Name	*Mass(ug)*	*rev/ug*	*Total(rev)*	*rev/ug*	*Total(rev)*
W88-DCM-B1	*90,968*	*0.35*	*31,839*	*0.51*	*46,394*
W88-DCM-A2	*47,123*	*0.78*	*36,756*	*0.56*	*26,389*
W88-DCM-B3	*35,220*	*0.71*	*25,006*	*0.70*	*24,654*
W88-DCM-A4	*41,705*	*0.63*	*26,274*	*0.30*	*12,512*
W88-DCM-N6	*227,770*	*0.34*	*77,442*	*0.60*	*136,662*
Total(1-6)	*442,787*	-	*197,317*	-	*246,611*
W88-DCM	*593,780*	*1.25*	*742,225*	*0.87*	*516,589*
Recovery	*74.57%*	-	*26.58%*	-	*47.74%*

The mass of each fraction is based upon the original 10 ml volume of W88-DCM whole extract.

Table 2-4b

Mass and Ames Assay Results for W88-DCM and Fractions

		TA98NR-S9		TA98DNP-S9	
Fraction Name	*Mass(ug)*	*rev/ug*	*Total(rev)*	*rev/ug*	*Total(rev)*
W88-DCM-B1	*90,968*	*0.23*	*20,923*	*0.11*	*10,006*
W88-DCM-A2	*47,123*	*0.27*	*12,723*	*0.10*	*4,712*
W88-DCM-B3	*35,220*	*0.61*	*21,484*	*0.25*	*8,805*
W88-DCM-A4	*41,705*	*0.48*	*20,018*	*0.13*	*5,422*
W88-DCM-N6	*227,770*	*0.23*	*52,387*	*0.13*	*29,610*
Total(1-6)	*442,787*	-	*127,535*	-	*58,555*
W88-DCM	*593,780*	*0.73*	*433,459*	*0.24*	*142,507*
Recovery	*74.57%*	-	*29.42%*	-	*41.09%*

The mass of each fraction is based upon the original 10 ml volume of W88-DCM whole extract.

Table 2-5a

Mass and Ames Assay Results for W88-DCM-N6 and Subfractions

		TA98-S9		TA98+S9	
Fraction Name	*Mass(ug)*	*rev/ug*	*Total(rev)*	*rev/ug*	*Total(rev)*
W88-DCM-N6-S1	*37,876*	*NEG*	*NEG*	*0.06*	*2,273*
W88-DCM-N6-S2	*9,379*	*0.64*	*6,003*	*1.81*	*16,976*
W88-DCM-N6-S3	*30,441*	*0.45*	*13,698*	*1.07*	*32,572*
W88-DCM-N6-S4	*57,107*	*0.30*	*17,132*	*0.70*	*39,975*
Total (1-4)	*134,802*	-	*36,833*	-	*91,796*
W88-DCM-N6	*178,800*	*0.34*	*60,792*	*0.60*	*107,280*
Recovery	*75.39%*	-	*60.59%*	-	*85.57%*

The mass of each subfraction is based upon the original 10 ml volume of neutral fraction, but this amount does not place on the original volume scale of W88-DCM extract.

Table 2-5b

Mass and Ames Assay Results for W88-DCM-N6 and Subfractions

		TA98NR-S9		TA98DNP-S9	
Fraction Name	*Mass(ug)*	*rev/ug*	*Total(rev)*	*rev/ug*	*Total(rev)*
W88-DCM-N6-S1	*37,876*	*NEG*	*NEG*	*NEG*	*NEG*
W88-DCM-N6-S2	*9,379*	*0.71*	*6,659*	*0.63*	*5,976*
W88-DCM-N6-S3	*30,441*	*0.14*	*4,262*	*0.21*	*6,393*
W88-DCM-N6-S4	*57,107*	*0.10*	*5,711*	*0.11*	*6,282*
Total (1-4)	*134,802*	-	*16,632*	-	*18,651*
W88-DCM-N6	*178,800*	*0.23*	*41,124*	*0.13*	*23,244*
Recovery	*75.39%*	-	*40.44%*	-	*80.24%*

The mass of each subfraction is based upon the original 10 ml volume of neutral fraction, but this amount does not place on the original volume scale of W88-DCM extract.

Table 2-6a

Mass and Ames Assay Results for S88-DCM and Fractions

		TA98-S9		TA98+S9	
Fraction Name	*Mass(ug)*	*rev/ug*	*Total(rev)*	*rev/ug*	*Total(rev)*
S88-DCM-B1	*10,016*	*1.19*	*11,919*	*0.68*	*6,811*
S88-DCM-A2	*36,675*	*0.64*	*23,472*	*0.55*	*20,171*
S88-DCM-B3	*10,403*	*0.52*	*5,410*	*0.58*	*6,034*
S88-DCM-A4	*34,371*	*0.54*	*18,560*	*0.27*	*9,280*
S88-DCM-N6	*227,241*	*0.29*	*65,900*	*0.29*	*65,900*
Total(1-6)	*318,704*	-	*125,261*	-	*108,196*
S88-DCM	*531,975*	*0.77*	*409,621*	*0.48*	*255,348*
Recovery	*59.91%*	-	*30.58%*	-	*42.37%*

The mass of each fraction is based upon the original 25 ml volume of S88-DCM whole extract.

Table 2-6b

Mass and Ames Assay Results for S88-DCM and Fractions

		TA98NR-S9		TA98DNP-S9	
Fraction Name	*Mass(ug)*	*rev/ug*	*Total(rev)*	*rev/ug*	*Total(rev)*
S88-DCM-B1	*10,016*	*0.22*	*2,204*	*0.15*	*1,502*
S88-DCM-A2	*36,675*	*0.13*	*4,768*	*0.12*	*4,401*
S88-DCM-B3	*10,403*	*0.46*	*4,785*	*0.16*	*1,664*
S88-DCM-A4	*34,371*	*0.28*	*9,624*	*0.06*	*2,062*
S88-DCM-N6	*227,241*	*0.13*	*29,541*	*0.03*	*6,817*
Total(1-6)	*318,704*	-	*50,922*	-	*16,446*
S88-DCM	*531,975*	*0.34*	*180,871*	*0.22*	*117,035*
Recovery	*59.91%*	-	*28.15%*	-	*14.05%*

The mass of each fraction is based upon the original 25 ml volume of S88-DCM whole extract.

Table 2-7a

Mass and Ames Assay Results for S88-DCM-N6 and Subfractions

		TA98-S9		TA98+S9	
Fraction Name	*Mass(ug)*	*rev/ug*	*Total(rev)*	*rev/ug*	*Total(rev)*
S88-DCM-N6-S1	*49,896*	*0.06*	*2,994*	*0.12*	*5,988*
S88-DCM-N6-S2	*5,616*	*1.76*	*9,884*	*2.51*	*14,096*
S88-DCM-N6-S3	*18,936*	*1.68*	*31,812*	*2.40*	*45,446*
S88-DCM-N6-S4	*100,824*	*0.28*	*28,231*	*0.27*	*27,222*
Total (1-4)	*175,272*	-	*72,921*	-	*92,752*
S88-DCM-N6	*216,788*	*0.29*	*62,869*	*0.29*	*62,869*
Recovery	*80.85%*	-	*115.99%*	-	*147.53%*

The mass of each subfraction is based upon the original 15 ml volume of neutral fraction, but this amount does not place on the original volume scale of W88-DCM extract.

Table 2-7b

Mass and Ames Assay Results for S88-DCM-N6 and Subfractions

		TA98NR-S9		TA98DNP-S9	
Fraction Name	*Mass(ug)*	*rev/ug*	*Total(rev)*	*rev/ug*	*Total(rev)*
S88-DCM-N6-S1	*49,896*	-	-	*NEG*	-
S88-DCM-N6-S2	*5,616*	-	-	*0.57*	*3,201*
S88-DCM-N6-S3	*18,936*	-	-	*0.30*	*5,681*
S88-DCM-N6-S4	*100,824*	-	-	*0.03*	*3,025*
Total (1-4)	*175,272*	-	-	-	*11,907*
S88-DCM-N6	*216,788*	-	-	*0.03*	*6,504*
Recovery	*80.85%*	-	-	-	*183.07%*

The mass of each subfraction is based upon the original 15 ml volume of neutral fraction, but this amount does not place on the original volume scale of W88-DCM extract.

Table 2-8 Ames Assay Results for W88-DCM and Fractions

	TA98-S9		TA98+S9	
Fraction Name	*Total(rev)*	*Percent(%)*	*Total(rev)*	*Percent(%)*
W88-DCM-B1	*119,786*	*16.14%*	*97,181*	*18.81%*
W88-DCM-A2	*138,284*	*18.63%*	*55,276*	*10.70%*
W88-DCM-B3	*94,078*	*12.67%*	*51,642*	*10.00%*
W88-DCM-A4	*98,849*	*13.32%*	*26,209*	*5.07%*
W88-DCM-N6	*291,354*	*39.25%*	*286,263*	*55.42%*
Total(1-6)	*742,351*	-	*516,571*	-

The total mutagenicity (rev) of each fraction is based upon the 100% of recovery, so the each mutagenicity should be divided by the original recovery.

Table 2-9 Ames Assay Results for W88-DCM-N6 and Subfractions

	TA98-S9		TA98+S9	
Fraction Name	*Total(rev)*	*Percent(%)*	*Total(rev)*	*Percent(%)*
W88-DCM-N6-S1	*NEG*	*NEG*	*2,656*	*2.48%*
W88-DCM-N6-S2	*9,908*	*16.30%*	*19,839*	*18.49%*
W88-DCM-N6-S3	*22,608*	*37.19%*	*38,065*	*35.48%*
W88-DCM-N6-S4	*28,275*	*46.51%*	*46,716*	*43.55%*
Total (1-4)	*60,791*	-	*107,276*	-

The total mutagenicity (rev) of fraction is based upon the 100% of recovery, so each mutagenicity should be divided by the original recovery.

Table 2-10 Ames Assay Results for S88-DCM and Fractions

	TA98-S9		TA98+S9	
Fraction Name	*Total(rev)*	*Percent(%)*	*Total(rev)*	*Percent(%)*
S88-DCM-B1	*38,976*	*9.52%*	*16,075*	*6.30%*
S88-DCM-A2	*76,756*	*18.74%*	*47,607*	*18.64%*
S88-DCM-B3	*17,691*	*4.32%*	*14,241*	*5.58%*
S88-DCM-A4	*60,693*	*14.82%*	*21,902*	*8.58%*
S88-DCM-N6	*215,500*	*52.61%*	*155,535*	*60.91%*
Total(1-6)	*409,616*	-	*255,360*	-

The total mutagenicity (rev) of each fraction is based upon the 100 by the original recovery.

Table 2-11 Ames Assay Results for S88-DCM-N6 and Subfractions

	TA98-S9		TA98+S9	
Fraction Name	*Total(rev)*	*Percent(%)*	*Total(rev)*	*Percent(%)*
S88-DCM-N6-S1	*2,581*	*4.11%*	*4,059*	*6.46%*
S88-DCM-N6-S2	*8,521*	*13.55%*	*9,555*	*15.20%*
S88-DCM-N6-S3	*27,427*	*43.63%*	*30,805*	*49.00*
S88-DCM-N6-S4	*24,339*	*38.71%*	*18,452*	*29.35%*
Total (1-4)	*62,868*	-	*62,871*	-

The total mutagenicity (rev) of each fraction is based upon the 100% of recovery, so the each mutagenicity should be divided by the original recovery.

Table 2-12 The Corrected Mutagenicity of W88 and S88 Fractions

	TA98-S9(rev)[a]		TA98+S9(rev)[b]	
Fraction Name	*W88-DCM*	*S88-DCM*	*W88-DCM*	*S88-DCM*
#B1 Weak Base	*119,800*	*39,000*	*174,600*	*22,300*
#A2 Weak Acid	*138,300*	*76,800*	*99,300*	*66,000*
#B3 Str. Base	*94,100*	*17,700*	*92,800*	*19,700*
#A4 Str. Acid	*98,800*	*60,700*	*47,000*	*30,400*
#N6 Neutral	*291,400*	*215,500*	*512,200*	*215,500*
Total (1-6)	*742,400*	*409,700*	*925,900*	*353,900*

[a]The TA98-S9 mutagenicity of each fraction is corrected for 100% recovery.

[b]The corrected TA98+S9 mutagenicity of each fraction is calculated from the corrected TA98-S9 multiplied by TA98+S9 over TA98-S9.

Table 2-13 The Corrected Mutagenicity of W88 and S88 Subfractions

	TA98-S9(rev)[a]		TA98+S9(rev)[b]	
Fraction Name	*W88-DCM*	*S88-DCM*	*W88-DCM*	*S88-DCM*
#S1 Hexane	*NEG*	*2,600*	*3,800*	*5,200*
#S2 Hex/Ben	*9,900*	*8,500*	*28,000*	*12,100*
#S3 DCM	*22,600*	*27,400*	*53,700*	*39,100*
#S4 Methanol	*28,300*	*24,300*	*66,000*	*23,400*
Total (1-4)	*60,600*	*62,800*	*151,500*	*79,800*

[a]The TA98-S9 mutagenicity of each subfraction is corrected for 100% recovery.
[b]The corrected TA98+S9 mutagenicity of each subfraction is calculated from the corrected TA98-S9 multiplied by TA98+S9 over TA98-S9.

Figure 2-1 Fractionation Scheme of DCM Extract

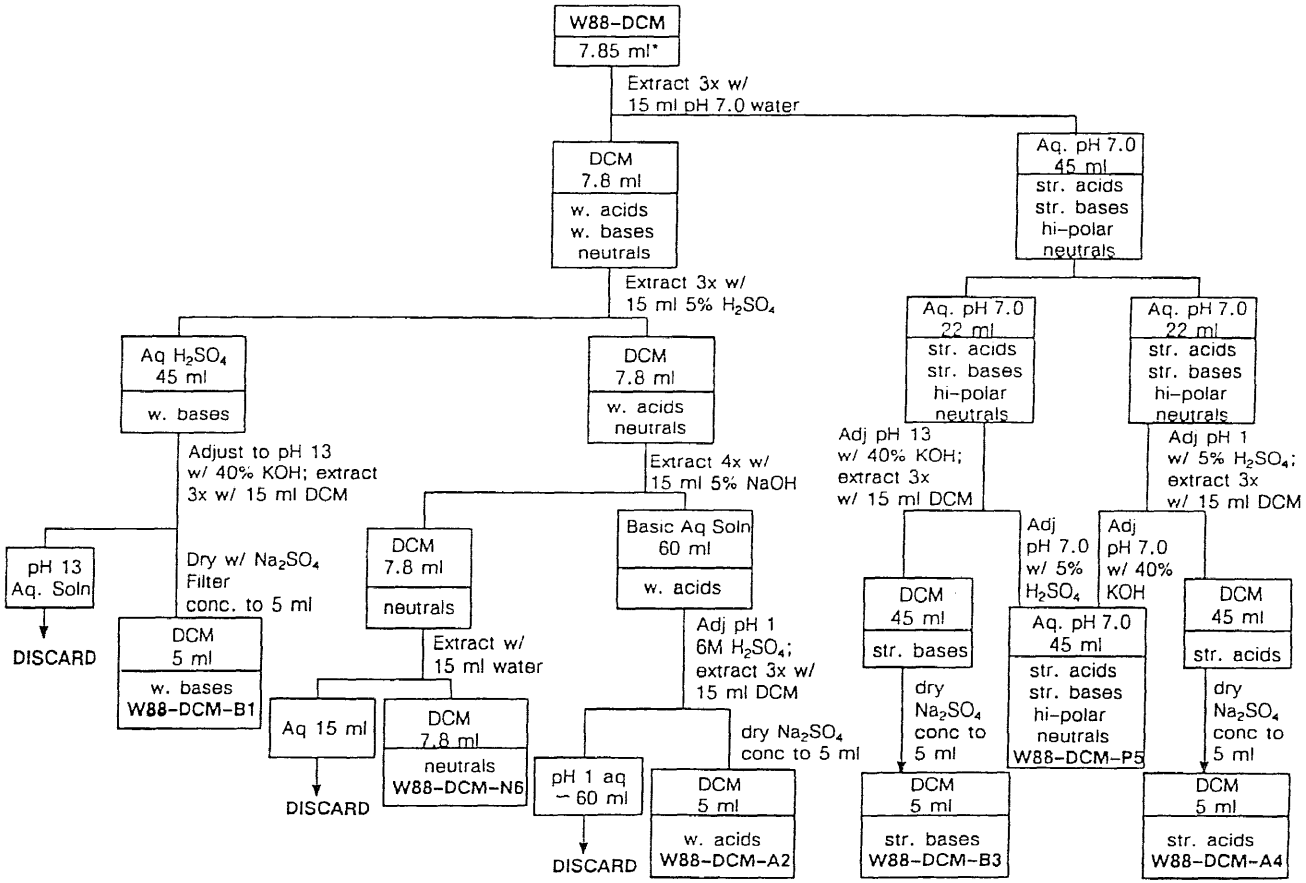


Figure 2-2 Distribution of Corrected Mass and Mutagenicity for Winter Fractions

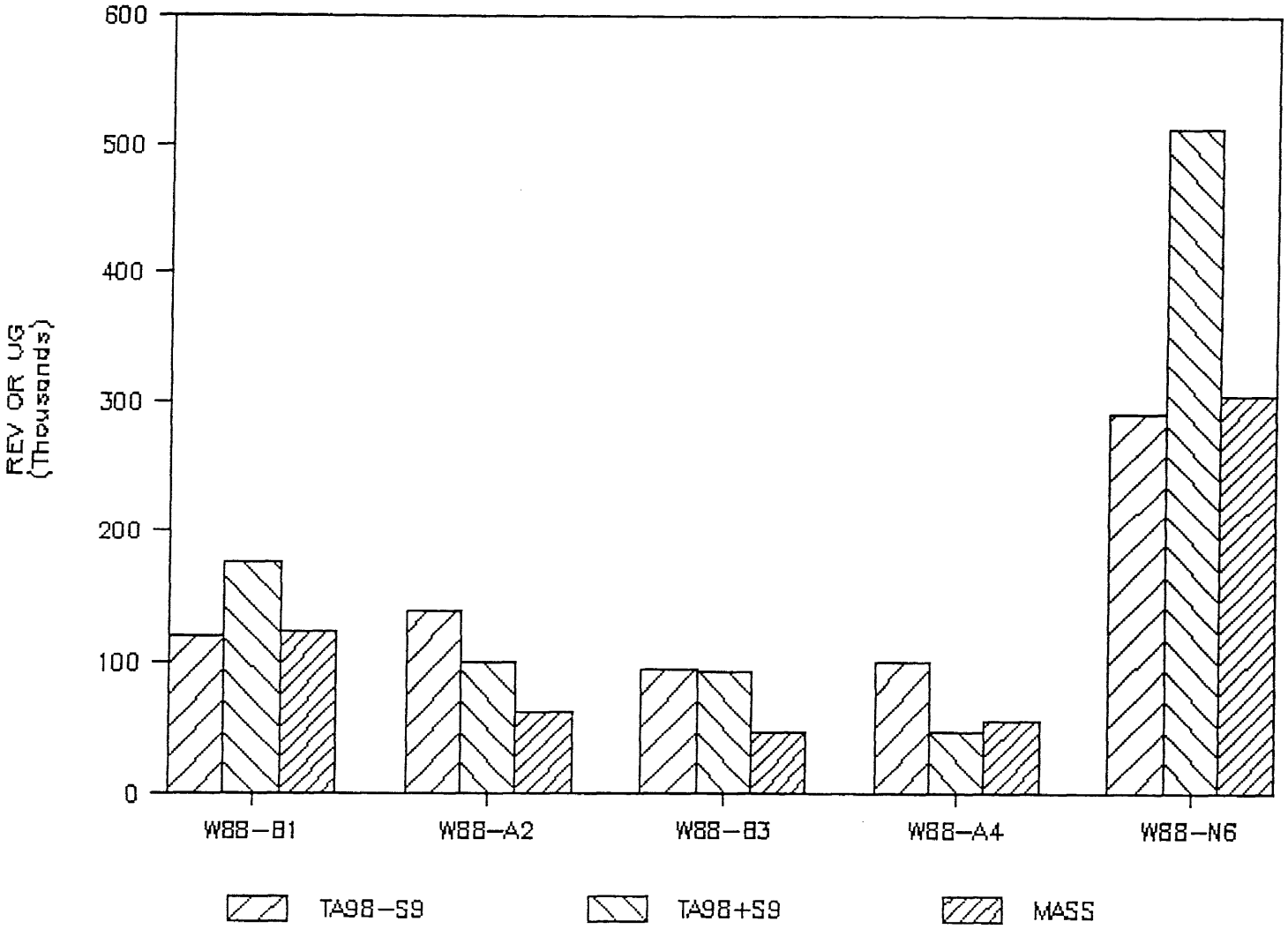


Figure 2-3 Distribution of Corrected Mass and Mutagenicity for Winter Subfractions

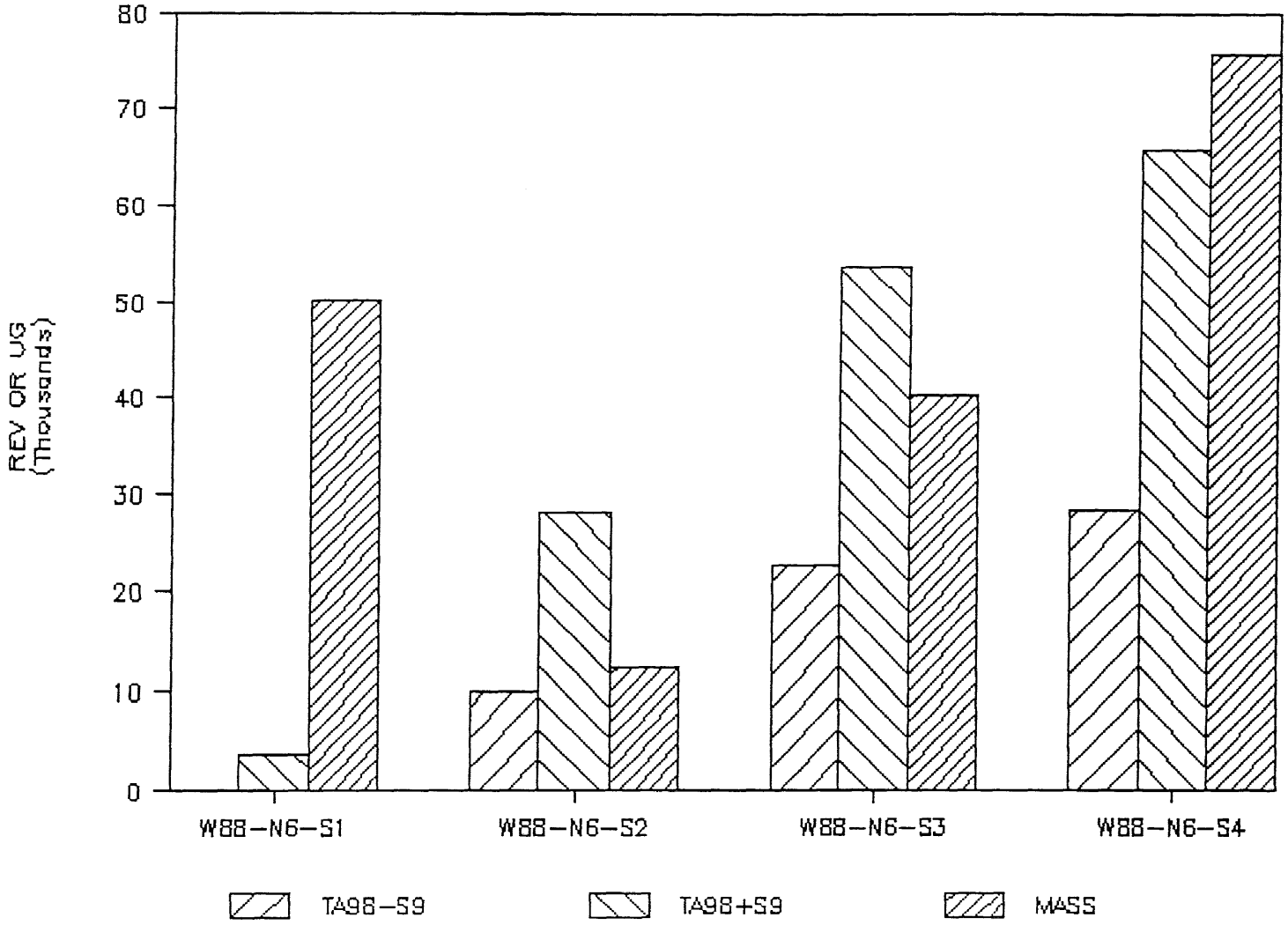


Figure 2-4 Distribution of Corrected Mass and Mutagenicity for Summer Fractions

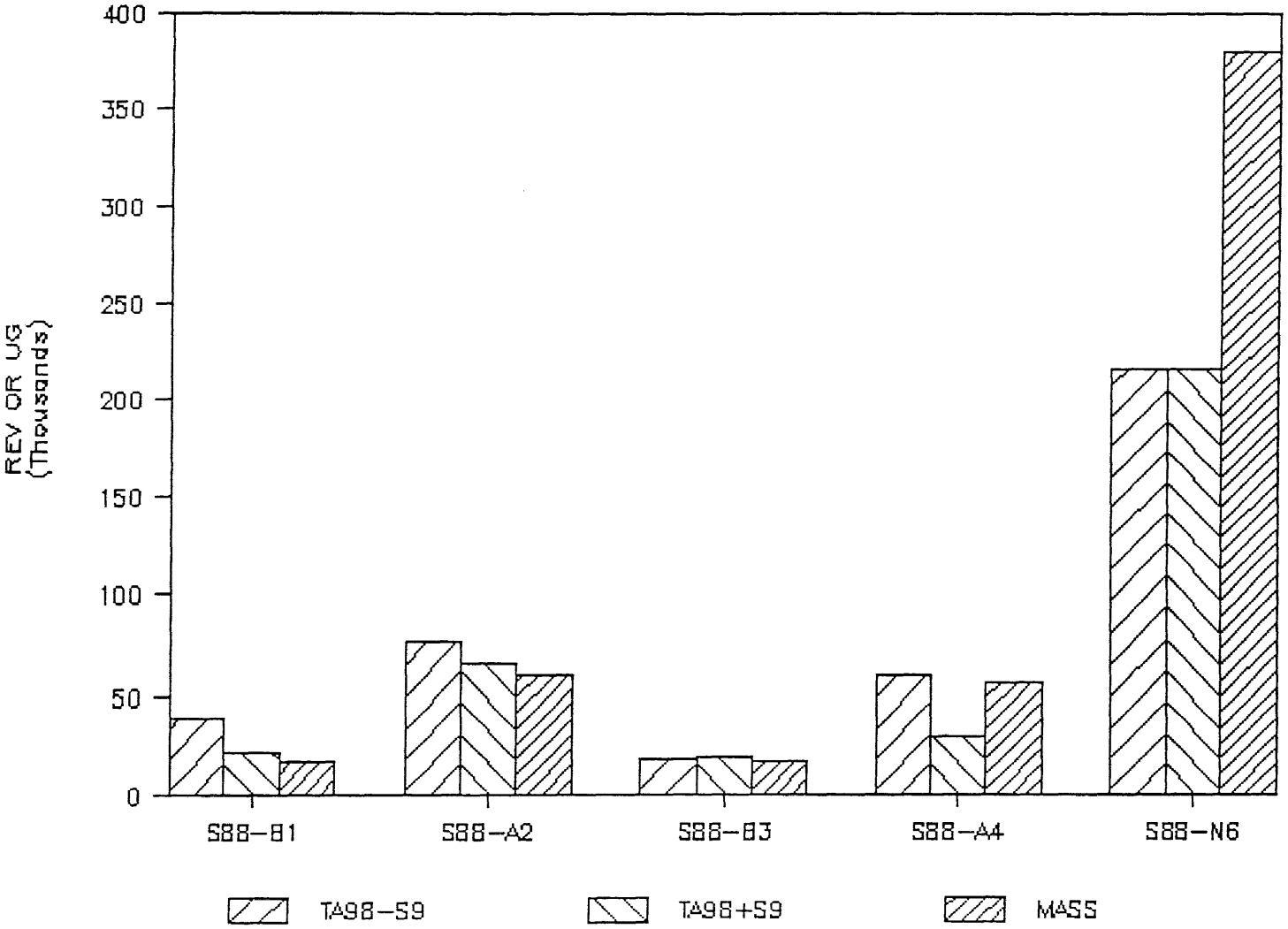


Figure 2-5 Distribution of Corrected Mass and Mutagenicity for Summer Subfractions

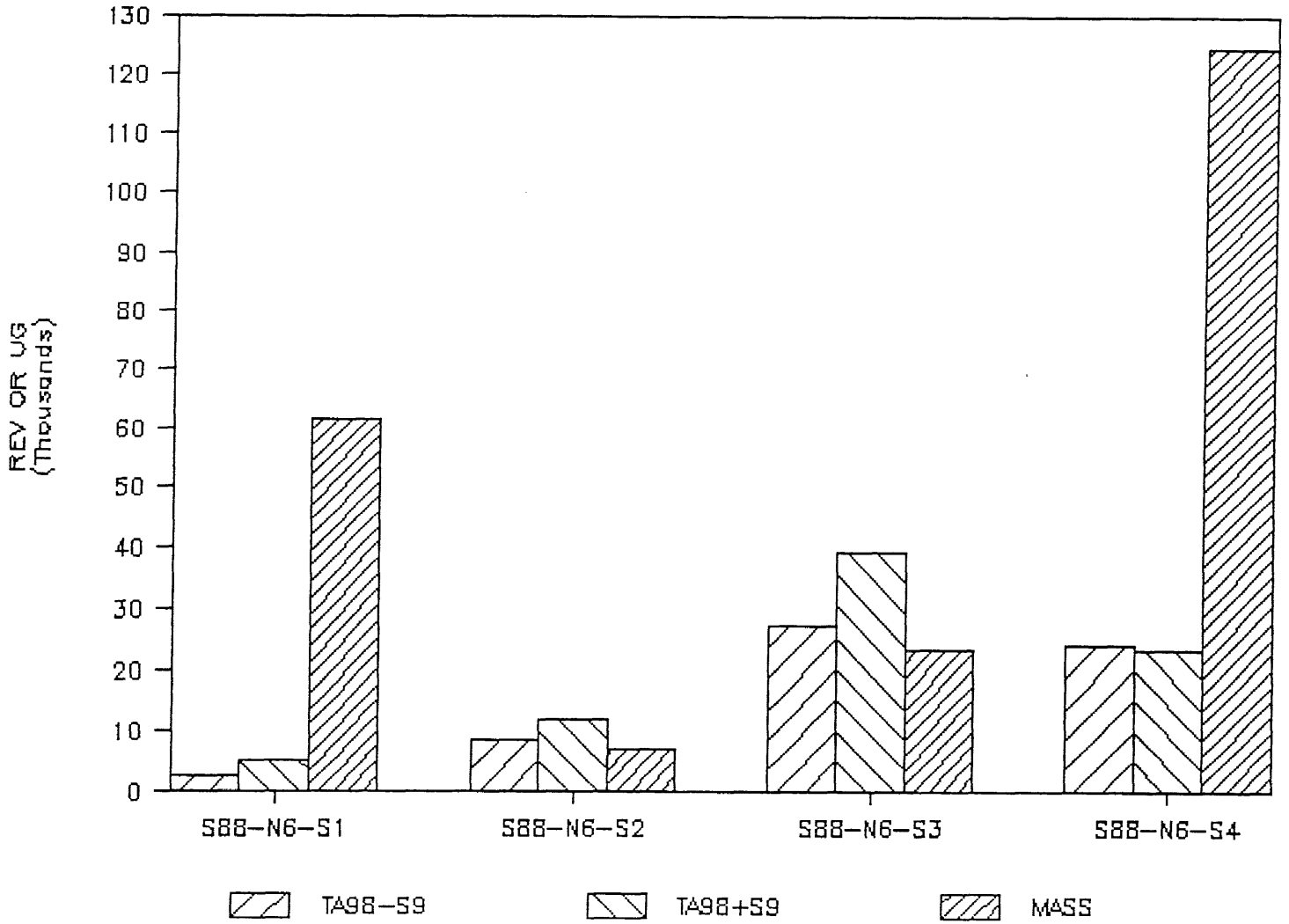


Figure 2-6 Percentage of Mass and Mutagenicity for Winter Fractions

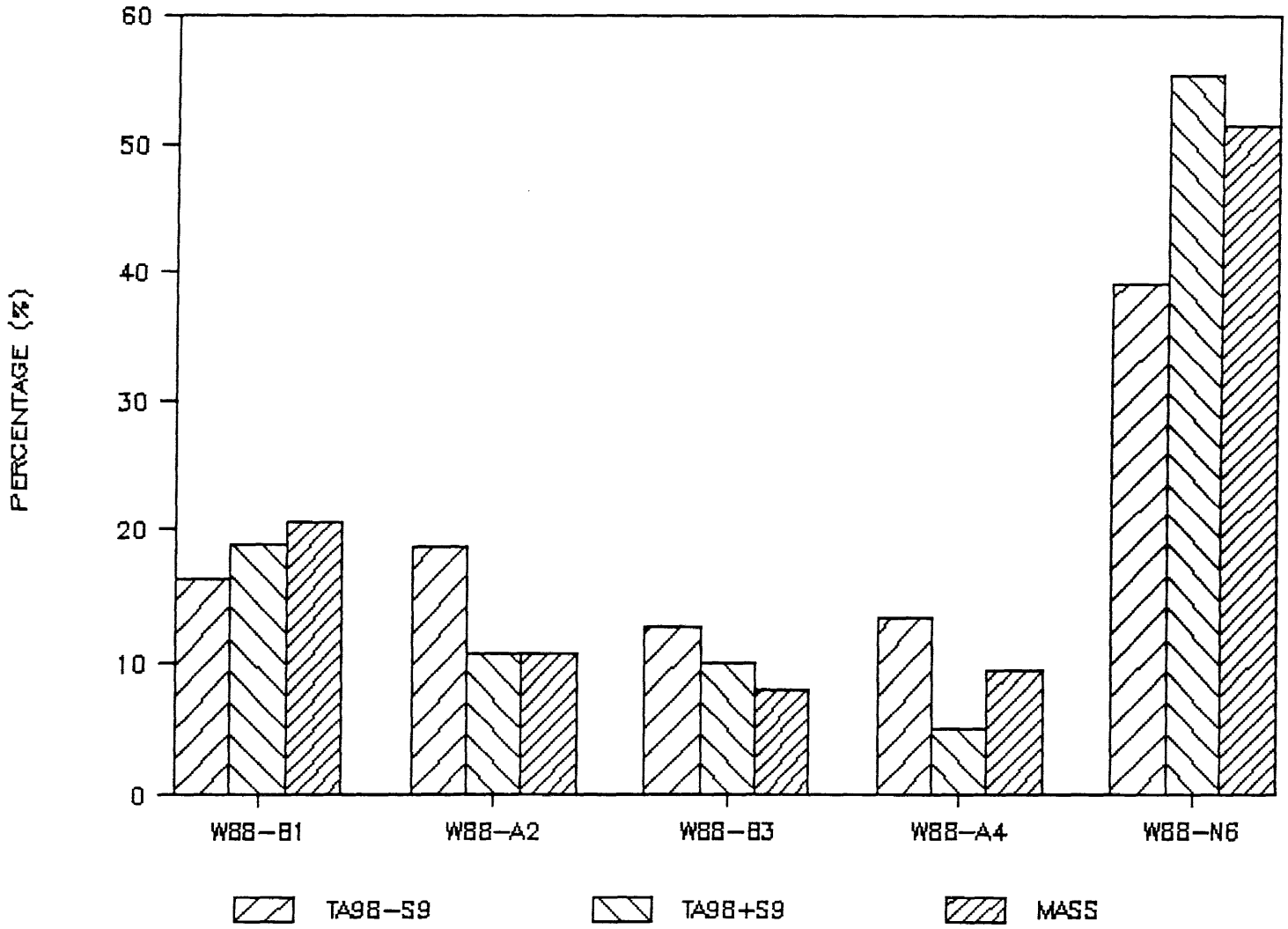


Figure 2-7 Percentage of Mass and Mutagenicity for Winter Subfractions

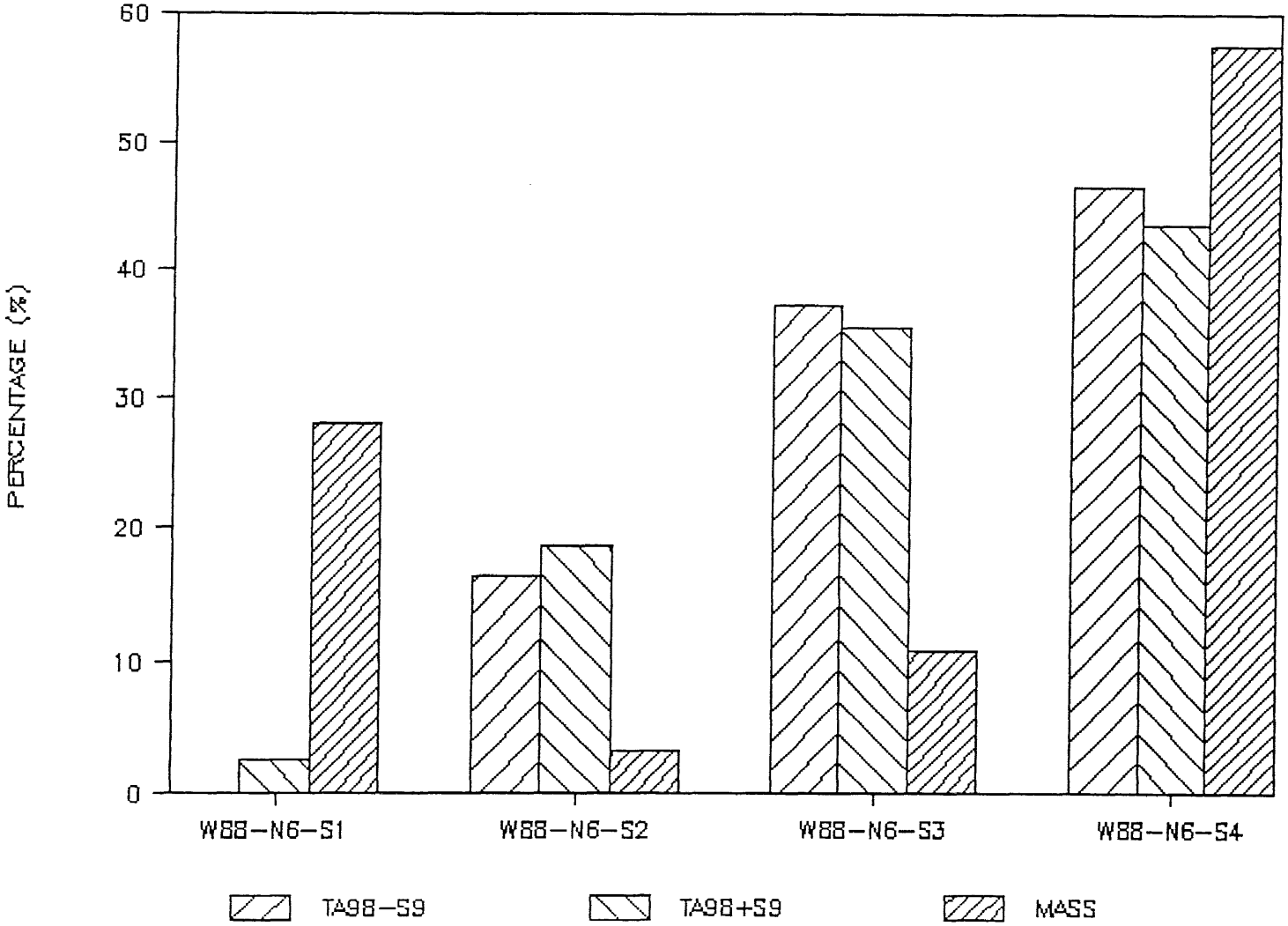


Figure 2-8 Percentage of Mass and Mutagenicity for Summer Fractions

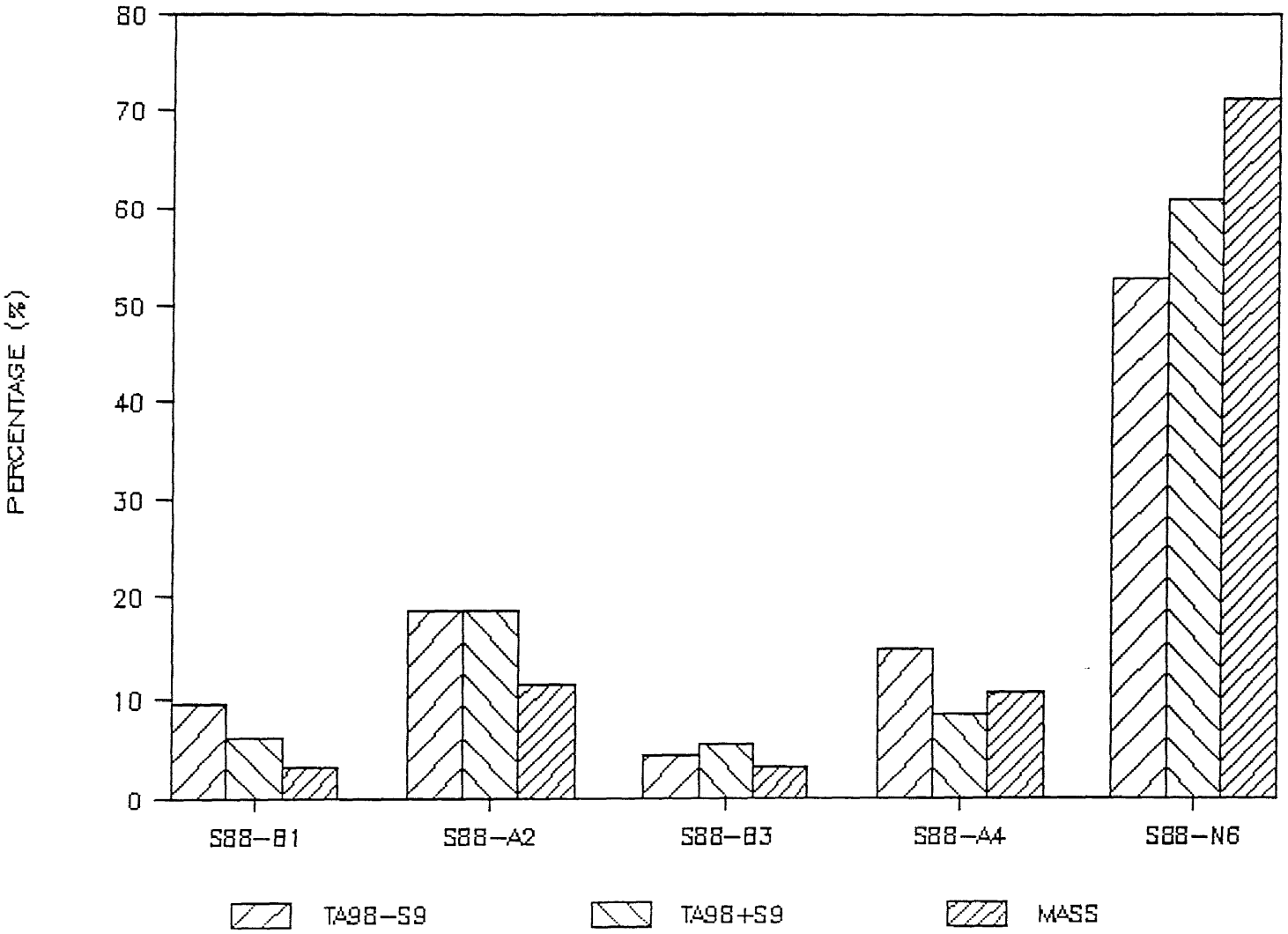
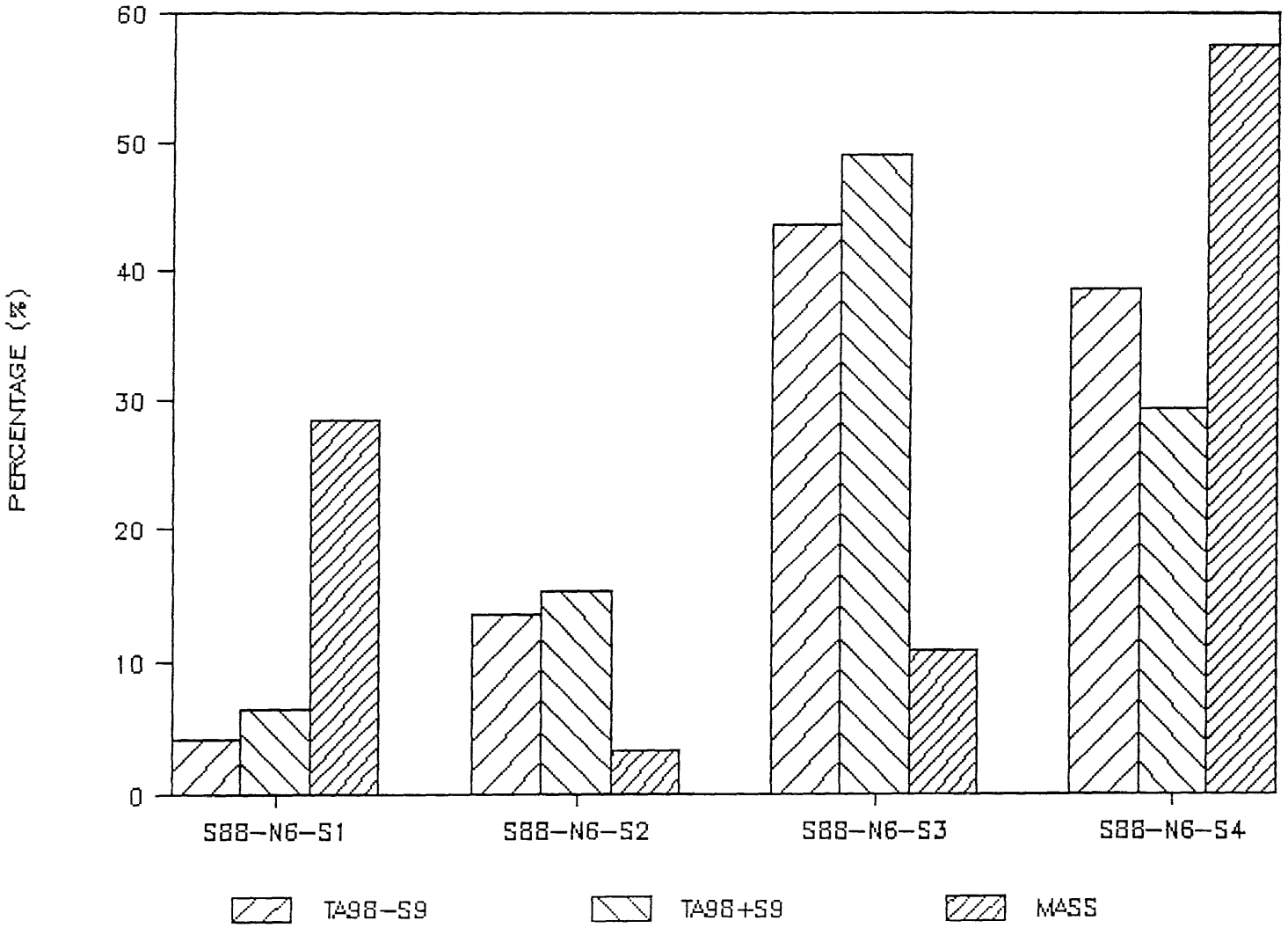


Figure 2-9 Percentage of Mass and Mutagenicity for Summer Subfractions



CHAPTER THREE

PAH Analysis and HPLC Subsubfractionation

3.1 Polycyclic Aromatic Hydrocarbon Analysis

3.1-1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are produced from incomplete combustion of fossil fuels. (1-6) Thus with the concommitant rise in industrial activity and population growth, these pollutants have become ubiquitous components of our environment. One of the major goals of the present study is to characterize the air environment for specific genotoxic pollutants, including PAH.

3.1-2 Experimental

The samples analyzed here are the appropriate eluent fractions using hexane/benzene (1:1 v/v) solvent through silica gel bed (DCM-N6-S2). A Waters Associates gradient High Performance Liquid Chromatography system was used consisting of two Model 501 pumps, a Model U6K injector, a Digital Professional 350 computer with a Waters system interface module, a Model 481 absorbance detector operated at 280 nm wavelength, and a Kratos Analytical spectroflow 980 programmable fluorescence detector operating at 290 nm (excitation) and 370 nm (emission). The column used was a Vydac 201 TP54 polymeric C_{18} (5 um, 4.6 mm

x 25 cm) column. The samples were analyzed by reversed-phase HPLC using an acetonitrile-water mobile phase and the solvent flow rate was 1 ml/min. The following gradient conditions were used for the separation: step (1) 3 minutes equilibration at 50% water: 50% acetonitrile, step (2) injection, step (3) 3 minutes hold at 50% water: 50% acetonitrile, step (4) 15 minutes linear gradient to 100% acetonitrile, step (5) 10 minutes hold at 100% acetonitrile, and step (6) 7 minutes linear gradient to 50% water: 50% acetonitrile.

NBS (National Bureau of Standards) Standard Reference Material (SRM) 1647a containing sixteen PAH compounds was first used to make the calibration curves (Figures 3-1a-3-1k) and a representative chromatogram of this mixture is shown in Figure 3- 2. In this investigation, a 25 or 50 microliter aliquot of the subsubfraction containing the PAH was injected after solvent exchange with tetrahydrofuran (THF) to replace the hexane/benzene eluant. Figures 3-3 and 3-4 represent the ultraviolet absorbance and fluorescence chromatograms for W88-DCM-N6-S2 and S88-DCM-N6- S2 subsubfractions, respectively. A Waters Model 990 photodiode array (PDA) UV detector was also used to identify compounds in these two samples. A typical chromatogram of W88-DCM-N6-S2 fraction using the PDA Detector is shown in Figure 3-5 and more detailed discussion related to data interpretation will be provided in Chapter Four.

3.1-3 Results and Discussion

Using the earlier HPLC analytic techniques, we assigned eleven PAH compounds in these two subfractions. In addition, the concentration of each compound identified was determined by comparing areas with those of the NBS standard and the results are shown in Tables 3-1 and 3-2. The winter levels are between ten and thirty-five times higher than the summer levels. Figures 3-6 and 3-7 show the profiles for eleven selected PAH compounds in winter and summer samples. Noteworthy, in Figure 3-8, is the drastic decrease in the concentrations of benz(a)anthracene and benzo(a)pyrene as one goes from winter to summer. This is due to volatility losses of the tetracyclic compound benz(a)anthracene, in warm weather, (7,8) and benzo(a)pyrene which is relatively reactive. (9,10)

Since BaP volatility losses are negligible, this might allow it to be the index compound. In Figure 3-9 we depict the PAH/BaP ratios for eleven selected PAH compounds. Of the eleven compounds, only benz(a)anthracene shows volatility losses and this is not consistent with our previous observation. According to our earlier study, phenanthrene (C_{14}), pyrene and fluoranthene (C_{16}) would be expected to display greater volatility losses than benz(a)anthracene (C_{18}). However, one of disadvantage of using BaP as the index compound for PAH profiles is that it is relatively reactive. (11) Thus, we prefer to use benzo(b)fluoranthene (BbF) as our index PAH since it is nonvolatile and very unreactive. (11) Figure 3-10 shows the PAH/BbF ratios for eleven selected PAH. Pyrene, benz(a)anthracene and benzo(a)pyrene show decreases in the PAH/BbF ratios upon going from winter to

summer. It is difficult to make anything of the trends in the other PAH/BbF ratios. Although some ratios increased from winter to summer there are not enough data to permit conclusions.

In spite of the long history of PAH studies in the air pollution literature, there still remains a rather incomplete knowledge of emission rates for selected PAH from specific sources and the factors which alter their emission rates. (6) In recent studies, much of the BaP emission data was summarized and generated. (12) The very high emissions due to winter wood use appear to suggest a winter/summer concentration ratio for BaP of about 50, somewhat higher than the maximum value of 13 observed in the earlier study (13) but our present study also show a ratio near 35 of winter/summer concentration ratio for BaP. The deduction that wood combustion is the major winter PAH source in New Jersey is at first somewhat surprising. However, the BaP emissions arising from residential wood combustion appear to be a factor of about 400-fold greater per BTU than emissions due to gasoline combustion and at least 10,000-fold greater than emissions from combustion of home-heating oil. (13) Even in the cities, where fireplaces and wood-burning stoves are not common and one might not expect wood combustion to be a significant PAH source, one could rationalize high PAH levels arising from wood combustion due to the presence of fairly densely populated inner suburbs surrounding the cities. (14)

Although the very high emissions due to winter wood use appear to suggest even higher winter/summer BaP factors (ca 50), as noted earlier, it is still

worthwhile to attempt to understand the possible role of chemical reactivity in establishing low levels during summer when photochemistry and presumably thermal chemistry should be more significant than in winter. In discussing the role of chemical reactivity of PAHs, it is important to differentiate, in principle at least, between losses occurring during sampling and atmospheric residence. Sampling losses due to volatilization are significant for tetracyclic species but negligible for pentacyclic and larger PAH. (15,16) Researchers have suggested that significant chemical losses occur as an artifact of sampling presumably due to reactions catalysed on the collection surface, particularly when glass-fiber filters are employed. (17-20) Reaction of PAH during sampling could also produce derivatives which are themselves powerful mutagens. For example, it has been estimated that 1-40% of the 1-nitropyrene collected from diesel exhaust on glass-fiber filters is artifactual. (21) Thus, as much as 30% of the TA-98 direct- acting mutagenicity of diesel particulate extract has been attributed to this compound. (22) However, a recent study employing levels of O_3, NO and SO_2 typical of urban environments suggested negligible losses of even such reactive PAH as BaP and perylene when adsorbed to a variety of substrates including airborne particulate matter. (23)

The issue of chemical reactivity in the atmosphere remains a significant question (5) since it relates to (a) atmospheric lifetime and thus atmospheric transport, (b) PAH profiles and the possibility of employing them in source assessment, (c) the nature of derivatives which may be direct mutagens or non- mutagens and (d)

a portion, perhaps small, of the winter/summer PAH concentration ratio. Thus the question remains whether physical disappearance of particles or chemical disappearance of the particle-bound PAH determines the atmospheric lifetime of these species.

3.2 Subsubfractionation of Subfraction S3 by HPLC

3.2-1 Introduction

Organic fractions extracted from airborne particles have been shown to exhibit mutagenic activity by in-vitro bioassays as reported by several investigations. (24-28) The search for major mutagens associated with airborne particulate matter has evolved from investigations of polycyclic aromatic hydrocarbons (PAH) through studies of nitro-PAH, PAH-quinones and other oxygenated derivatives. It has been shown that the PAH class itself only accounts for a minor part of the mutagenicity in ambient airborne particulate matter. (29) Therefore, interest in nitro-PAH, PAH-quinones and the oxygenated derivatives of PAH has grown in recent years.

Nitro-PAH have been a source of increasing health-related concern due to their direct-acting mutagenic response in the salmonella test, (30-32) positive mutagenic responses in mammalian cells, (33) and carcinogenic activity in animal experiments. (34) In addition to nitro-PAHs, many oxygenated PAH may be associated

with particulate matter in ambient air. For instance, model experiments on chemical and, particularly, photochemical PAH stability suggest that several of the PAH that are emitted in sizable amounts from various natural and anthropogenic sources are degraded in the atmosphere by sunlight or by interactions with other reactive airborne species. (35-44)

The discovery of potent bacterial mutagenicity of some nitro-PAH, coupled with the recognition of the almost ubiquitous distribution of these chemicals in the environment, has generated a great deal of interest in their properties. (45,46) The mutagenicity of nitro-PAH was found to be optimal in *Salmonella typhimurium* TA98, a plasmid-containing strain which detects frameshift mutations. Therefore, in this present study, we also tried to develop better analytical method for the separation and identification of nitro-PAH in ambient air samples.

According to Nishioka's studies (47) and our mutagenicity results, obviously, most of the mutagenic activity (TA98$\pm$S9) was found in the most polar DCM-N6-S3 and S4, especially in DCM-N6-S3. At the same time, the contributions of nitro-PAH in these subfractions appear to be much greater. However, detection of the specific compounds responsible for this mutagenic and carcinogenic activities is limited by the complexity of air samples. In other words, separation into substantially less complex fractions to facilitate detection of the mutagenic compounds should be very important for us. Thus, the further fractionation of these subfractions has been adopted in this study.

3.2-2 Experimental

This fractionation procedure was done by High Performance Liquid Chromatography (HPLC) system as that described previously, except that a Du Pont zorbax silica semi-prep column (9.4 mm id x 25 cm) and a UV absorbance detector operating at 280 nm as well as a fluorescence detector operating at 290 nm (excitation) and 370 nm (emission) were used here. The mobile phase used are 2% methanol/98% methylene chloride (solvent A) as well as hexane (solvent B) and the solvent flow rate is set up at 4.0 ml/min. The gradient conditions are: (1) 10 minutes hold at 8% solvent A: 92% solvent B, (2) 30 minutes linear gradient to 35% solvent A: 65% solvent B, (3) 35 minutes hold at 35% solvent A: 65% solvent B, (4) 5 minutes linear gradient to 8% solvent A: 92% solvent B.

A mixed standard containing NBS 1647a (PAHs), NBS 1587 (nitro- PAHs), PAH-quinones and hydroxypyrenes as well as nitrohydroxypyrenes (1-nitro-3;6;8-hydroxypyrene isomers) was first injected to make sure what conditions were best for separation in order to decrease the complexity of the samples. A typical chromatogram is shown in Figure 3-11. 1 ml aliquots of environmental subfraction sample were injected each time, and the four subsubfractions were separated and collected from the outlet of the HPLC system. Representative chromatograms of the winter (DCM-N6-S3) and summer (DCM-N6-S3) subfraction samples are shown in Figures 3-12 and 3-13, respectively. After each subsubfraction was concentrated

with nitrogen and the same subsubfractions were combined, one-third of the sample was tested for mutagenicity, one-third of the sample was run on FTIR and another one-third of the sample was identified by GC/MS.

3.2-3 Results and Discussion

The mass balance results are shown in Table 3-3. Total recoveries for winter and summer samples are around 82% and 92% respectively. It is better than those of acid-base partitioning and open column chromatography fractionations. Tables 3-4 and 3-5 represent the mutagenic activities of winter and summer DCM-N6-S3 subsubfractions. We also depict the trends of corrected mutagenicities in winter and summer subsubfractions in Figures 3- 14 and 3-15.

In fact, there are some difference between these two trends. Of winter subsubfractions, obviously, the most potent direct-acting mutagenic fractions should be L1, L2 and L3, and Indirect-acting mutagens were present in the first subsubfraction (L1) only. However, for summer samples, we would reasonably expect that subsubfraction L3 contained about 70% masses should respond the most potent mutagenic activity. Actually, of all W88 and S88 subsubfractions assayed, the highest specific activity was observed in S88-DCM-N6-S3-L3 subsubfraction, and the specific activity of this subsubfraction was higher than the activity of the equivalent subsubfraction from the Winter campaign. Meanwhile, we cannot get optimal quality

data for the other three subsubfractions (L1, L2 and L4) due to the small amount of material available for Ames assays. In addition, of the direct- acting mutagens, there was clear-cut evidence that some or most were nitro group-containing mutagens in this subsubfraction. Therefore, the further identification of these major mutagenic subsubfractions is necessary. The results of FTIR and GC/MS will be described in the following two chapters.

3.3 Subsubfractionation of Subfraction S4 by HPLC

3.3-1 Introduction

Beside the subfractions, DCM-N6-S3, corresponding to most of mutagenic activity, the subfractions, DCM-N6-S4, also exhibited important roles in the contributions of nitro-PAH. Thus, a fractionation by HPLC was also employed here.

3.3-2 Experimental

The same Waters HPLC chromatography system and silica semi-prep column were used but we employed 5% methanol/95% methylene chloride (solvent A) and hexane (solvent B) as the mobile phase. The running conditions, also changed, are: (1) 15 minutes hold at 100% solvent B, (2) 15 minutes linear gradient to 35% solvent A: 65% solvent B, (3) 40 minutes hold at 35% solvent A: 65% solvent B, then (4)

10 minutes linear gradient to 100% solvent B. The UV absorbance detector is set at 254 nm and the wavelength of fluorescene detector are 290 nm (excitation) and 370 nm (emission).

3.3-3 Results and Discussion

We also cut the subfractions to four subsubfractions and the chromatograms of a mixed standard and these two subfractions are shown in Figure 3-16 to Figure 3-18. After collection in the same way as above, a portion of these samples was also bioassayed in TA98 strains. Table 3-6 shows about mass balance results and the Ames assay data are depicted in Table 3-7 and Table 3-8. The mass recoveries of both samples are approximately 60% and similar mass distributions are displayed in these two subfractions. From Figure 3-19 and Figure 3-20, it is obvious that the subsubfractions with the highest specific activity were L2 for winter and summer samples, followed by L1 and L3, then L4. At the same time, the summer L2 subsubfraction had a lower specific activity than did the equivalent fraction from the Winter campaign. In fact, these L2 mutagens appeared to be nitro mutagens, so the further identification is positively necessary to be achieved. Moreover, although the winter L4 had the lowest specific activity of direct-acting mutagens of the four winter subsubfractions, it was the only fraction of the four which appeared to contain indirect-acting mutagens. This is also an interesting point.

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Table 3-1

Concentrations of Compounds Identified from W88- DCM Extract

Compounds Identified	[a] *Conc. by HPLC (ug/ml)*	[b] *Conc. in Air (ng/m^3)*
Phenanthrene	-	-
Fluoranthene	*1.33*	*0.29*
Pyrene	*1.45*	*0.32*
Benzo(a)anthracene	*1.92*	*0.42*
Chrysene	*2.88*	*0.63*
Benzo(b)fluoranthene	*4.43*	*0.96*
Benzo(k)fluoranthene	*2.01*	*0.44*
Benzo(a)pyrene	*4.29*	*0.93*
Dibenz(a,h)anthracene	*0.53*	*0.11*
Benzo(ghi)perylene	*8.76*	*1.91*
Indeno(1,2,3-cd)pyrene	*6.87*	*1.50*

[a]The concentrations determined by HPLC are based upon W88-DCM-N6-S2.

[b]The concentration in the air is calculated by:

1. The concentration determined by HPLC times 10 ml, total volume of W88-DCM-N6-S2 fraction
2. The masses got from step 1. are divided by 0.885 (8.85 ml/10 ml) to place an original 10 ml scale in neutral composite and then divided by 0.785 (7.85 ml/10 ml) to reflect an original 10 ml scale in whole DCM extract.
3. Those masses in DCM extract are finally divided by 66,115 m^3 of total air volume collected for 40 days.

Table 3-2

Concentrations of Compounds Identified from S88- DCM Extract

Compounds Identified	[a] *Conc. by HPLC (ug/ml)*	[b] *Conc. in Air (ng/m³)*
Phenanthrene	*0.10*	*0.03*
Fluoranthene	*0.12*	*0.03*
Pyrene	*0.06*	*0.01*
Benzo(a)anthracene	*0.04*	*0.01*
Chrysene	*0.17*	*0.04*
Benzo(b)fluoranthene	*0.18*	*0.05*
Benzo(k)fluoranthene	*0.09*	*0.02*
Benzo(a)pyrene	*0.10*	*0.03*
Dibenz(a,h)anthracene	*0.03*	*0.01*
Benzo(ghi)perylene	*0.37*	*0.10*
Indeno(1,2,3-cd)pyrene	*0.29*	*0.08*

[a]The concentrations determined by HPLC are based upon S88-DCM-N6-S2.

[b]The concentration in the air is calculated by:

1. The concentration determined by HPLC times 10 ml, total volume of S88-DCM-N6-S2 fraction
2. The masses got from step 1. are divided by 0.4167 (6.25 ml/15 ml) to place an original 15 ml scale in neutral composite and then divided by 0.954 (23.85 ml/25 ml) to reflect an original 25 ml scale in whole DCM extract.
3. Those masses in DCM extract are finally divided by 96,664 m^3 of total air volume collected for 60 days.

Table 3-3 Mass Results of Winter and Summer S3 Subsubfractions

	Total Masses (mg)*		Percentage (%)	
Fraction Name	*W88-DCM*	*S88-DCM*	*W88-DCM*	*S88-DCM*
DCM-N6-S3-L1	*4.95*	*0.81*	*22.34*	*11.10*
DCM-N6-S3-L2	*10.30*	*0.72*	*46.49*	*9.86*
DCM-N6-S3-L3	*4.80*	*5.07*	*21.66*	*69.45*
DCM-N6-S3-L4	*2.11*	*0.70*	*9.52*	*9.59*
Sum of Masses	*22.16*	*7.30*	*100.01*	*100.00*
Original Mass	*26.94*	*7.89*	-	-
Recovery	*82.26%*	*92.52%*	-	-

*Total masses of each subsubfraction were placed on original volume scale for winter and summer samples.

Table 3-4

Ames Assay Results for W88-DCM-N6-S3 and Subsubfractions

		TA98-S9		TA98+S9	
Fraction Name	*Mass(ug)*	*rev/ug*	*Total(rev)*	*rev/ug*	*Total(rev)*
W88-N6-S3-L1	*6,020*	*0.49*	*2,950*	*0.91*	*5,478*
W88-N6-S3-L2	*12,521*	*0.59*	*7,387*	*0.60*	*7,513*
W88-N6-S3-L3	*5,831*	*0.68*	*3,965*	*0.46*	*2,682*
W88-N6-S3-L4	*2,567*	*0.28*	*719*	*NEG*	*NEG*
Total(1-4)	-	-	*15,021*	-	*15,673*
W88-N6-S3	*26,939*	*0.45*	*12,123*	*1.07*	*28,825*
Recovery	-	-	*123.90%*	-	*54.37%*

The mass of each subsubfraction is based upon the original 10 ml volume of W88-DCM-N6-S3 subfraction and EOM recovery is assumped at 100%.

Table 3-5

Ames Assay Results for S88-DCM-N6-S3 and Subsubfractions

		TA98-S9		TA98+S9	
Fraction Name	*Mass(ug)*	*rev/ug*	*Total(rev)*	*rev/ug*	*Total(rev)*
S88-N6-S3-L1	*875*	-	-	-	-
S88-N6-S3-L2	*778*	-	-	-	-
S88-N6-S3-L3	*5,480*	*2.60*	*14,248*	*1.50*	*8,220*
S88-N6-S3-L4	*757*	-	-	-	-
Total(1-4)	-	-	*14,248*	-	*8,220*
S88-DCM-N6-S3	*7,890*	*1.68*	*13,255*	*2.40*	*18,936*
Recovery	-	-	*107.54%*	-	*43.41%*

The mass of each subsubfraction is based upon the original 10 ml volume of S88-DCM-N6-S3 subfraction and EOM recovery is assumped at 100%. Additionally, L1, L2 and L4 did not run bioassays since too low mass was not available for testing.

Table 3-6 Mass Results of Winter and Summer S4 Subsubfractions

	Total Masses (mg)*		Percentage (%)	
Fraction Name	*W88-DCM*	*S88-DCM*	*W88-DCM*	*S88-DCM*
DCM-N6-S4-L1	*6.56*	*2.20*	*20.74*	*9.03*
DCM-N6-S4-L2	*19.74*	*16.27*	*62.41*	*66.79*
DCM-N6-S4-L3	*2.31*	*0.71*	*7.30*	*2.91*
DCM-N6-S4-L4	*3.02*	*5.18*	*9.55*	*21.26*
Sum of Masses	*31.63*	*24.36*	*100*	*99.99*
Original Mass	*50.54*	*42.01*	-	-
Recovery	*62.58%*	*57.99%*	-	-

*Total masses of each subsubfraction were placed on original volume scale for winter and summer samples.

Table 3-7

Ames Assay Results for W88-DCM-N6-S4 and Subsubfractions

		TA98-S9		TA98+S9	
Fraction Name	*Mass(ug)*	*rev/ug*	*Total(rev)*	*rev/ug*	*Total(rev)*
W88-N6-S4-L1	*10,483*	*0.39*	*4,088*	*0.55*	*5,766*
W88-N6-S4-L2	*31,544*	*0.93*	*29,336*	*0.67*	*21,134*
W88-N6-S4-L3	*3,691*	*0.37*	*1,366*	*0.38*	*1,403*
W88-N6-S4-L4	*4,826*	*0.26*	*1,255*	*0.63*	*3,040*
Total(1-4)	-	-	*36,045*	-	*31,343*
W88-DCM-N6-S4	*50,540*	*0.30*	*15,162*	*0.70*	*35,378*
Recovery	-	-	*237.73%*	-	*88.59%*

The mass of each subsubfraction is based upon the original 10 ml volume of W88-DCM-N6-S4 subfraction and EOM recovery is assumped at 100%.

Table 3-8

Ames Assay Results for S88-DCM-N6-S4 and Subsubfractions

		TA98-S9		TA98+S9	
Fraction Name	*Mass(ug)*	*rev/ug*	*Total(rev)*	*rev/ug*	*Total(rev)*
S88-N6-S4-L1	*3,794*	*0.32*	*1,214*	-	-
S88-N6-S4-L2	*28,057*	*0.43*	*12,065*	-	-
S88-N6-S4-L3	*1,224*	-	-	-	-
S88-N6-S4-L4	*8,933*	*0.12*	*1,072*	-	-
Total(1-4)	-	-	*14,351*	-	-
S88-DCM-N6-S4	*42,010*	*0.28*	*11,763*	-	-
Recovery	-	-	*122.00%*	-	-

The mass of each subsubfraction is based upon the original 10 ml volume of S88-DCM-N6-S4 subfraction and EOM recovery is assumped at 100%. Additionally, L3 did not run bioassays since too low mass was not available for testing.

Figure 3-1a Fluoranthene Calibration Using NBS SRM 1647a, A Mixture of 16 PAH

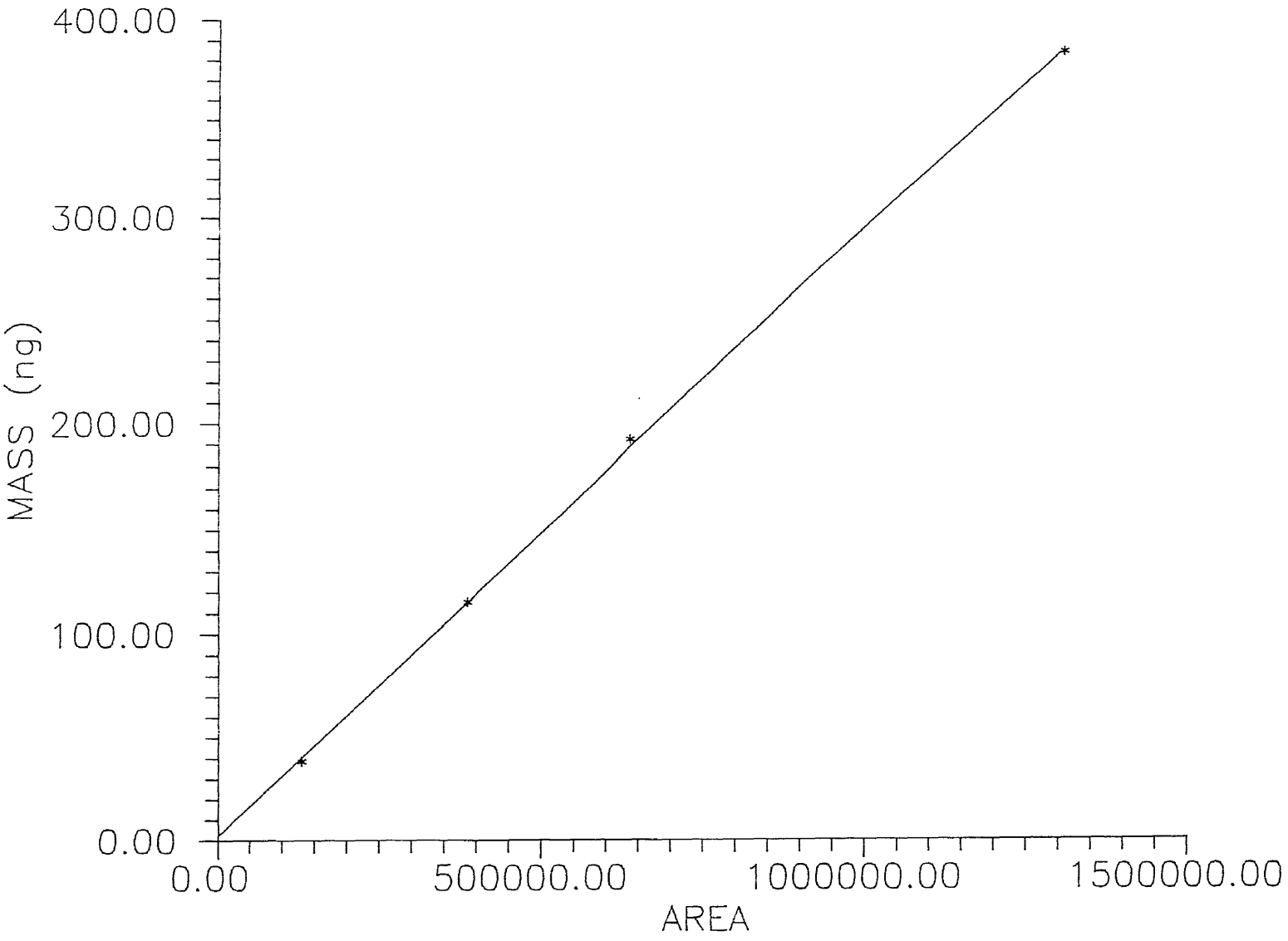


Figure 3-1b Pyrene Calibration Using NBS SRM 1647a, A Mixture of 16 PAH

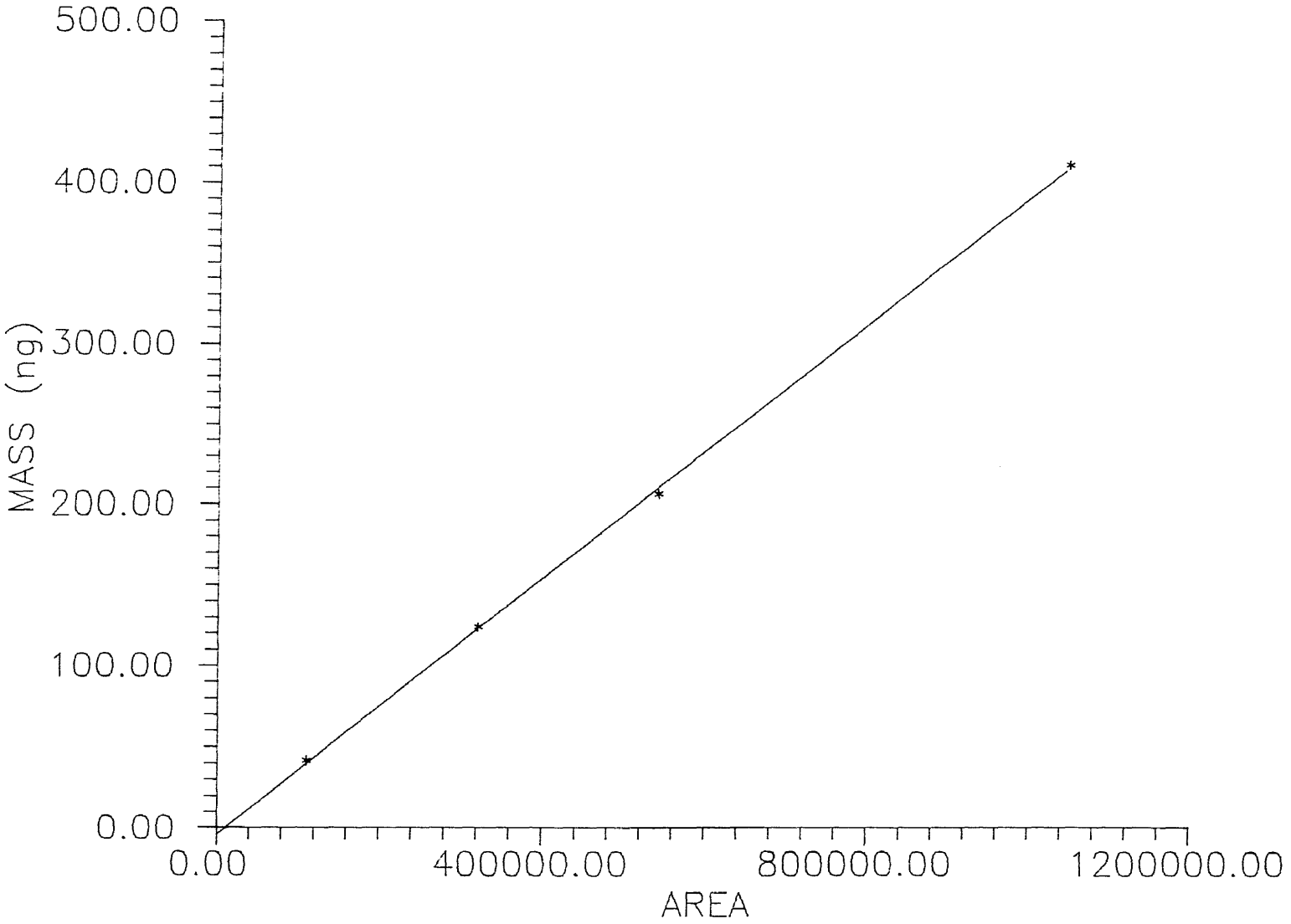


Figure 3-1c Benz(a)anthracene Calibration Using NBS SRM 1647a, A Mixture of 16 PAH

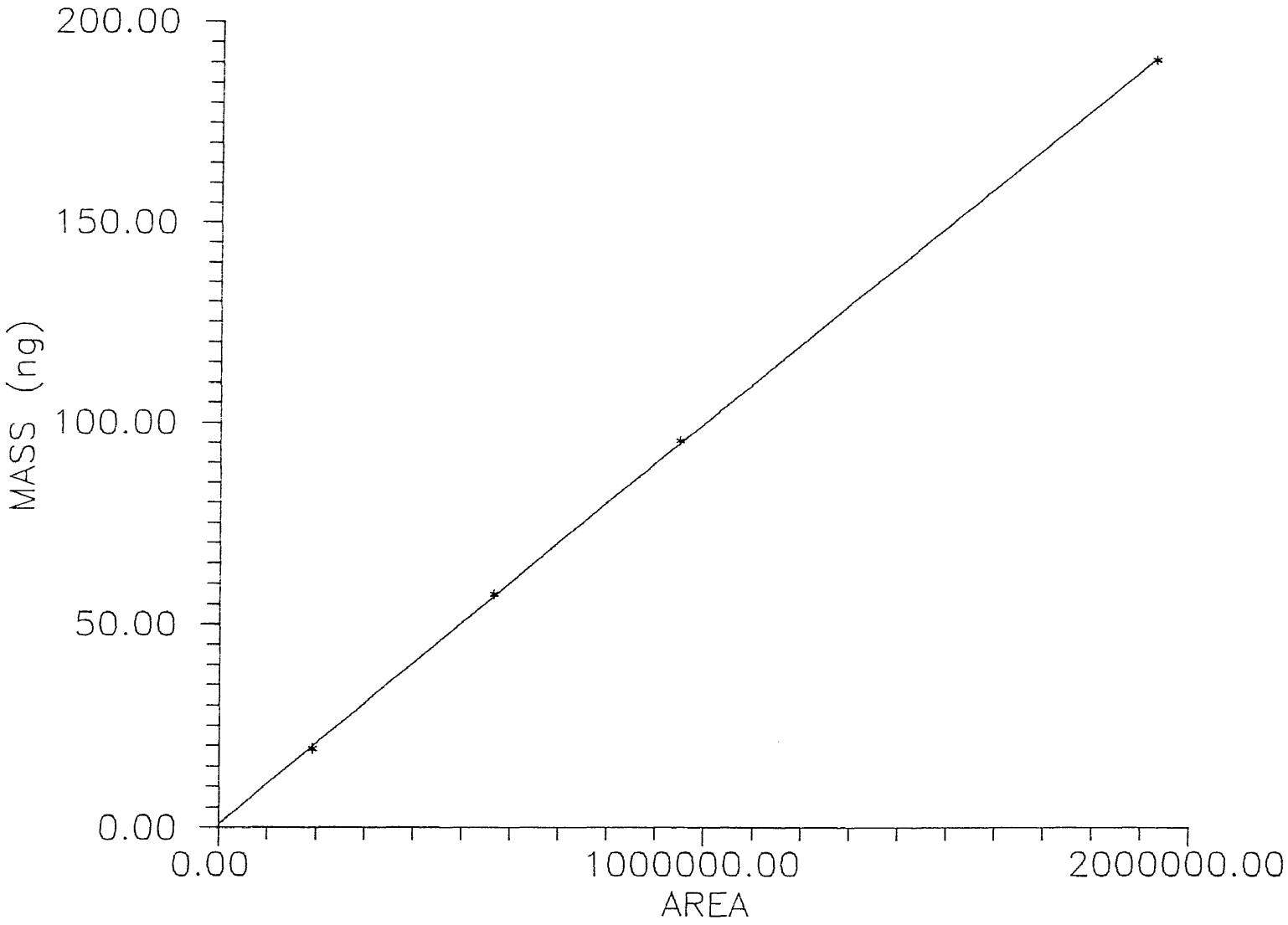


Figure 3-1d Chrysene Calibration Using NBS SRM 1647a, A Mixture of 16 PAH

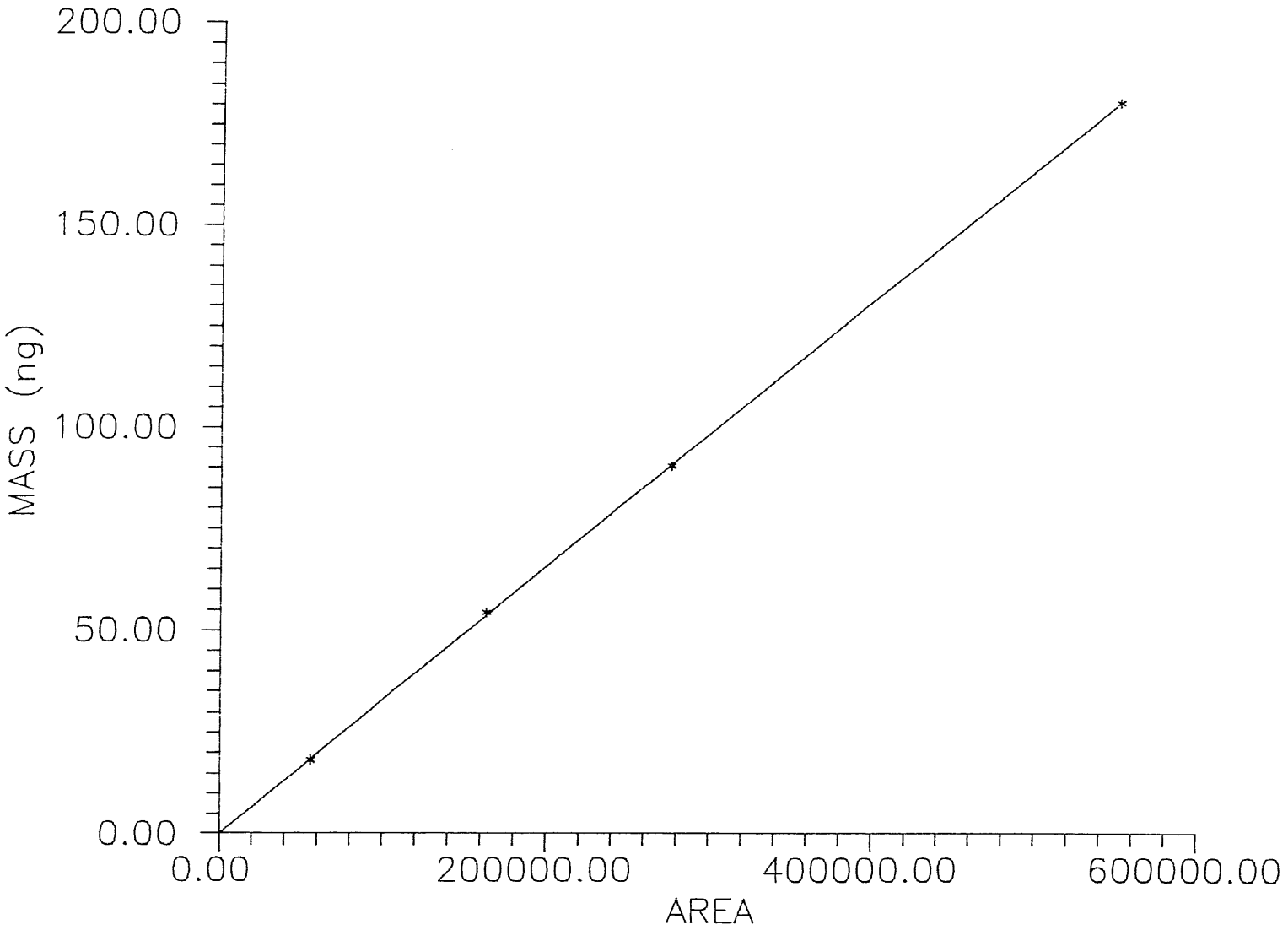


Figure 3-1e Benzo(b)fluoranthene Calibration Using NBS SRM 1647a, A Mixture of 16 PAH

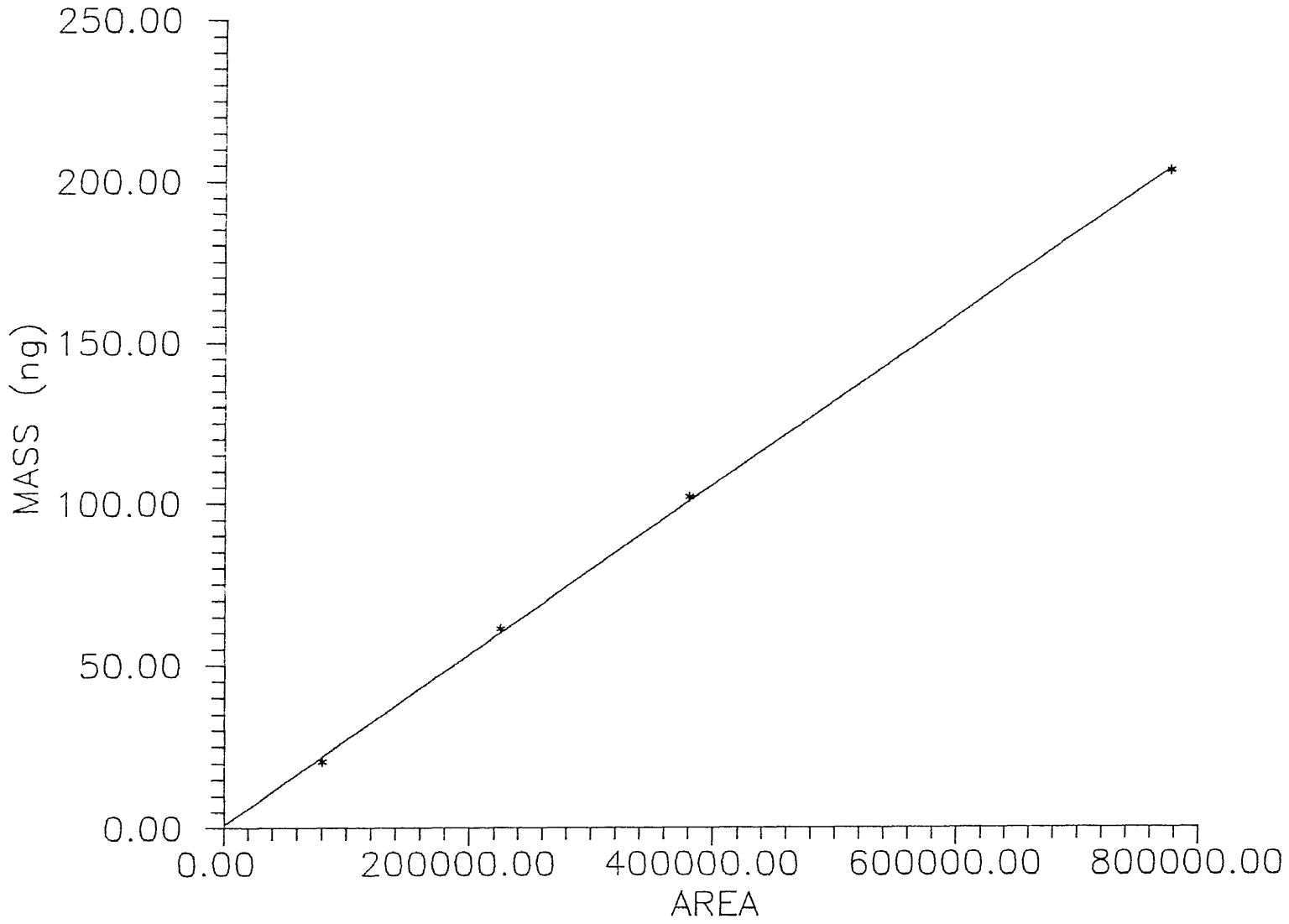


Figure 3-1f Benzo(k)fluoranthene Calibration Using NBS SRM 1647a, A Mixture of 16 PAH

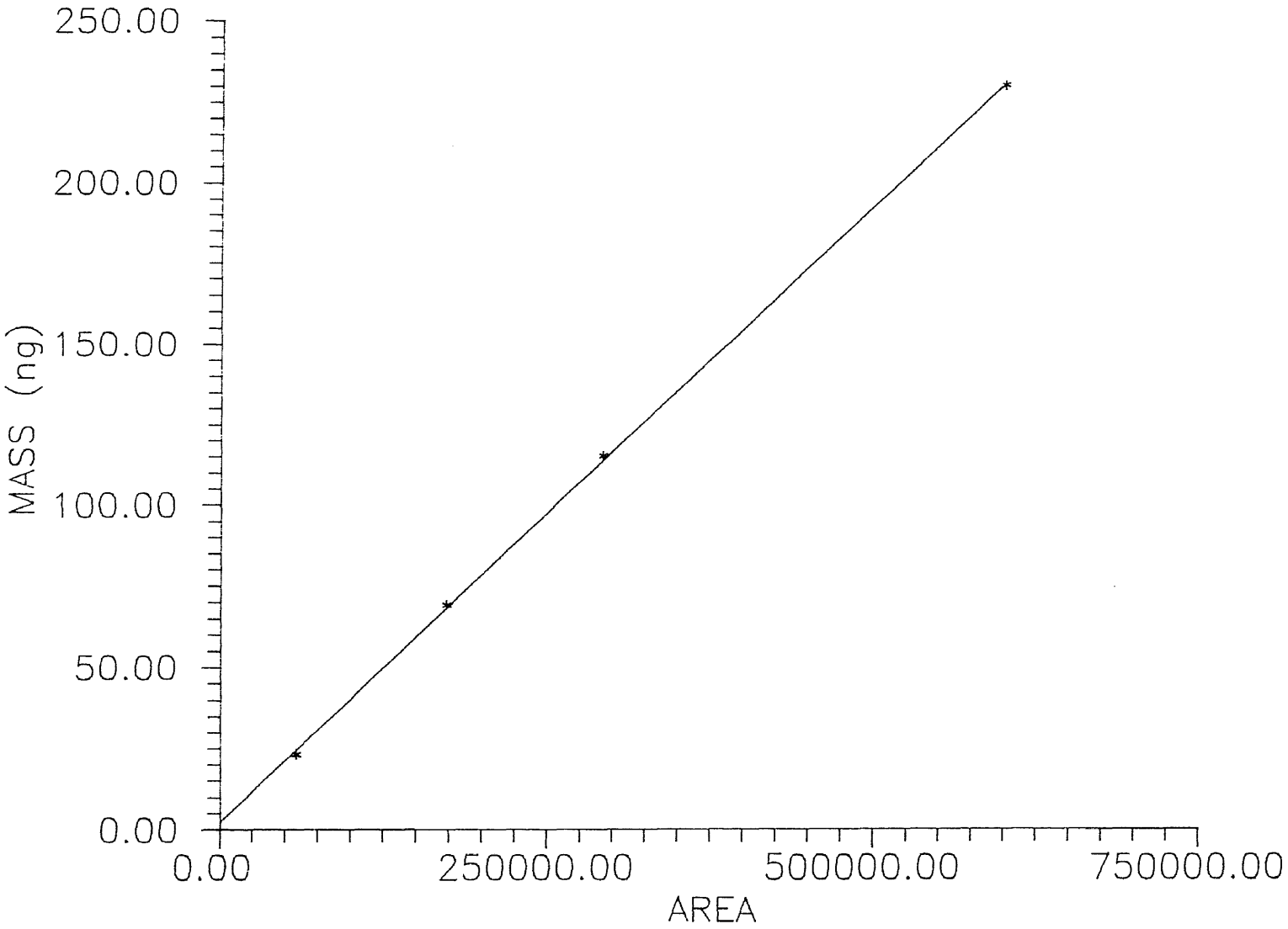


Figure 3-1g Benzo(a)pyrene Calibration Using NBS SRM 1647a, A Mixture of 16 PAH

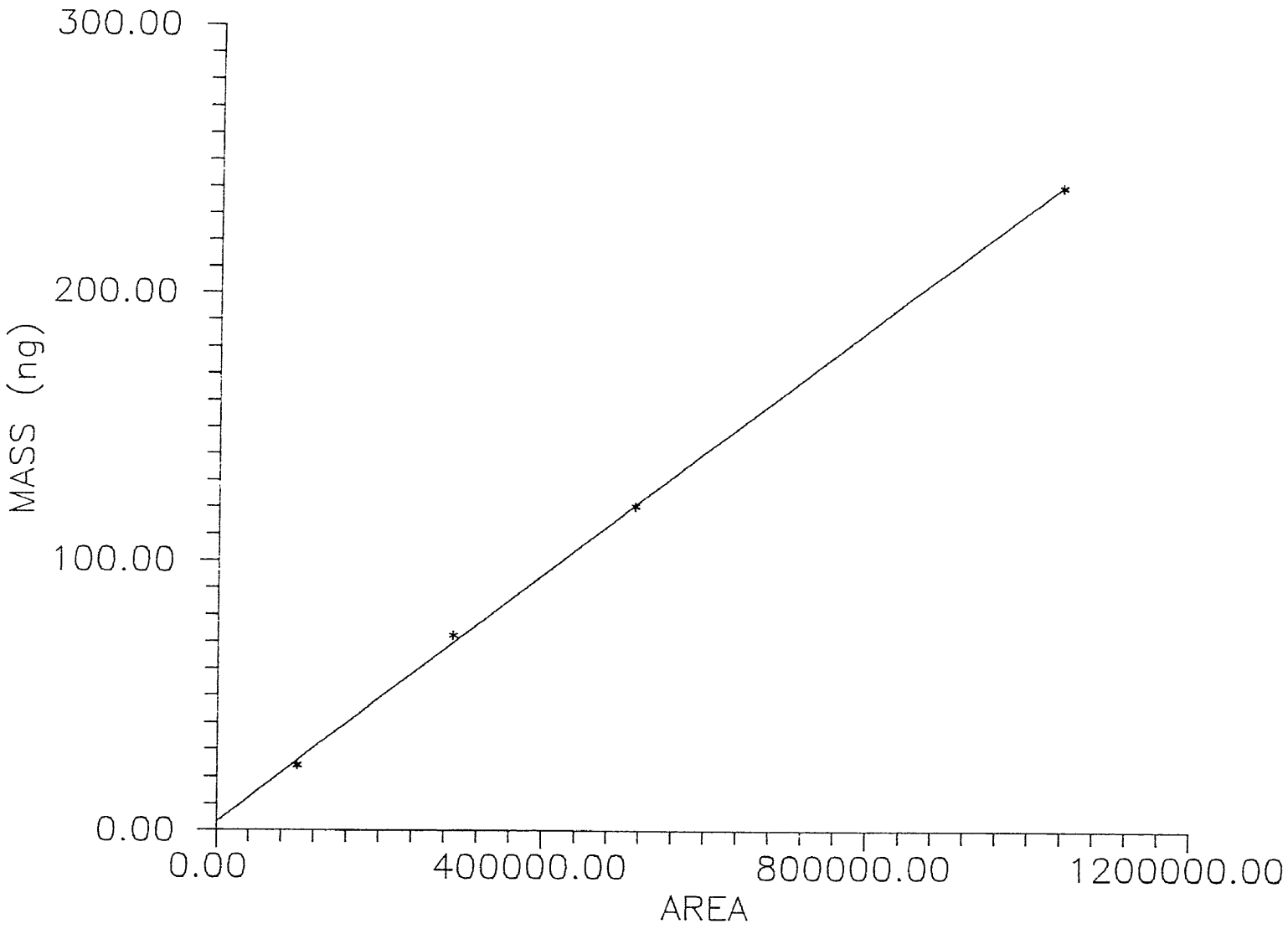


Figure 3-1h Dibenz(a,h)anthracene Calibration Using NBS SRM 1647a, A Mixture of 16 PAH

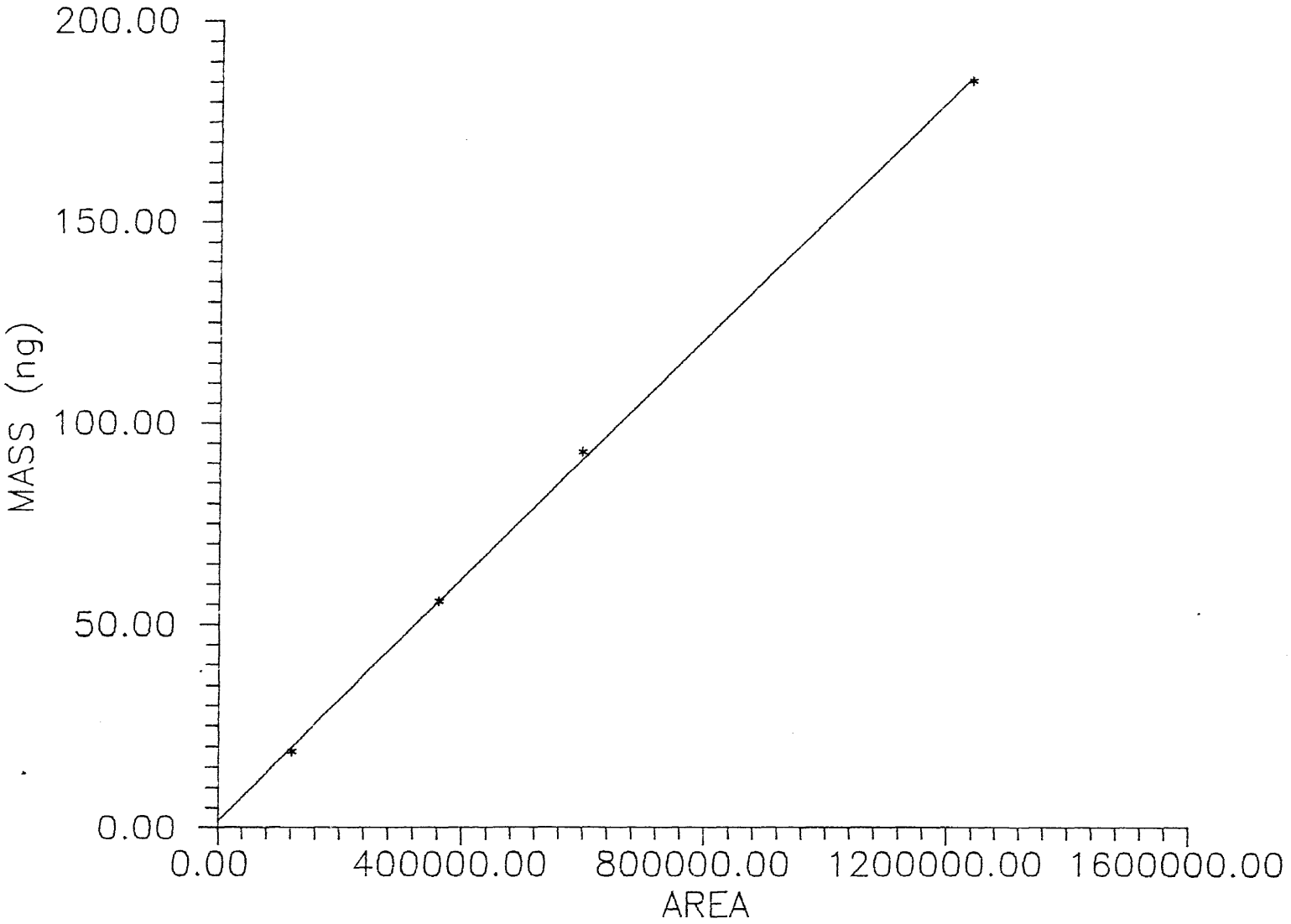


Figure 3-1i Benzo(ghi)perylene Calibration Using NBS SRM 1647a, A Mixture of 16 PAH

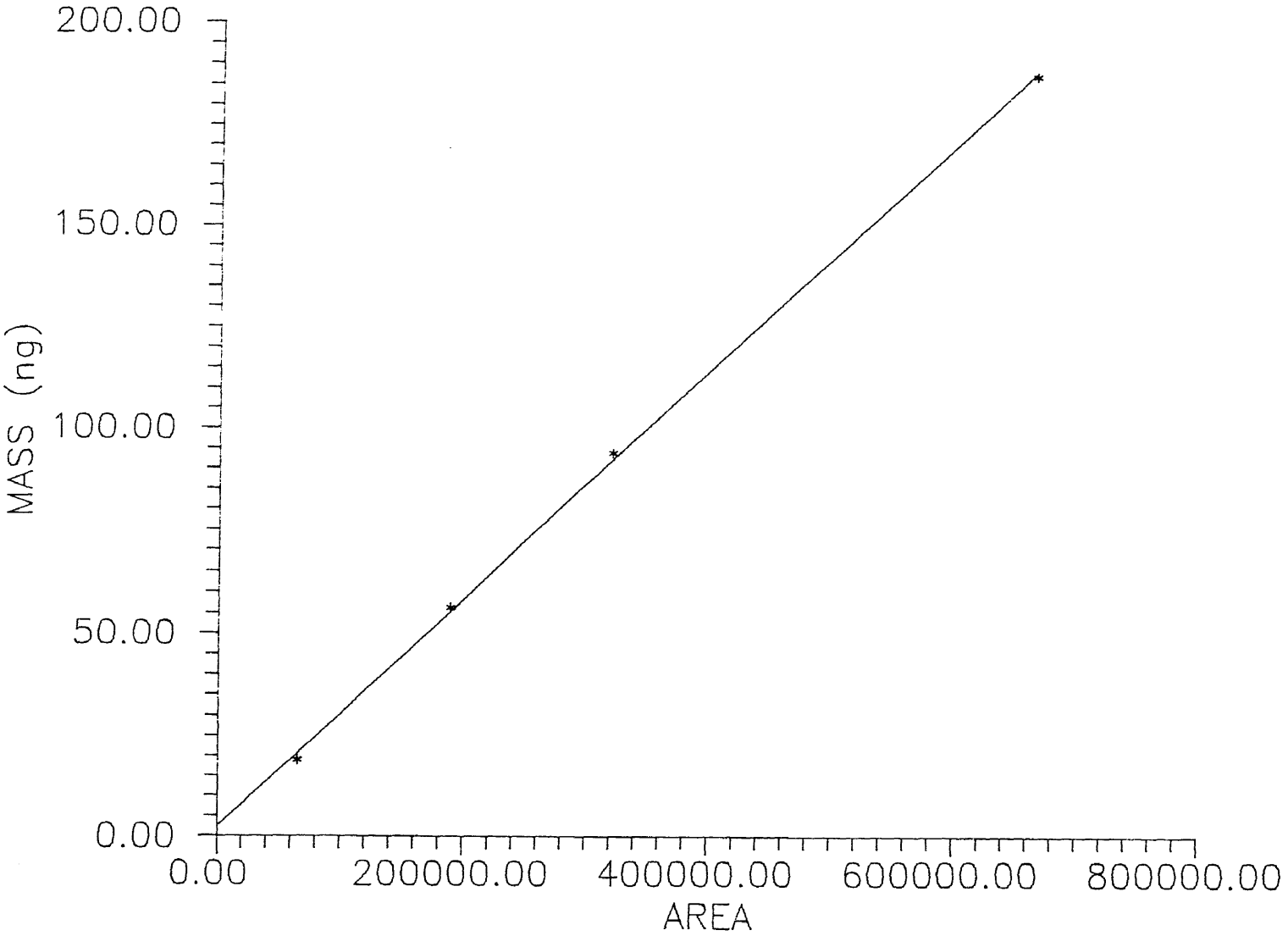


Figure 3-1j Indeno(1,2,3-cd)Pyrene Calibration Using NBS SRM 1647a, A Mixture of 16 PAH

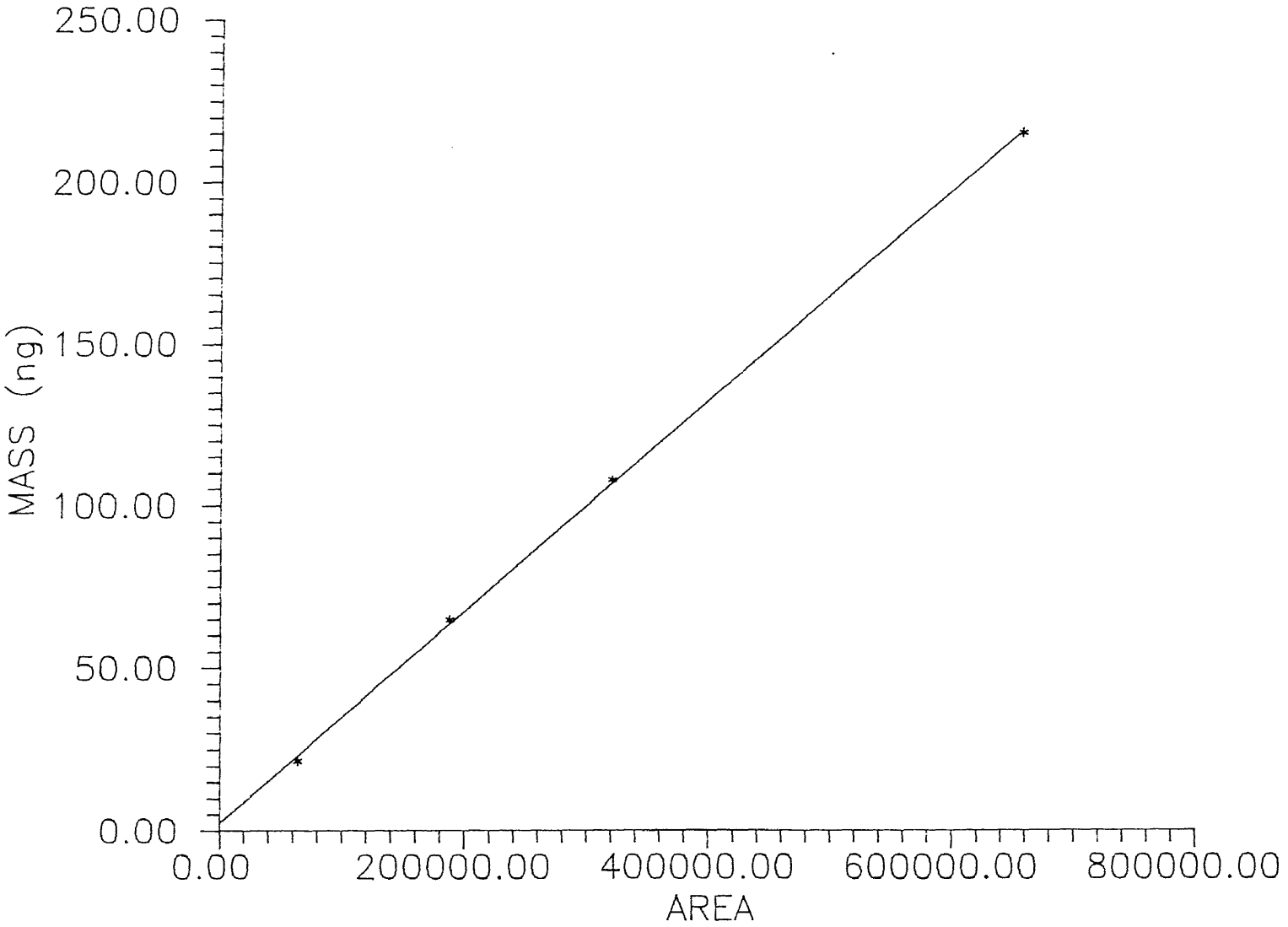


Figure 3-1k Phenanthrene Calibration Using NBS SRM 1647a, A Mixture of 16 PAH

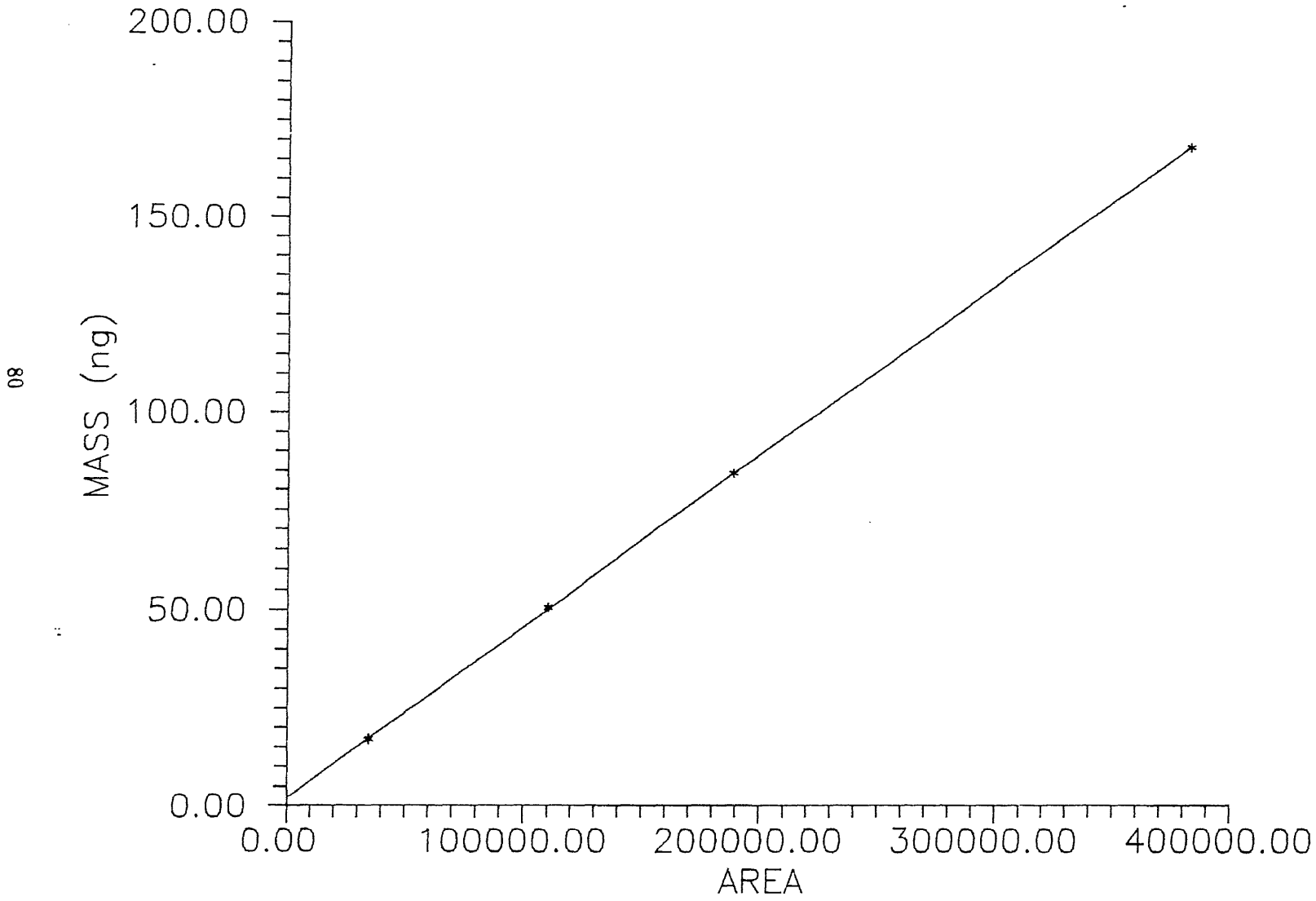
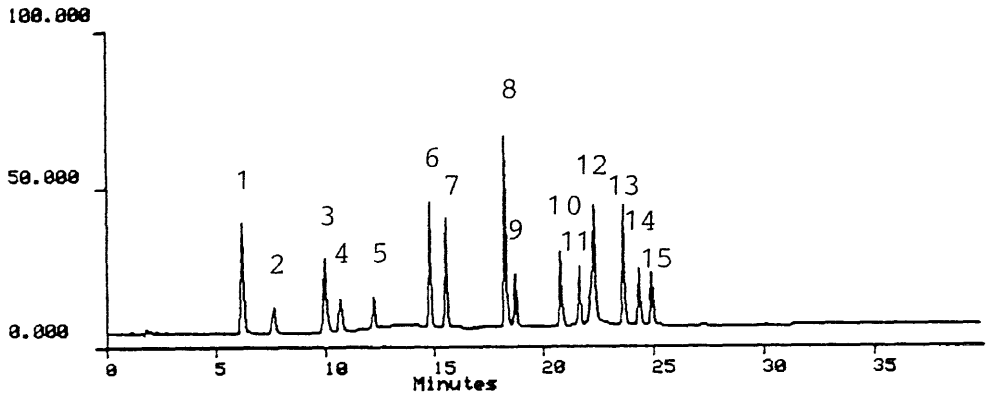
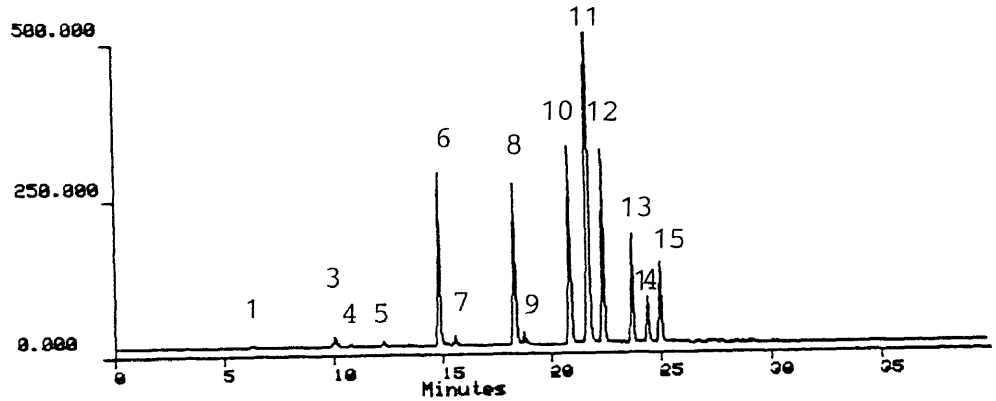


Figure 3-2 The HPLC Chromatogram of NBS 1647a



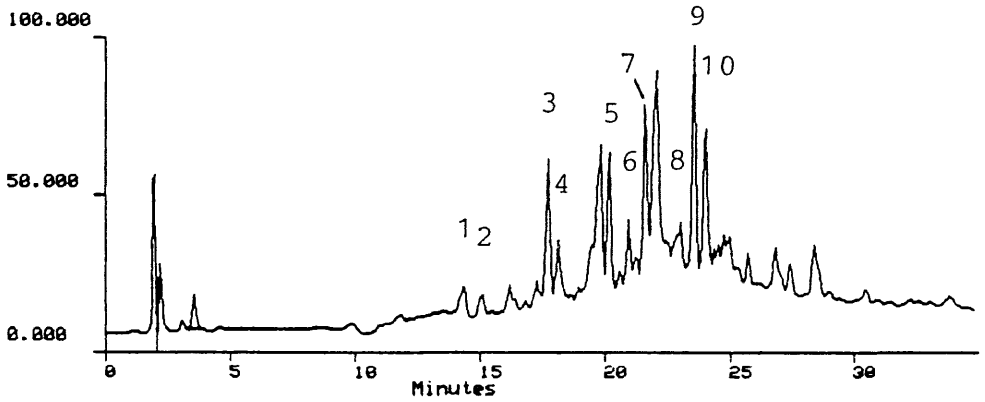
(a) Ultraviolet detector



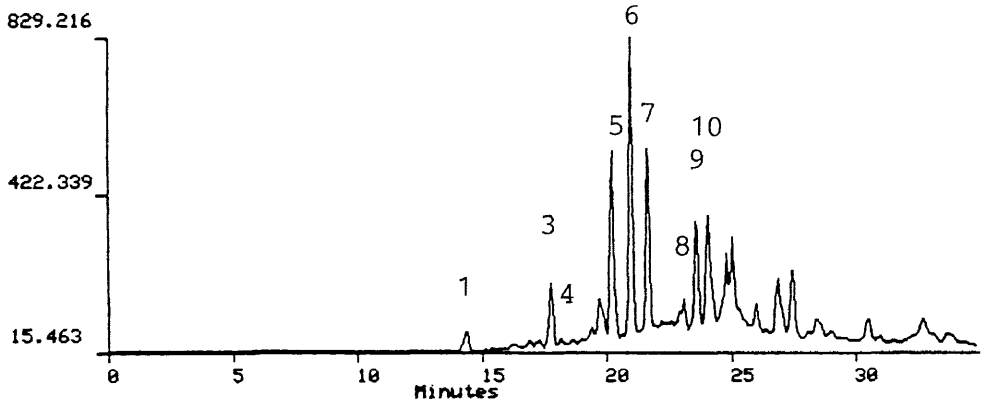
(b) Fluorescene detector

1. naphthalene
2. acenaphthylene
3. acenaphthene
4. fluorene
5. phenanthrene
6. fluoranthene
7. pyrene
8. benz(a)anthracene
9. chrysene
10. benzo(b)fluoranthene
11. benzo(k)fluoranthene
12. benzo(a)pyrene
13. dibenz(a,h)anthracene
14. benzo(ghi)perylene
15. indeno(1,2,3-cd)pyrene

Figure 3-3 The HPLC Chromatogram of W88-DCM-N6-S2



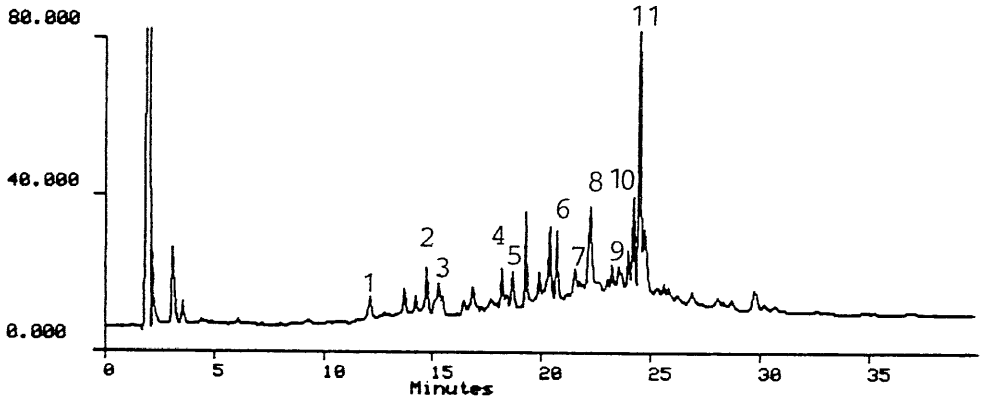
(a) Ultraviolet detector



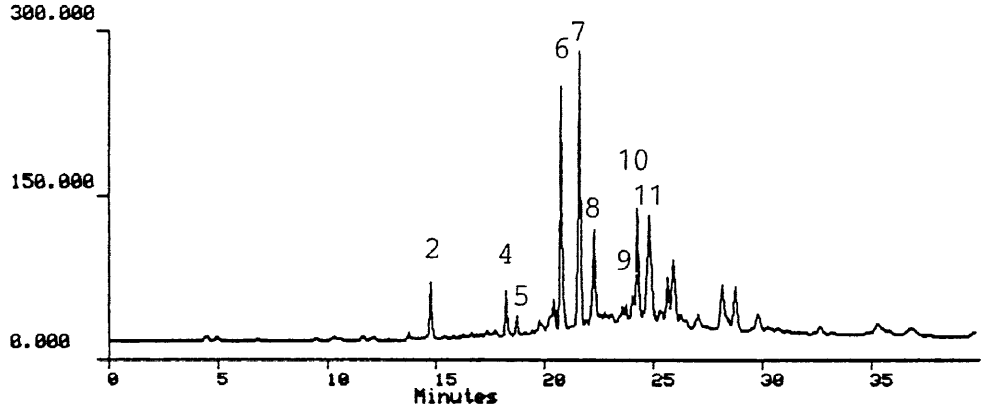
(b) Fluorescene detector

1. fluoranthene
2. pyrene
3. benz(a)anthracene
4. chrysene
5. benzo(b)fluoranthene
6. benzo(k)fluoranthene
7. benzo(a)pyrene
8. dibenz(a,h)anthracene
9. benzo(ghi)perylene
10. indeno(1,2,3-cd)pyrene

Figure 3-4 The HPLC Chromatogram of S88-DCM-N6-S2



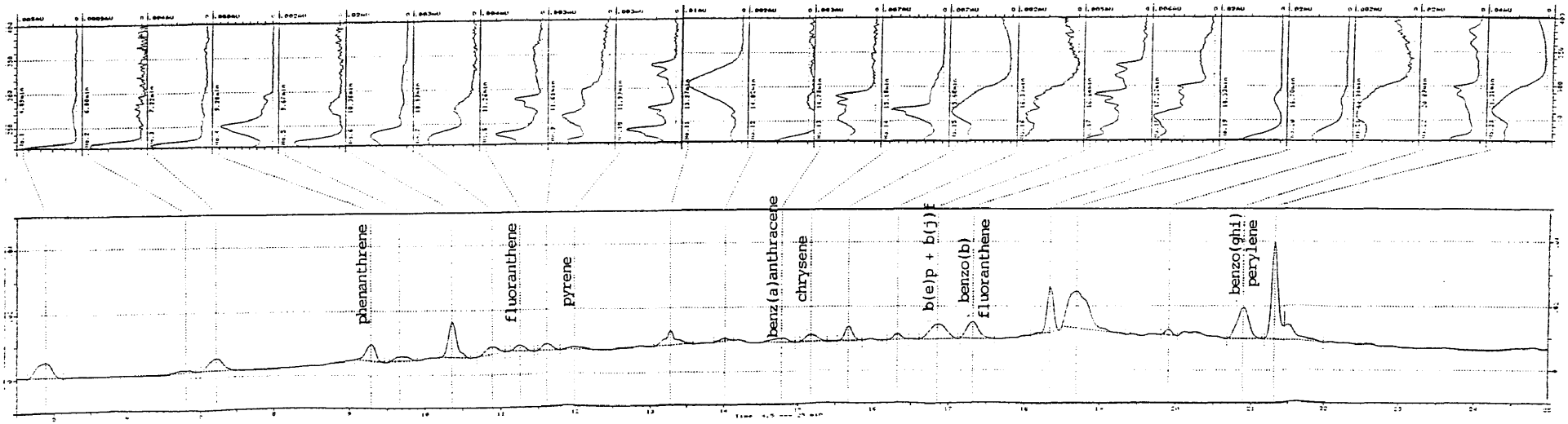
(a) Ultraviolet detector



(b) Fluorescene detector

1. phenanthrene
2. fluoranthene
3. pyrene
4. benz(a)anthracene
5. chrysene
6. benzo(b)fluoranthene
7. benzo(k)fluoranthene
8. benzo(a)pyrene
9. dibenz(a,h)anthracene
10. benzo(ghi)perylene
11. indeno(1,2,3-cd)pyrene

Figure 3-5 The UV Chromatogram done by HPLC with Photodiode Array Detector



W88-DCM-N6-S2 Sample

Figure 3-6 The Concentrations of Ten Selected PAH Identified in W88-DCM-N6-S2

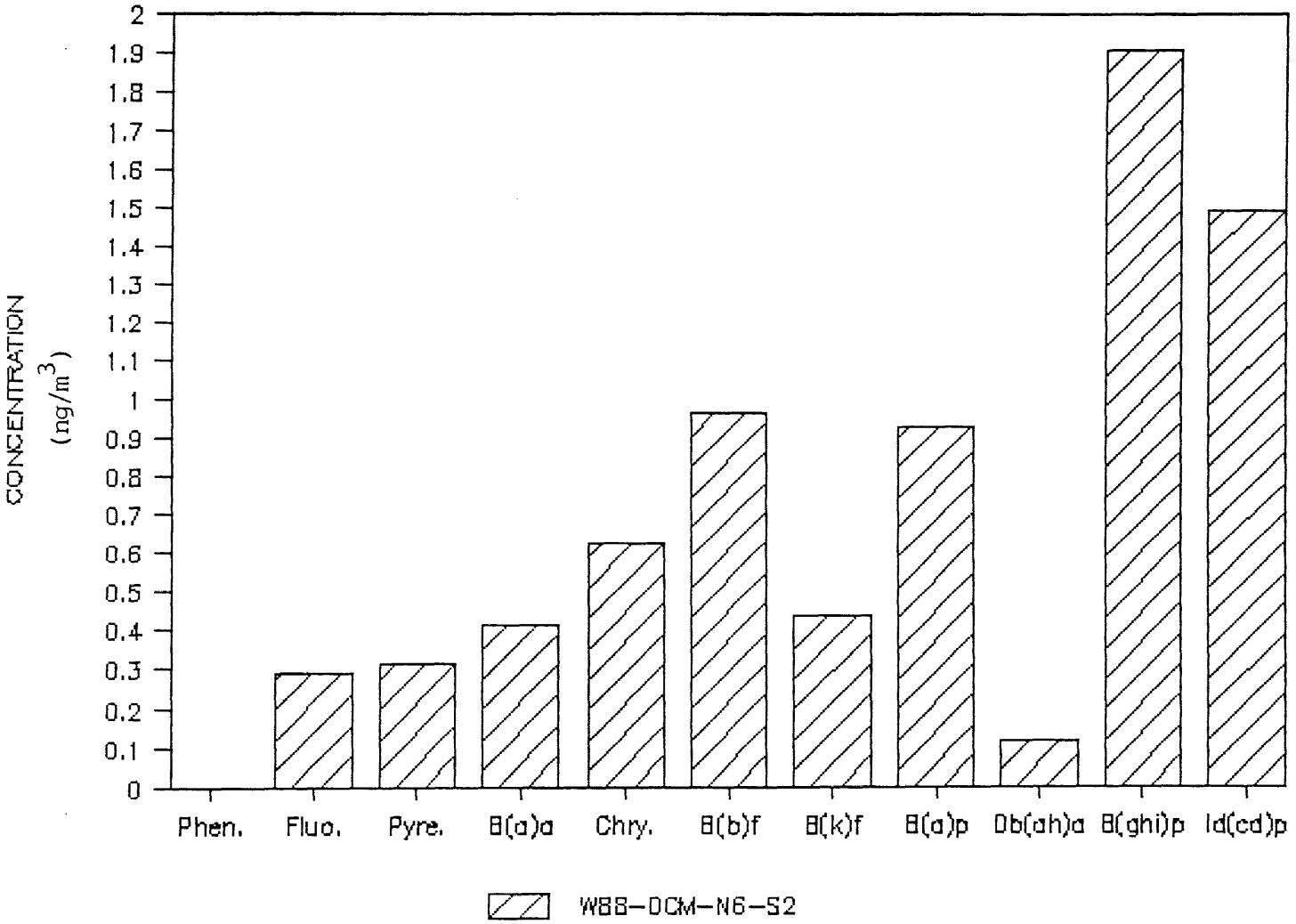


Figure 3-7 The Concentrations of Eleven Selected PAH Identified in S88-DCM-N6-S2

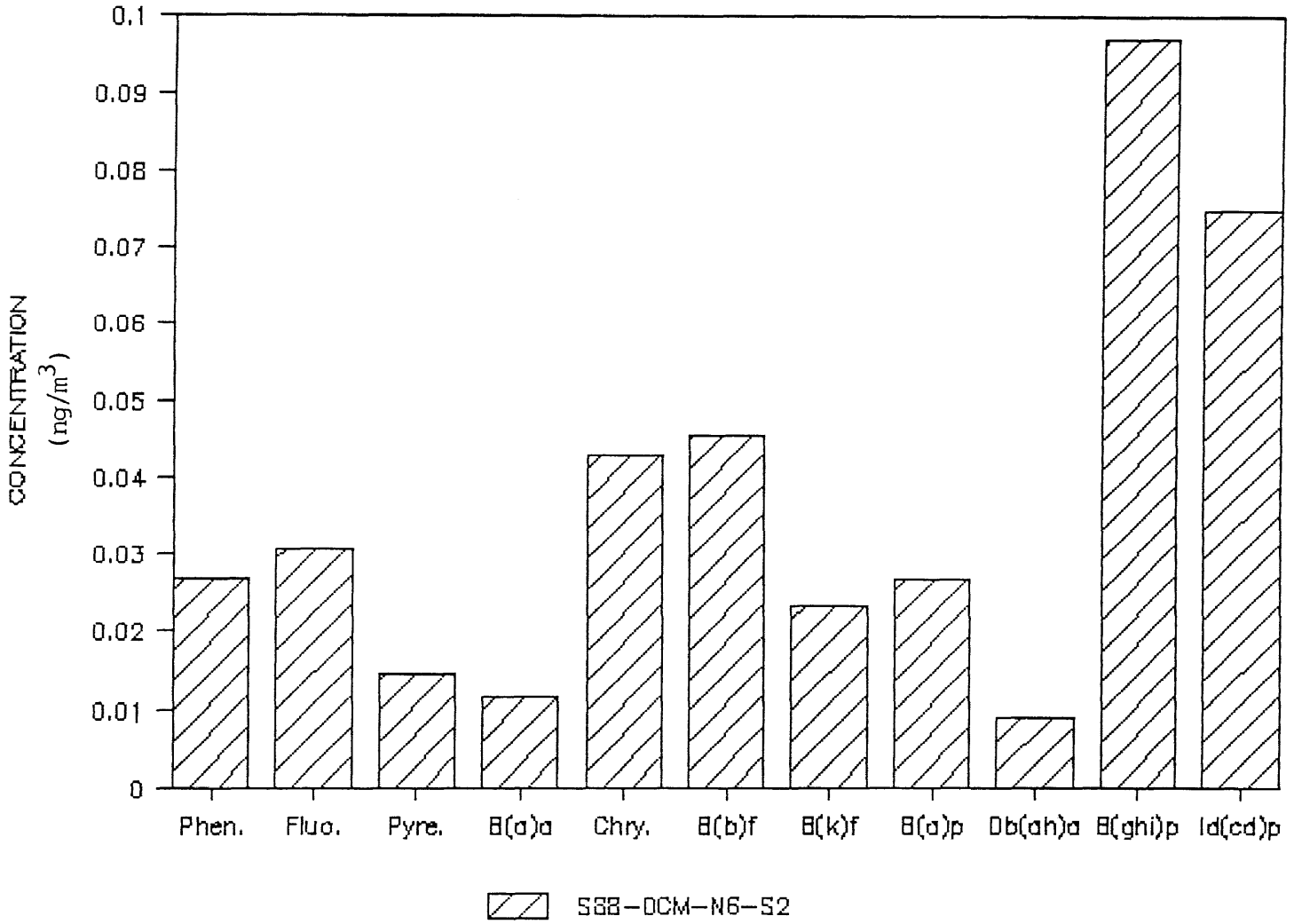


Figure 3-8 Concentration Comparison of Selected PAH between W88- DCM-N6-S2 and S88-DCM-N6-S2

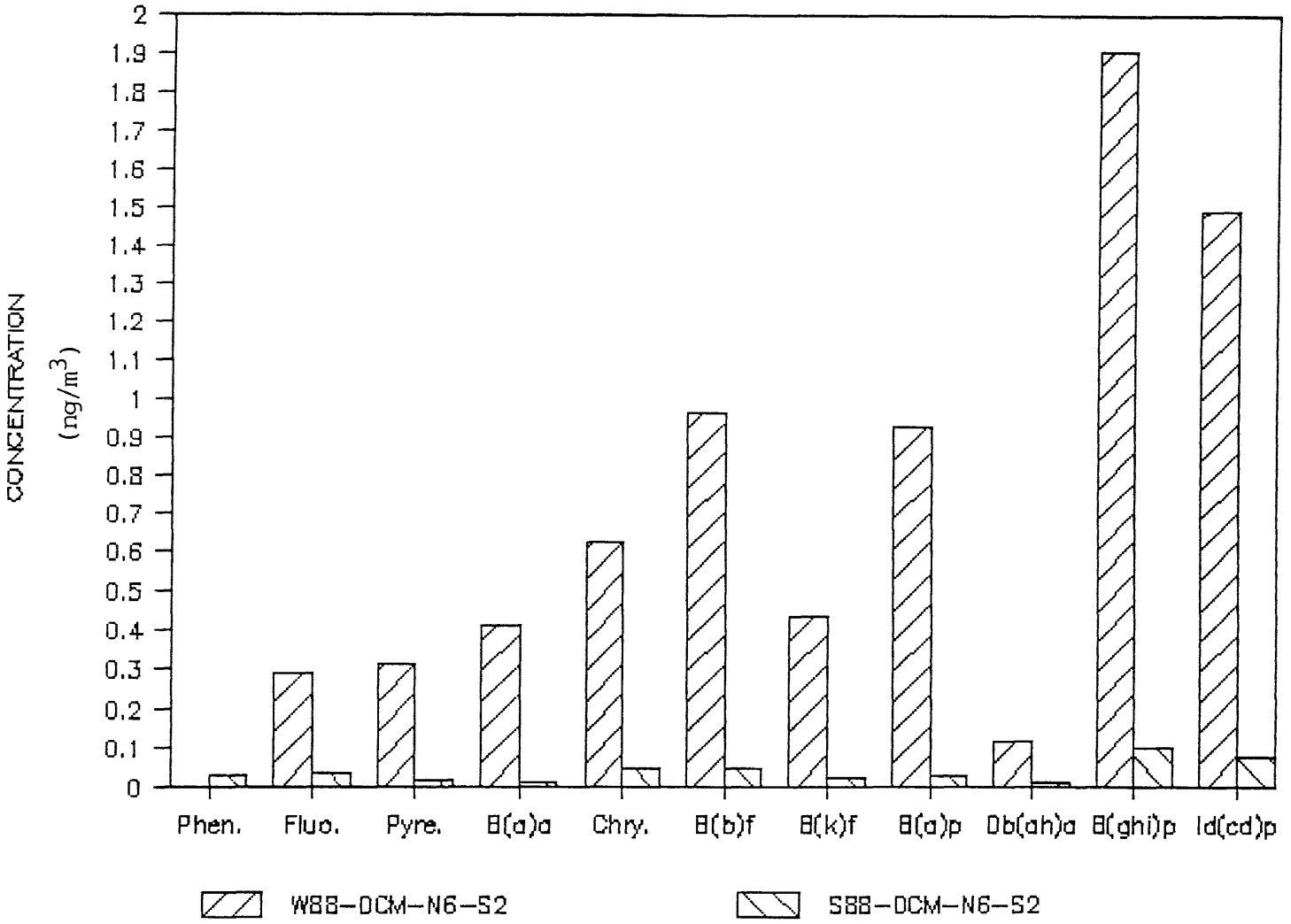


Figure 3-9 The Ratio (PAH/BaP) Comparison between W88-DCM-N6-S2 and S88-DCM-N6-S2

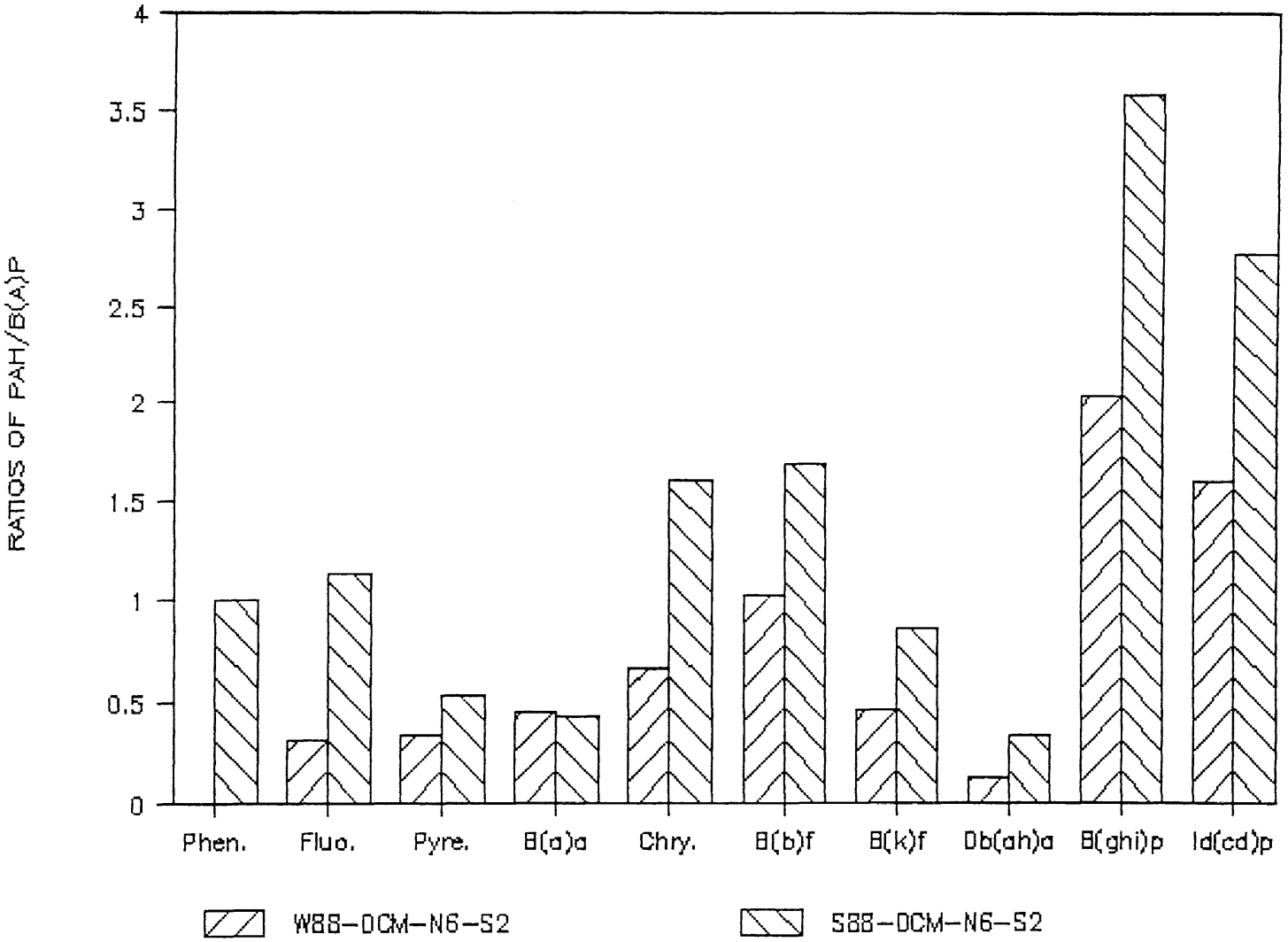


Figure 3-10 The Ratio (PAH/BbF) Comparison between W88-DCM-N6-S2 and S88-DCM-N6-S2

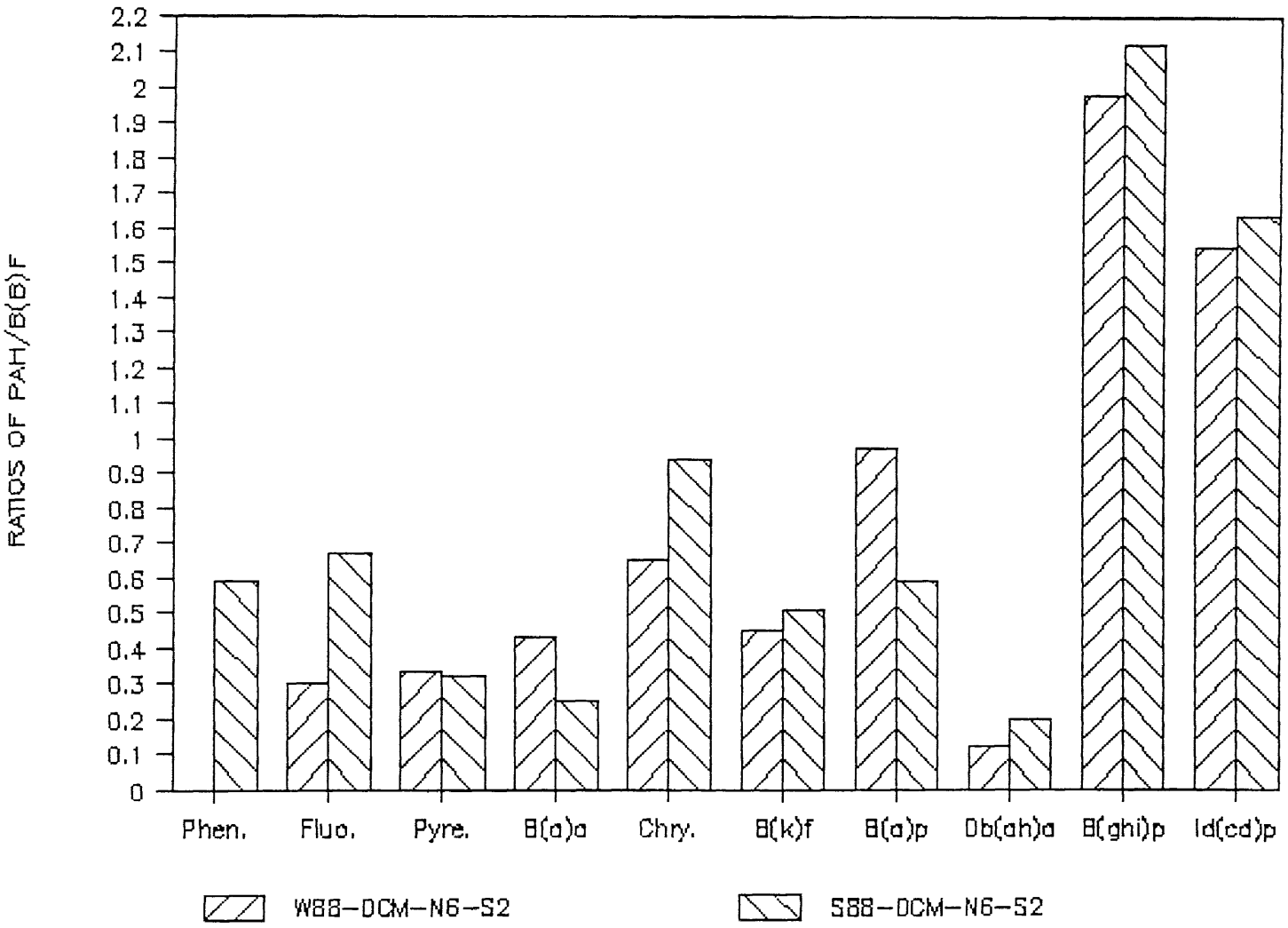
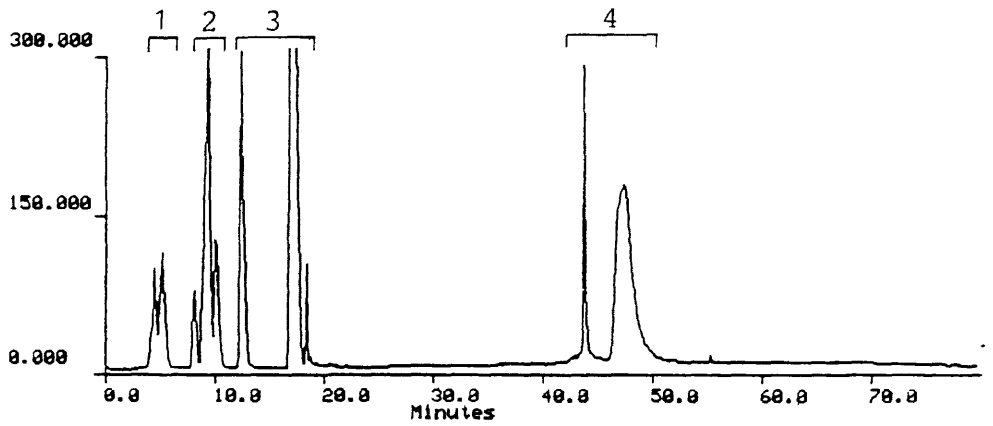
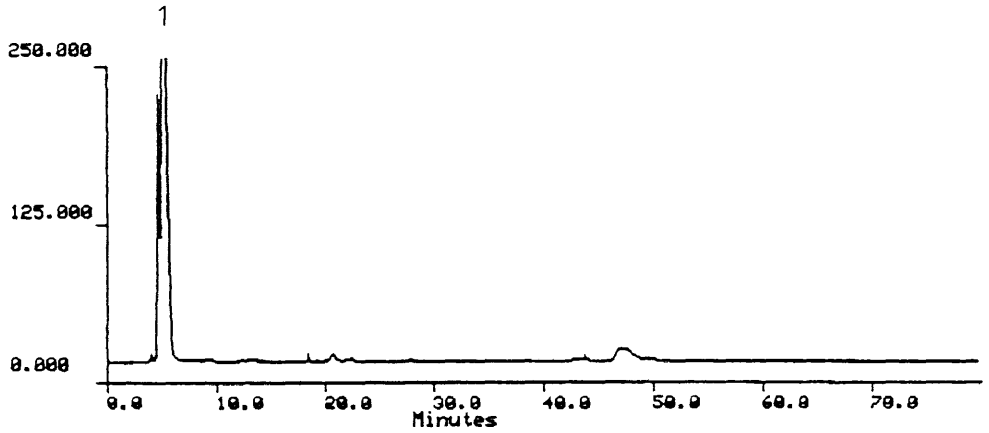


Figure 3-11 The HPLC Chromatogram of Mixed Standard



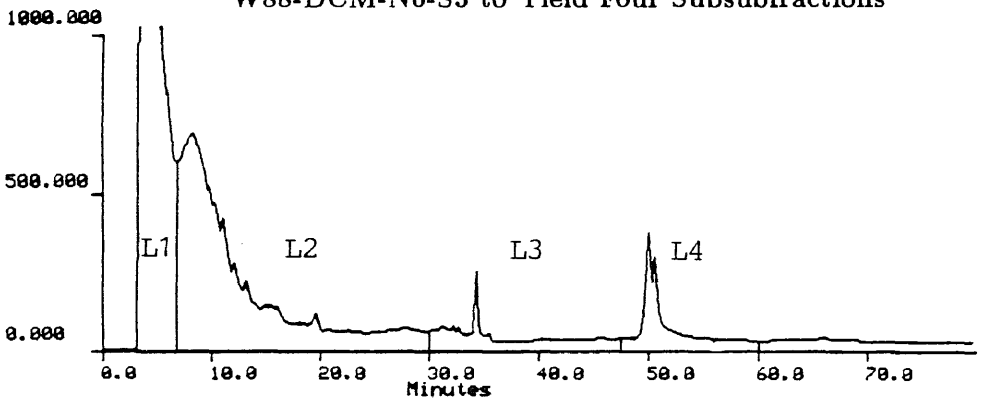
(a) Ultraviolet detector

1. polycyclic aromatic hydrocarbon 2. nitro-PAH
3. PAH-quinone 4. 3;6;8-hydroxy-1-nitropyrene

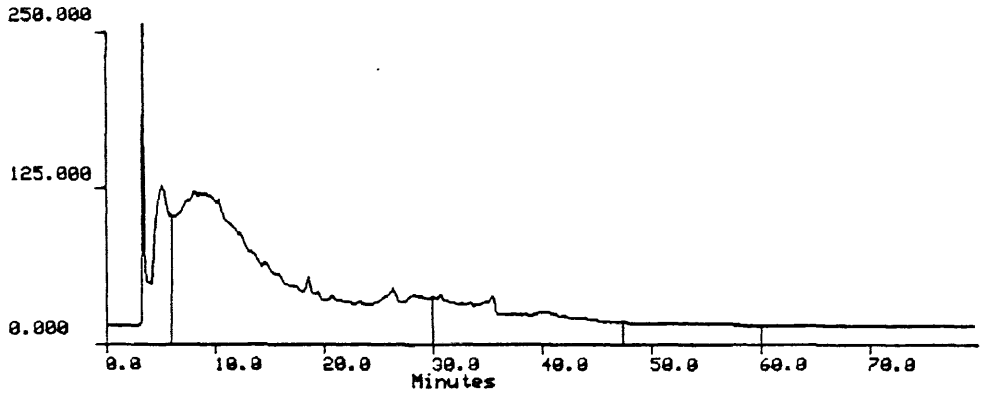


(b) Fluorescene detector

Figure 3-12 The Semi-preparative HPLC Chromatogram of Subfraction W88-DCM-N6-S3 to Yield Four Subsubfractions

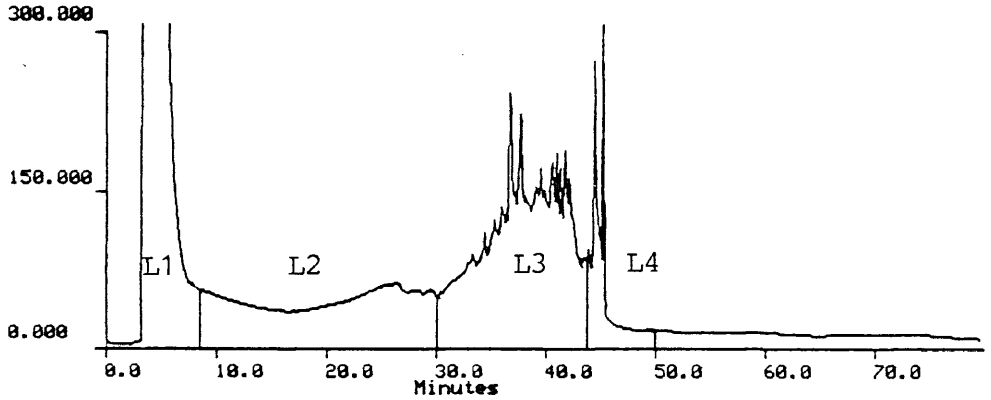


(a) Ultraviolet detector

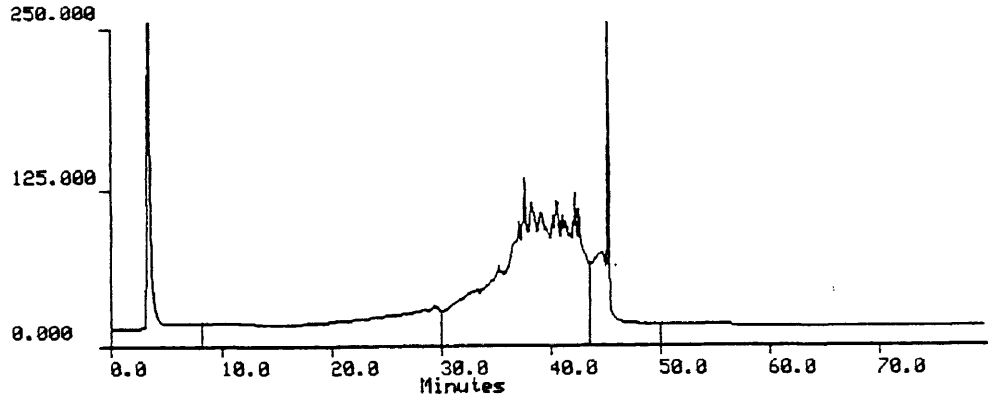


(b) Fluorescene detector

Figure 3-13 The Semi-preparative HPLC Chromatogram of Subfraction S88-DCM-N6-S3 to Yield Four Subsubfractions



(a) Ultraviolet detector



(b) Fluorescene detector

Figure 3-14 Distribution of Corrected Mass and Mutagenicity for W88-DCM-N6-S3-L1 to L4

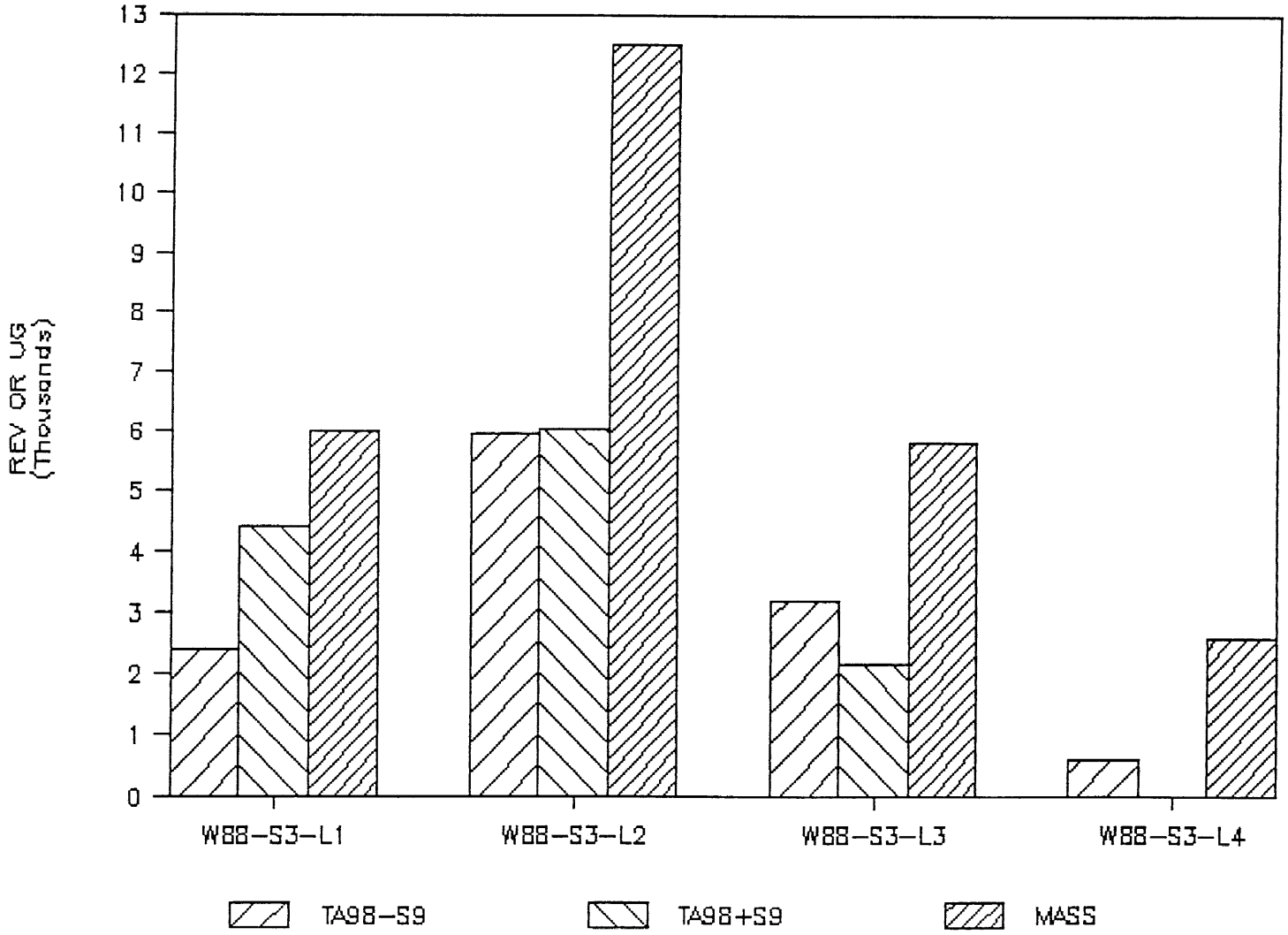


Figure 3-15 Distribution of Corrected Mass and Mutagenicity for S88-DCM-N6-S3-L1 to L4

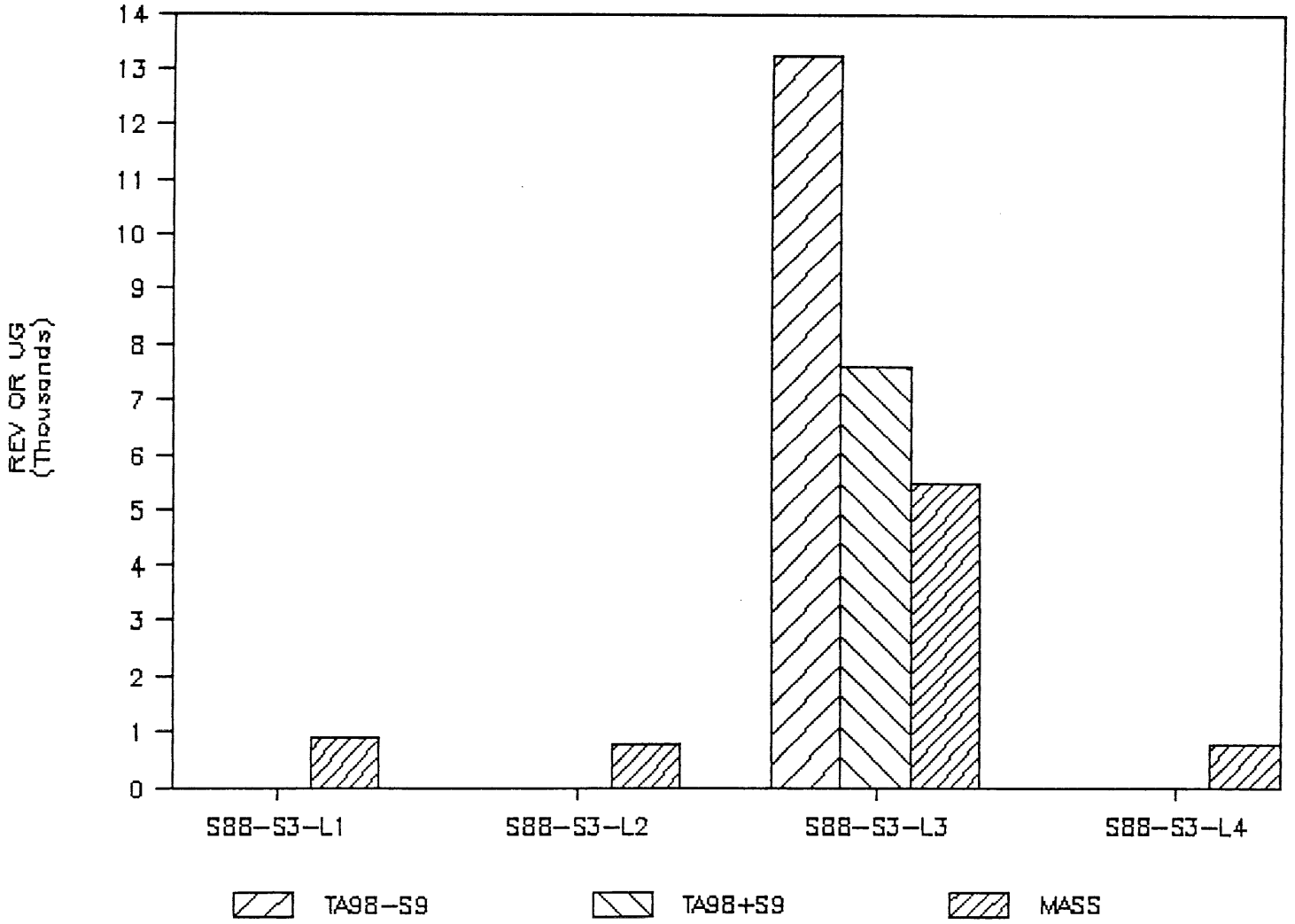
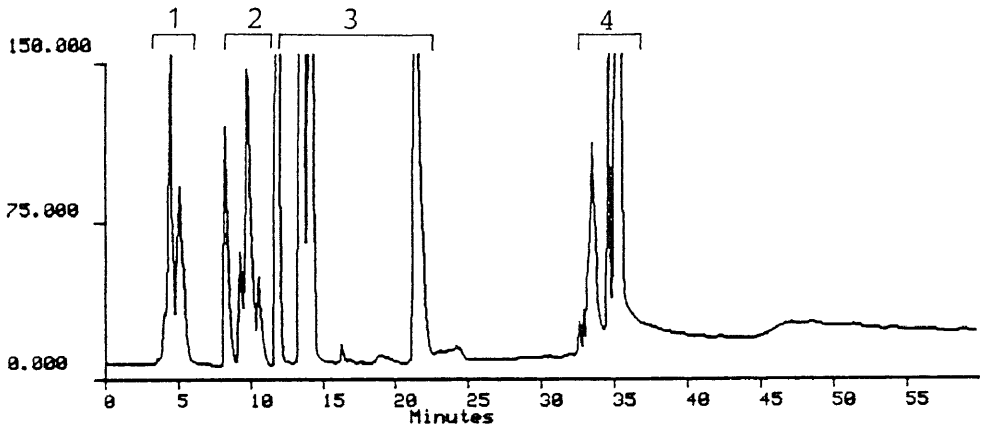
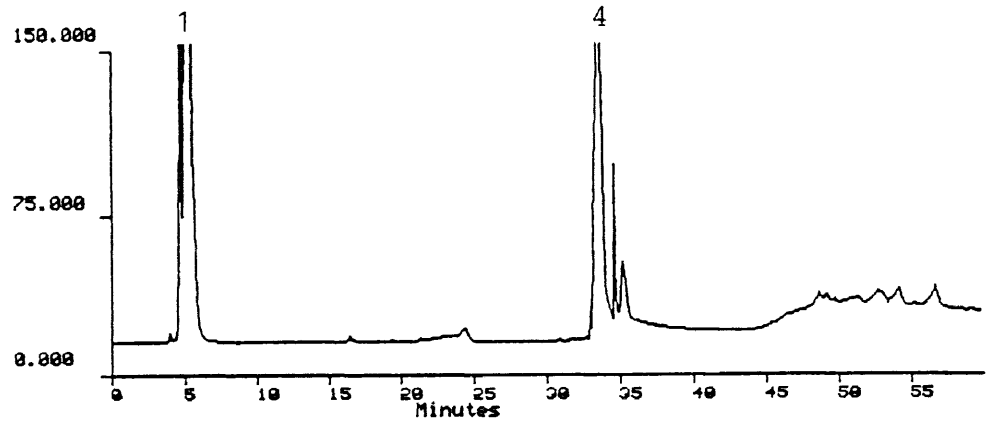


Figure 3-16 The HPLC Chromatogram of Mixed Standard



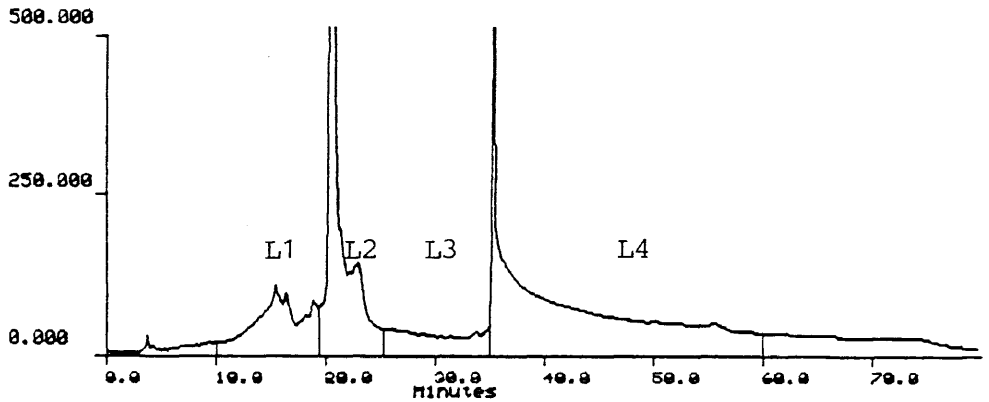
(a) Ultraviolet detector

1. polycyclic aromatic hydrocarbon 2. nitro-PAH
3. PAH-quinone 4. hydroxypyrene and hydroxynitropyrene

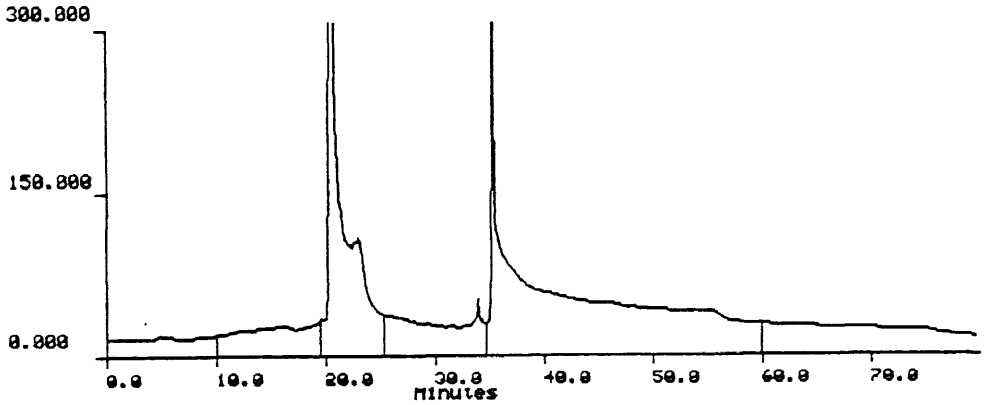


(b) Fluorescene detector

Figure 3-17 The Semi-preparative HPLC Chromatogram of Subfraction W88-DCM-N6-S4 to Yield Four Subsubfractions

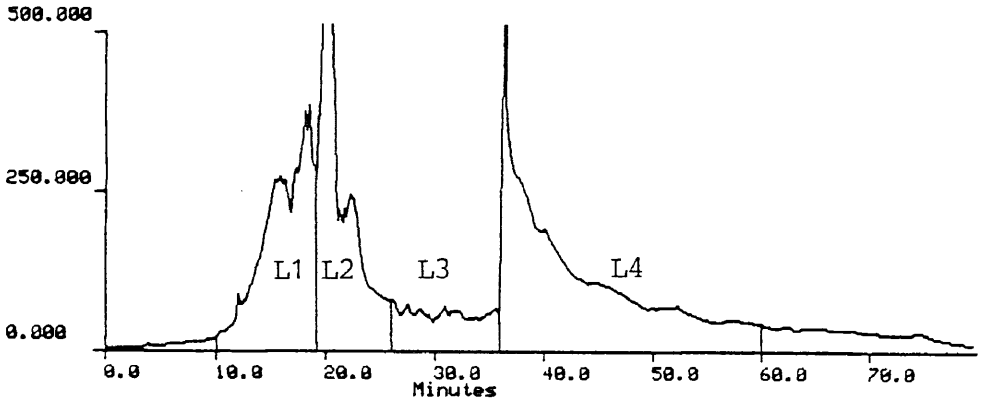


(a) Ultraviolet detector

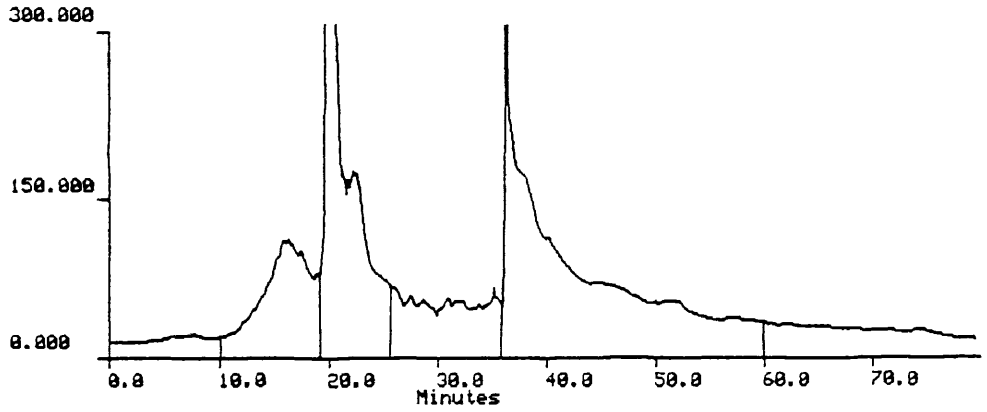


(b) Fluorescene detector

Figure 3-18 The Semi-preparative HPLC Chromatogram of Subfraction S88-DCM-N6-S4 to Yield Four Subsubfractions



(a) Ultraviolet detector



(b) Fluorescene detector

Figure 3-19 Distribution of Corrected Mass and Mutagenicity for W88-DCM-N6-S4-L1 to L4

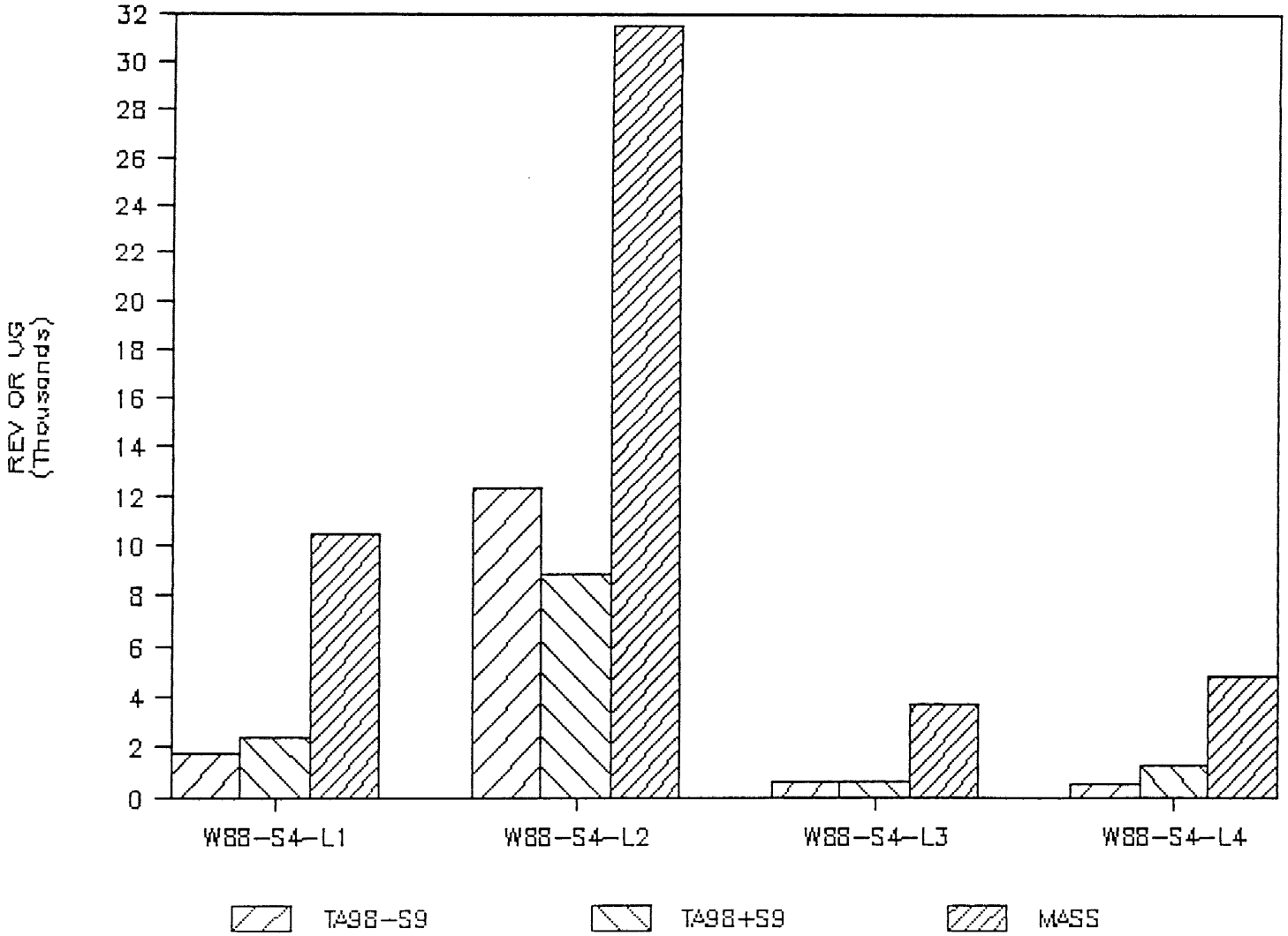
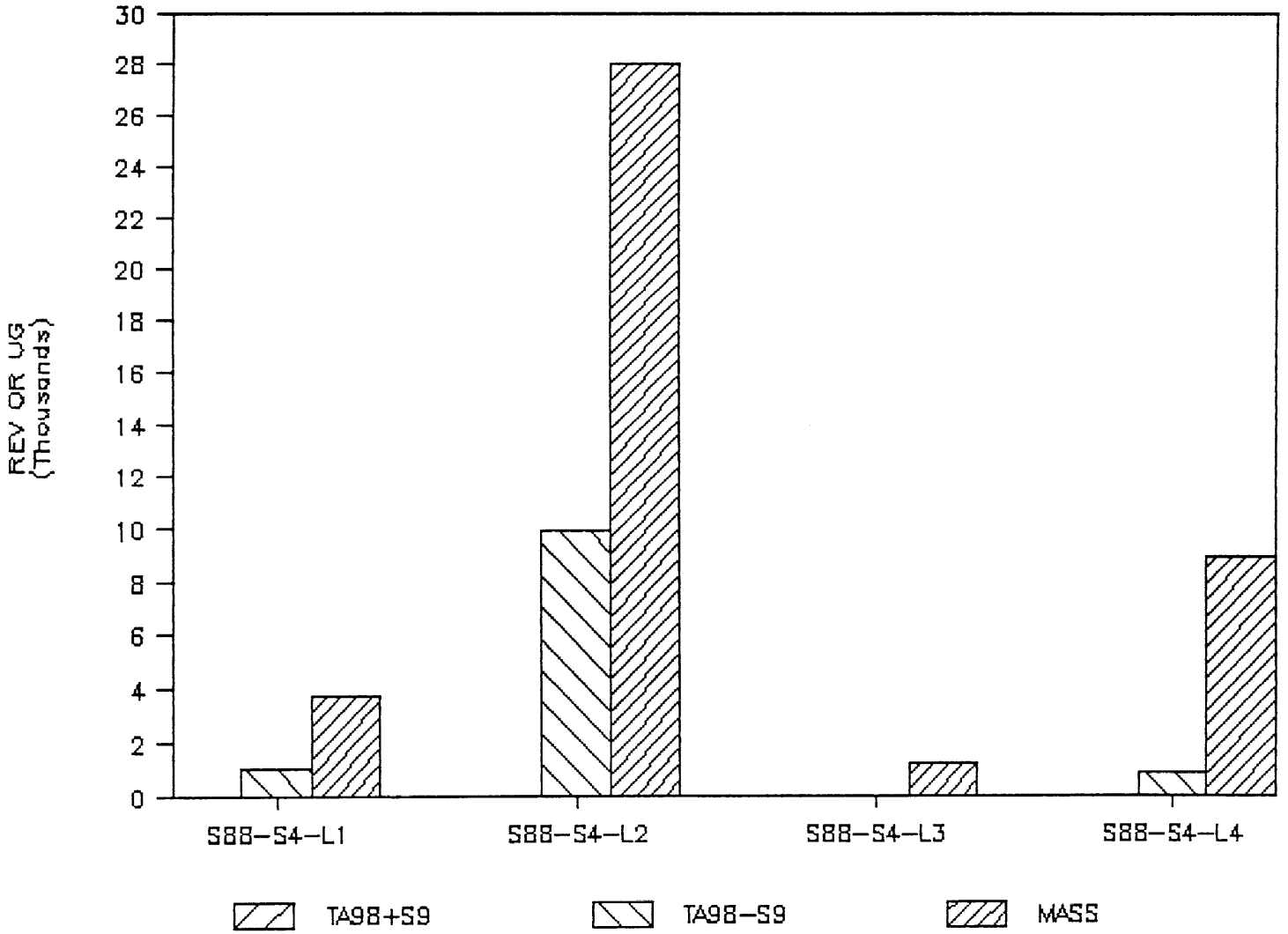


Figure 3-20 Distribution of Corrected Mass and Mutagenicity for S88-DCM-N6-S4-L1 to L4



CHAPTER FOUR

Fourier Transform Infrared and Diode Array UV

4.1 Fourier Transform Infrared (FTIR) Spectroscopy

4.1-1 Introduction

We have attempted to gain insight into the nature of functional groups and classes of organic compounds in our airborne particulate extracts by employing Fourier Transform Infrared (FTIR) spectroscopy on 8 fractions obtained by acid-base partition, 8 subfractions obtained by open column chromatography and 7 sub-subfractions from HPLC semi-preparative column chromatography.

Fourier Transform Infrared (FTIR) Spectroscopy is a potentially powerful technique for analyzing classes of pollutants on ambient particulates. In the present study, a portion of each fraction, subfraction and subsubfraction was brought down to around 50 ul. Then, all samples were layered on a KBr window and the solvent evaporated.

4.1-2 Experimental

The FTIR technique has been discussed elsewhere. (1) For the present study, samples dissolved in different solvents were evaporated to dryness on KBr (13 x 2

mm) windows and scanned 32 times on a Nicolet System 740 FTIR spectrophotometer equipped with a sensitive MCT detector. Interferograms are collected with an optical velocity of 20 scans per second and Fourier transformed to yield a resolution of 0.3 cm^{-1} and data encoded every 0.2 cm^{-1}. The frequencies were determined with an uncertainty of less than ± 0.004 cm^{-1}. The spectra were obtained under the supervision of Dr. David Bugay at the Squibb Medical Research Institute in New Brunswick, N.J. Interpretation of FTIR spectra is based on standard sources. (2) We will discuss the FTIR spectra of the fractions, subfractions and "subsubfractions" in turn.

4.1-3 Results and Discussion

A. Weak Base Fractions

A quick glance at the transmittance scales of Figure 4-1 and Figure 4-2 indicates that there are very strong bands at 2925 cm^{-1}, 2854 cm^{-1}, 1463 cm^{-1}, 1377 cm^{-1} and 1277 cm^{-1}, which could correspond to alkanes. However, there is a hint of unsaturated and/or aromatic hydrocarbons as shown by the small shoulder around 3058 cm^{-1} and the small peak around 1600 cm^{-1}. Two weak absorption bands: one near 3430 cm^{-1}, the other near 3360 cm^{-1} represent, respectively, the "free" asymmetrical and symmetrical N-H stretching modes, and a shoulder around 3200 cm^{-1} display Fermi resonance band of aromatic amines with overtone of 1074 cm^{-1}

band. Moreover, some of the intense carbonyl absorptions around 1713-1728 cm^{-1} are likely to be phthalates because our mass spectrometric results indicated that samples were contaminated with phthalates. Acctually, phthalates have carbonyl absorption around 1720 cm^{-1}. For the winter weak base fraction (W88-DCM-B1), the band at 1123 cm^{-1} could correspond, along with the 3430 cm^{-1} band, to an alcohol or to an ester. This is a significant difference between winter and summer weak base fractions, since the summer fraction lacks this. At the same time, the S88-DCM-B1 fraction has more bands in the 1600-1730 cm^{-1} area than does W88-DCM-B1 fraction. This could be the resonance effect increases the C=O band length and reduces the frequency of absorption, such as NH_2 or S groups in R(CO)X compounds.

B. Weak Acid Fractions

The winter (Figure 4-3) and summer (Figure 4-4) weak acid fractions have very similar IR spectra: both have carbonyl bands at 1710 cm^{-1} and weak aromatic band above 3000 cm^{-1} as well as aromatic C-C ring stretch around 1600 cm^{-1}. In addition, the O-H absorption peak around 3280 cm^{-1} is probably due to some fatty acid contribution although moisture is possible. It is interesting that asymmetric stretching (1515-1550 cm^{-1}) and symmetric stretching (1345-1385 cm^{-1}) for the nitro group were found in these two fractions. In this regard it is worthwhile remembering the earlier-cited observations of a number of research groups that most of the muta-

genic activity is associated with nitrated compounds. This is, perhaps, the reason why these weak acid fractions were the second most mutagenic at the first level of separation.

C. Strong Base Fractions

The IR spectra of strong base fractions are shown in Figure 4-5 and Figure 4-6. There is a significant level of alkanes which appear to compose most of its mass. Thus, there is less mutagenicity in these two samples. It appears, as in the weak base fractions, that levels of nitro group can not be obviously found in these fractions but the Ames assay results showed more important TA98NR-S9 and TA98DNP-S9 response in W88-DCM-B3 fraction. This could be due to very few nitro compounds in the samples. There might be a carbonyl band (1712-1728 cm^{-1}) which is likely to be associated with an ester. Actually, a lot of esters of fatty acids have been found (using GC/MS) in these two fractions. In addition, the associated N-H bands at 3400-3330 cm^{-1} and 3330-3250 cm^{-1}, which are weaker but frequently sharper than the corresponding O-H bands, were also found in these strong base samples. A weak aromatic band around 3000 cm^{-1} was also displayed in the spectra of the winter strong base fraction but not in that of summer fraction.

D. Strong Acid Fractions

In Figure 4-7 and Figure 4-8, the spectra of strong acid fractions displayed

intense and wide carbonyl absorption bands at 1720 cm^{-1} which could correspond to carboxylic C=O stretch, 1715 cm^{-1}, and the bands around 1280 cm^{-1} might represent the C-O, dimer, stretch in carboxylic acids. The aromatic absorption band above 3000 cm^{-1} was also seen in the winter fraction only. At the same time, samples were contaminated by a lot of phthalates when analyzed by GC/MS, but the results still show the presence of carboxylic acid. Furthermore, the weak peak around 1550 cm^{-1} and the bands at 1377 cm^{-1} corresponding to nitro group were observed in these fractions. This is interesting and surprising to us. In fact, a nitrosomorpholine compound was found in GC/MS results. The stronger band at 1638 cm^{-1} in S88-DCM-A4 but weaker in W88-DCM-A4 could be due to alkene C=C stretch or PAH-quinones since extended quinones are known to absorb at around 1645 cm^{-1}.

E. First Neutral Subfraction (S1)

These two subfractions (W88-DCM-N6-S1 and S88-DCM-N6-S1) eluted from an open silica column with hexane are the non-polar and second most massive fractions at the second level of separation. Those FTIR spectra (Figure 4-9 and Figure 4-10) show the alkane bands at 2970 cm^{-1} and 2842 cm^{-1} corresponding to C-H stretch of alkanes, as well as at 1463 cm^{-1} and 1377 cm^{-1} corresponding to C-H bend of alkanes. The absorption band around 720 cm^{-1} also represent the CH_2 rock. However, the peak around 1640 cm^{-1} display the C=C stretch, too. Therefore, there

could be some other aliphatic compounds, such as alkenes, in these subfractions. The absorption band around 1600 cm^{-1}, along with the strong band at 1460 cm^{-1}, should well be the aromatic C-C ring stretch. GC/MS results, in fact, show some alkene and aromatic compounds found in these samples.

F. Second Neutral Subfraction (S2)

These hexane/benzene (1:1 v/v) eluant neutral subfractions should correspond to polycyclic aromatic hydrocarbon (PAH) compounds. The aromatic C-H stretching band at 3050 cm^{-1} is evident and the most characteristic absorption of polycyclic aromatics resulting from C-H out-of-plane bending in the 900-675 cm^{-1} region is also found in Figure 4-11 and Figure 4-12. Thus, consistent with HPLC and GC/MS results, most of the parent PAH compounds are in these subfractions. At the same time, a major indirect-acting mutagenic activity was also found in these two subfractions. Two peaks at 1090-1030 cm^{-1} displayed unconjugated straight chain anhydrides, and cyclic anhydride C- CO-O-CO-C stretch near 952-909 cm^{-1} as well as 1299-1176 cm^{-1} was shown in these samples.

G. Third Neutral Subfraction (S3)

The aromatic C-H stretching bands are still observable at 3050 cm^{-1} in Figure 4-13 and Figure 4-14. Weak C-C ring stretch occurs at 1580, 1487 and 1466 cm^{-1}. Unexpectedly, it seems to us that these spectra do not clearly show asym-

metrical and symmetrical stretching of the nitro group in the regions of 1550- 1515 cm^{-1} and 1385-1345 cm^{-1}. However, a C-N stretching vibration of nitro aromatic compounds appears near 860 cm^{-1}. Asymmetrical stretching in the NO_2 group of organic nitrates results in strong absorption in the 1660-1625 cm^{-1} region and the symmetrical vibration absorbs strongly near 1300-1255 cm^{-1}. In addition, the carbonyl band at 1729 cm^{-1} could correspond to benzoates because conjugation of an aryl group or other unsaturation with the carbonyl group causes this C=O stretch to be at lower than normal frequency (e.g. benzoates absorb at ca. 1724 cm^{-1}). The band around 1635 cm^{-1} could well be due to PAH-quinones since extended quinones are known to absorb in 1655-1635 cm^{-1} region. In fact, we found some ketones in these subfractions using GC/MS techniques.

H. Fourth Neutral Subfraction (S4)

The characteristic bands observed in these two spectra (Figure 4- 15 and Figure 4-16) are shown at 3350-3360 cm^{-1} which might be intermolecular hydrogen bonded O-H stretch. We feel that this band is likely to be associated with phenols. Furthermore, for winter sample, the bands near 3430 cm^{-1} and 3350 cm^{-1} represent N-H stretching modes and the shoulder around 3200 cm^{-1} with overtone of 1073 cm^{-1} could be aromatic amines. This is the difference between these two methanol eluant subfractions. Moreover, the absorption bands at 1721 cm^{-1} and 1122 cm^{-1} should correspond to benzoates and alcohols. Our GC/MS results indicate the presence of

phenols and alcohols as the major contributor in these more polar samples. The clear band at 1278 cm^{-1} might represent the organic phosphate compounds which display P=O stretch near 1299-1250 cm^{-1}. The evidence can be also observed from the GC/MS results.

I. Neutral Subsubfractions (S3-L1 to S3-L4)

The IR spectra of subsubfractions (L1-L4) separated from neutral subfraction S3 using HPLC techniques are shown in Figure 4-17 to Figure 4-23, respectively. Basically, these spectra have similar peak positions but more intense absorption band and more obvious aromatic C-H stretch were found in winter samples. It means these four subsubfractions could contain the same classes of compounds and the only difference is individual concentration. This can be evidenced from our GC/MS results.

There is a clear carbonyl band at 1730 cm^{-1} but no O-H stretching band was seen in these subsubfractions except summer L4 sample. We feel that this carbonyl band could correspond to ketones or PAH-quinones. The aliphatic C-H stretch between 2956 cm^{-1} and 2855 cm^{-1} as well as aliphatic C-H bend at 1466 cm^{-1} and 1378 cm^{-1} displayed a lot of aliphatic CH_3 and CH_2 groups included in these samples. An absorption band around 3060 cm^{-1} corresponding to O-H stretch found in summer L4 subsubfraction might represent some polar compounds such as phenols or alcohols. Actually, there is not enough L4 sample for GC/MS test

and the Ames assay results exhibited no significant mutagenic activity in these two samples.

In addition, as our earlier description about the neutral S3 subfraction, the nitro group stretch seemed negligible to be shown in all of these four subsubfractions. However, a little N-H stretching peak around 3055 cm^{-1} and the N-H bend (scissoring) at 1620 cm^{-1} as well as N-H wag between 900-700 cm^{-1} indicated that some amines might exist in these samples.

It is worth noting that the first three subsubfractions of winter samples and the third subsubfraction of summer samples show the major masses and mutagenic activities in this level of separation. Therefore, the further identification should be made, obviously.

4.2 Photodiode Array Ultraviolet

4.2-1 Introduction

High performance liquid chromatography (HPLC) with photodiode array ultraviolet detector is a advanced technique to identify individual compounds from mixed organic solution samples. Thus, in this study, a Waters Model 990 photodiode array HPLC system was employed to determine the specific compounds in the

subfraction S2 and subsubfractions (L1-L3) separated from subfraction S3. Subsubfraction L4 and summer subsubfraction L1 as well as L2 were not run on this system because no significant mutagenic activity was found in these samples.

4.2-2 Experimental

A Vydac 201 TP54 polymeric C_{18} (5 um, 4.6 mm x 25 cm) column was used and the separation condition for subsubfraction L1 to L3 is: step (1) 3 minutes equilibration at 60% acetonitrile: 40% water, step (2) 30 minutes linear gradient to 90% acetonitrile: 10% water, step (3) 15 minutes hold at 90% acetonitrile: 10% water. The separation condition for subfraction S2 has been described in Chapter Three and the chromatogram was shown in Figure 3-5.

Photodiode array detector which can provide rapid scan from 200 nm to 800 nm once a second is a very powerful ultraviolet spectroscopy. Dr. Edward Aig and Mr. John Van Antwerp, Waters Associates, ran this advanced HPLC system for us. They set up UV wavelength at the range from 220 nm to 400 nm. Each sample was injected using a automatic injector and all data were collected by Digital computer data system. At the same time, one selected wavelength ultraviolet chromatogram was also graphically shown in a printer.

4.2-3 Results and Discussion

Figure 4-24 to Figure 4-27 represent the whole chromatogram of winter sub-subfraction L1, L2, L3 and summer L3, respectively. The same chromatographic runs with complete UV spectra at the beginning, top and end of each peak were displayed in Figure 4-28 to Figure 4-31. From analysis of these chromatograms with UV spectra, one can clearly establish whether each peak corresponds to one pure compound or includes more than one component. Furthermore, the UV spectrum of each compound can provide the identification from matching of these compounds in the environmental sample with those in the library of knowns. In Figure 4-32 through Figure 4-35, the same chromatogram with UV spectra from 220 nm to 400 nm were used to indicate what compounds exist in these subsubfractions. Figure 4-33a to Figure 4-33d show four examples of matching and mismatching of PAH compounds found in W88-DCM-N6-S2 samples. Unfortunately, there is no clear evidence for the nitro-PAH compounds identified in these four subsubfractions. However, three similar hydroxynitro-PAH compounds were found in W88-DCM-N6-S3-L3 samples. Thus, even having not confirmed the presence of nitro derivatives in these most mutagenic polar subsubfractions yet, we believe they are present but at low enough levels that they are obscured by abundant substances.

Reference

1. P.R. Griffiths and J.A. De Haseth, *Fourier Transform Infrared Spectrometry*, Wiley, New York, 1986.
2. K. Nakanishi, *Infrared Absorption Spectroscopy Practical*, Holden-Day Inc., San Francisco, 1962.

Figure 4-1

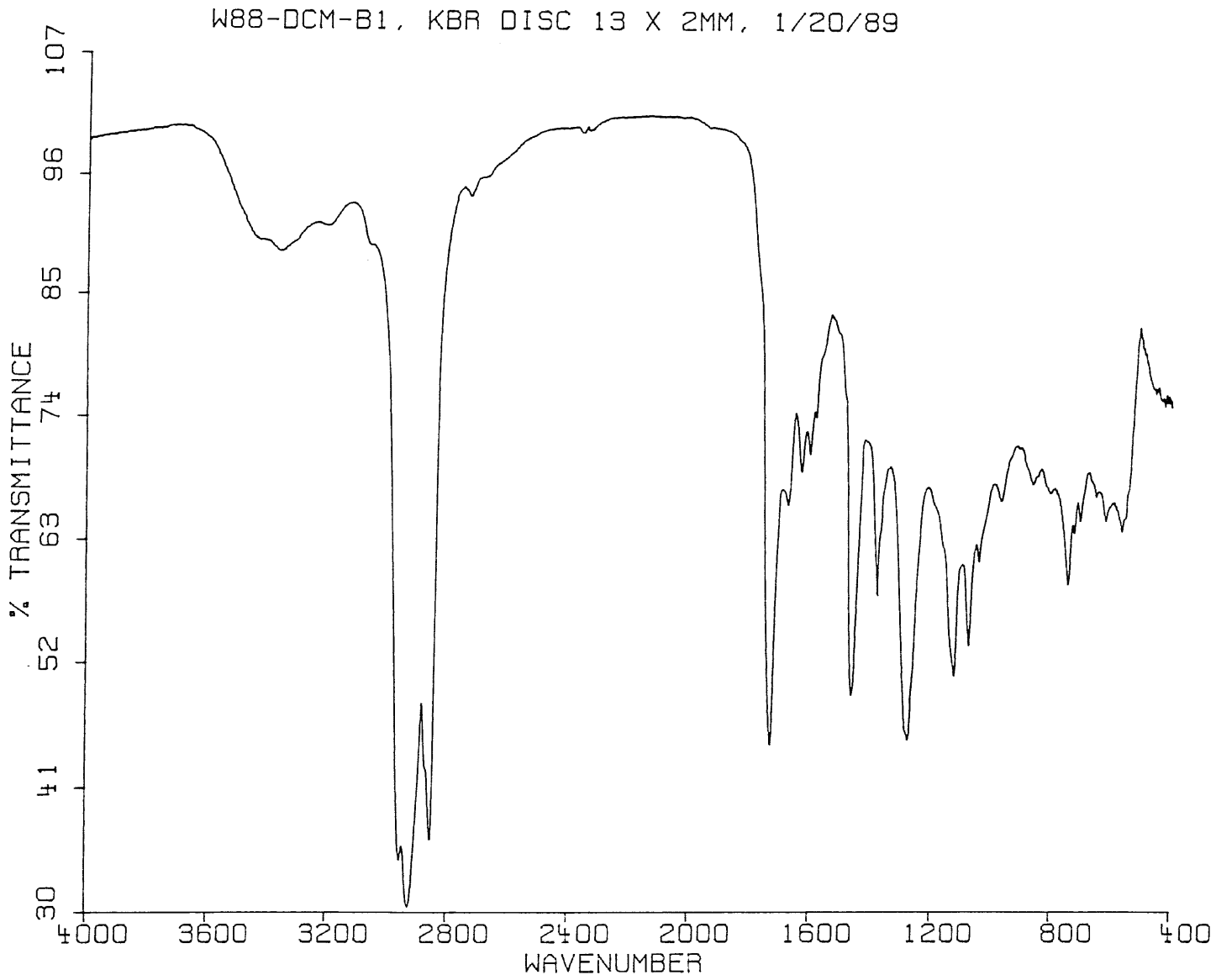

W88-DCM-B1, KBR DISC 13 X 2MM, 1/20/89
% TRANSMITTANCE
107
96
85
74
63
52
41
30
4000
3600
3200
2800
2400
2000
1600
1200
800
400
WAVENUMBER

Figure 4-2

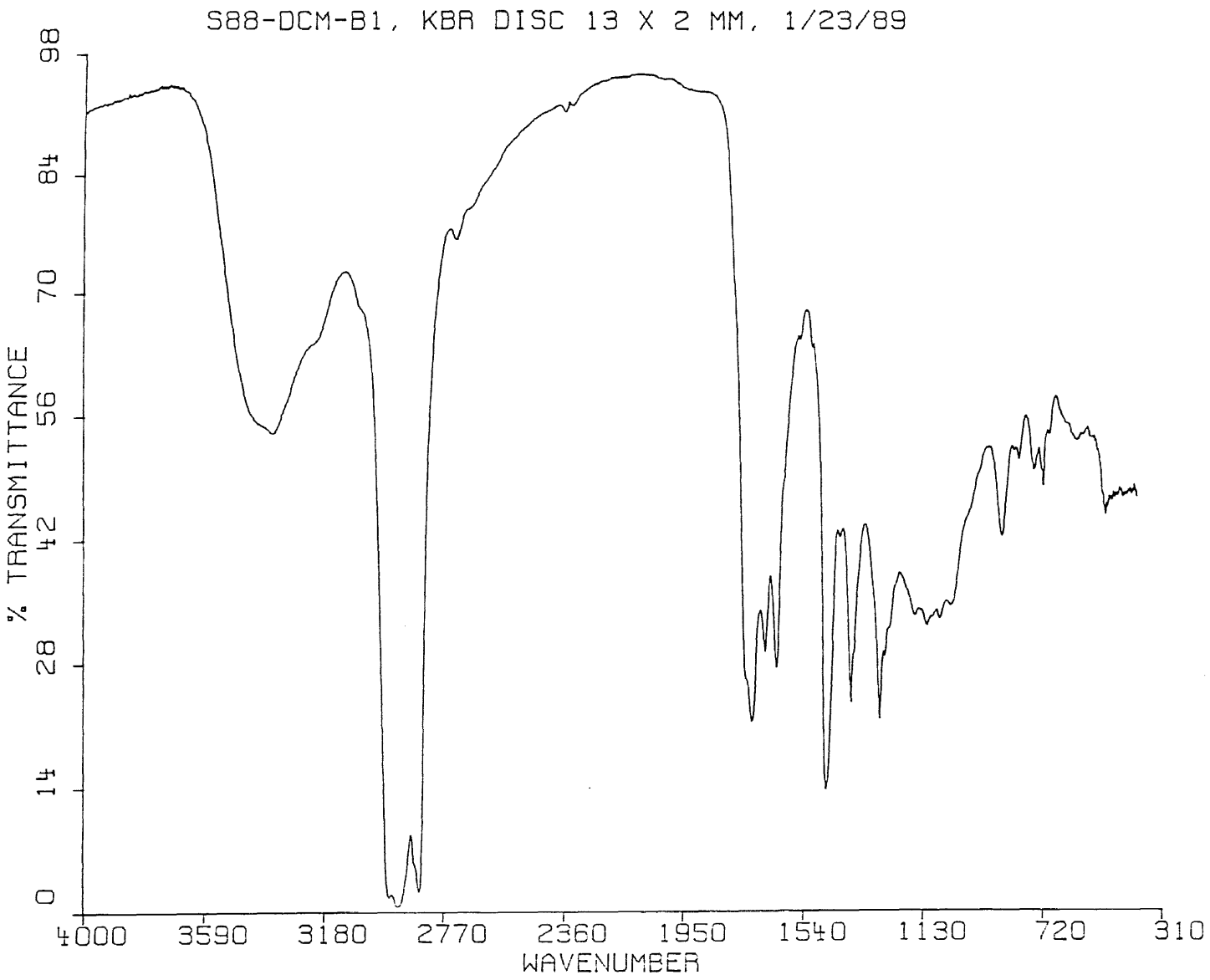

S88-DCM-B1, KBR DISC 13 X 2 MM, 1/23/89
% TRANSMITTANCE
0
14
28
42
56
70
84
98
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

Figure 4-3

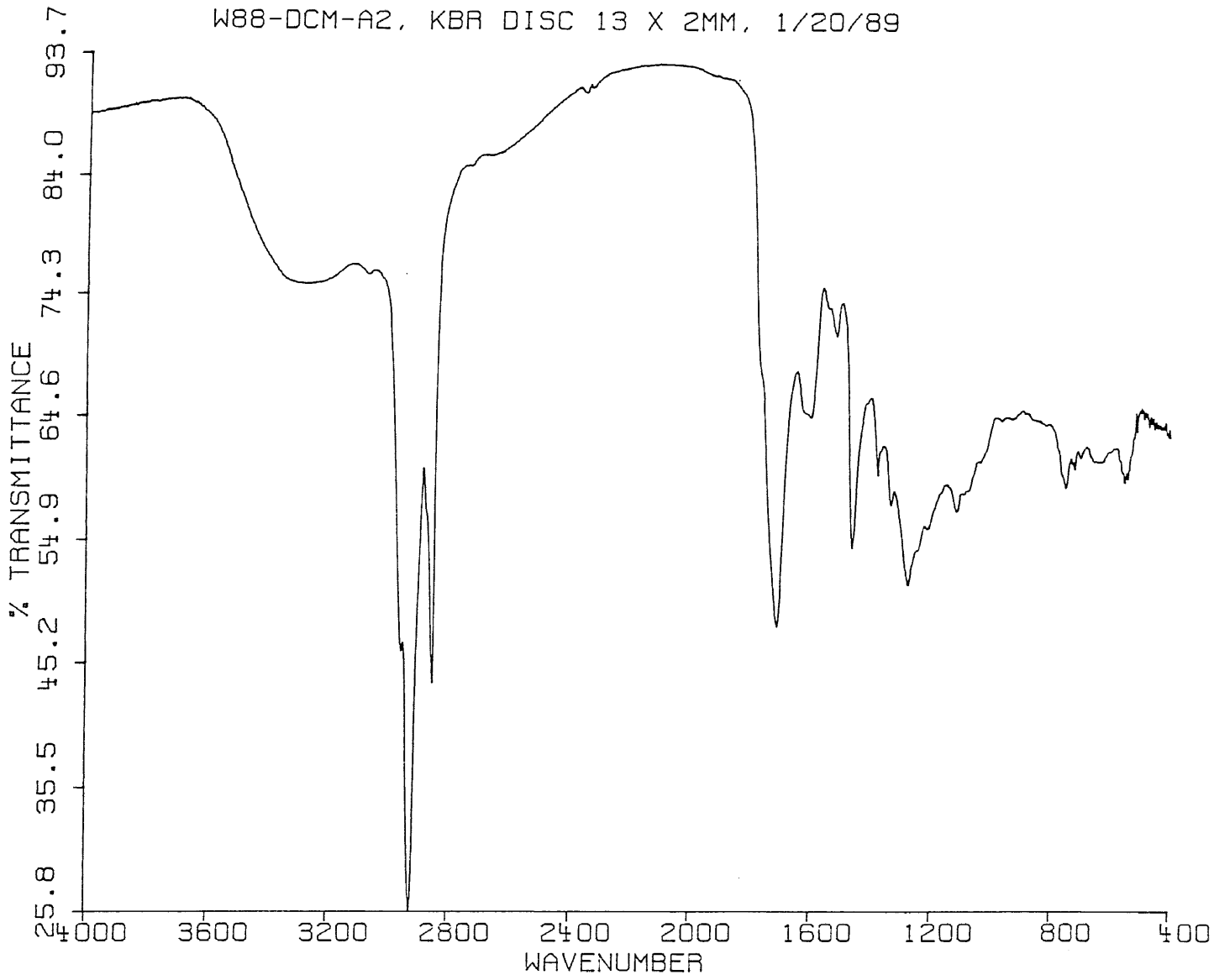

W88-DCM-A2, KBR DISC 13 X 2MM, 1/20/89
% TRANSMITTANCE
93.7
84.0
74.3
64.6
54.9
45.2
35.5
25.8
4000
3600
3200
2800
2400
2000
1600
1200
800
400
WAVENUMBER

Figure 4-4

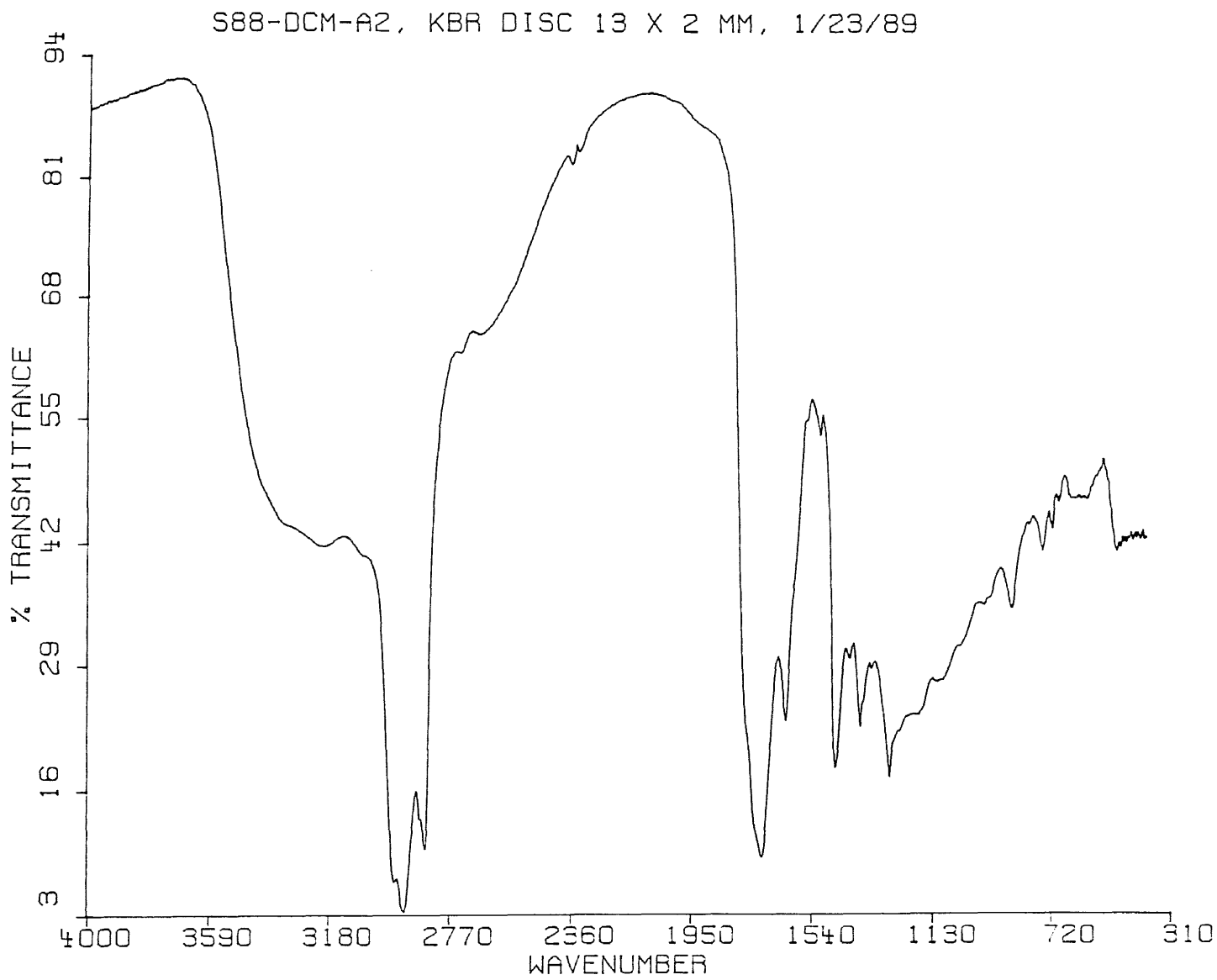

S88-DCM-A2, KBR DISC 13 X 2 MM, 1/23/89
% TRANSMITTANCE
94
81
68
55
42
29
16
3
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

Figure 4-5

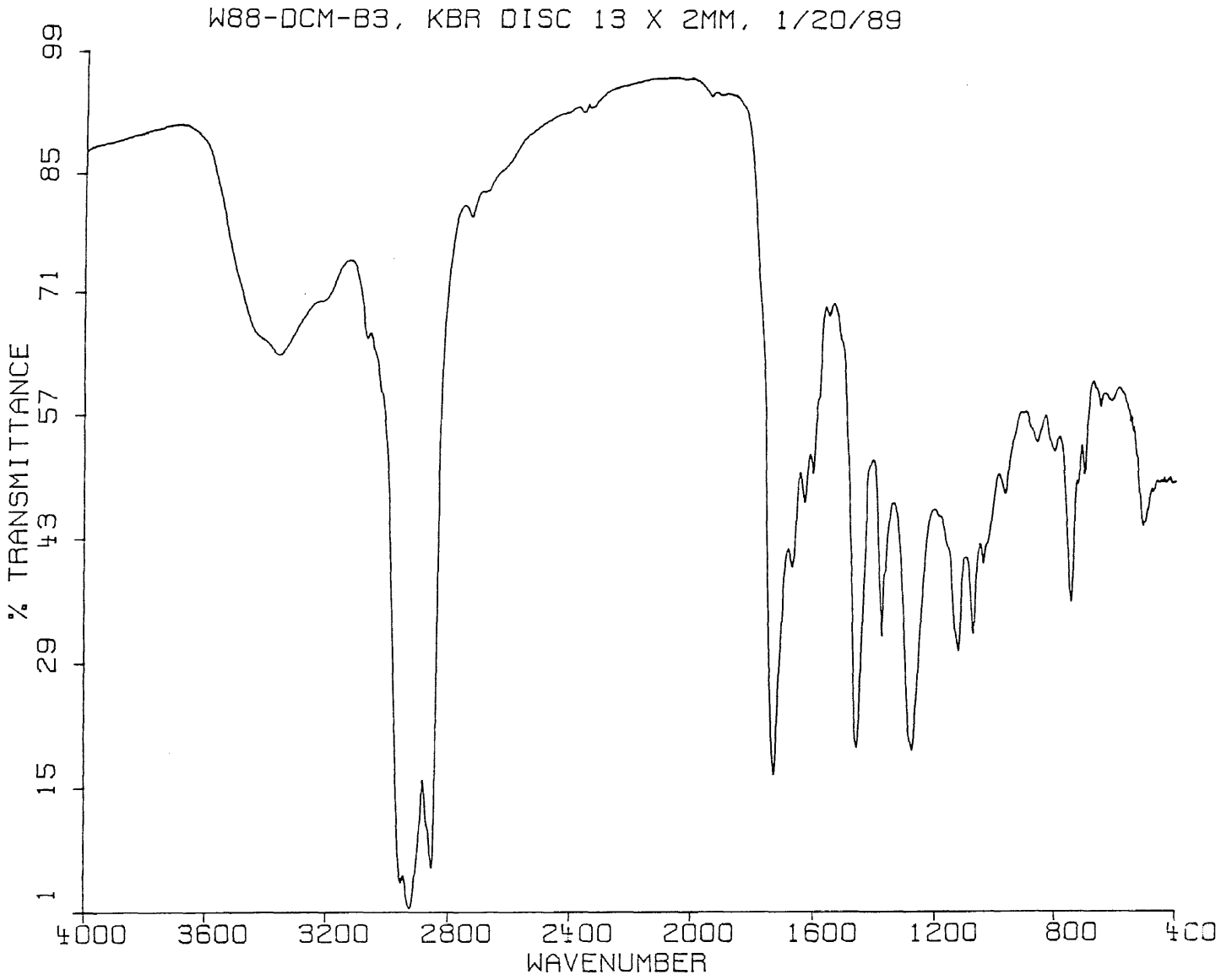

W88-DCM-B3, KBR DISC 13 X 2MM, 1/20/89
% TRANSMITTANCE
99
85
71
57
43
29
15
1
4000
3600
3200
2800
2400
2000
1600
1200
800
400
WAVENUMBER

Figure 4-6

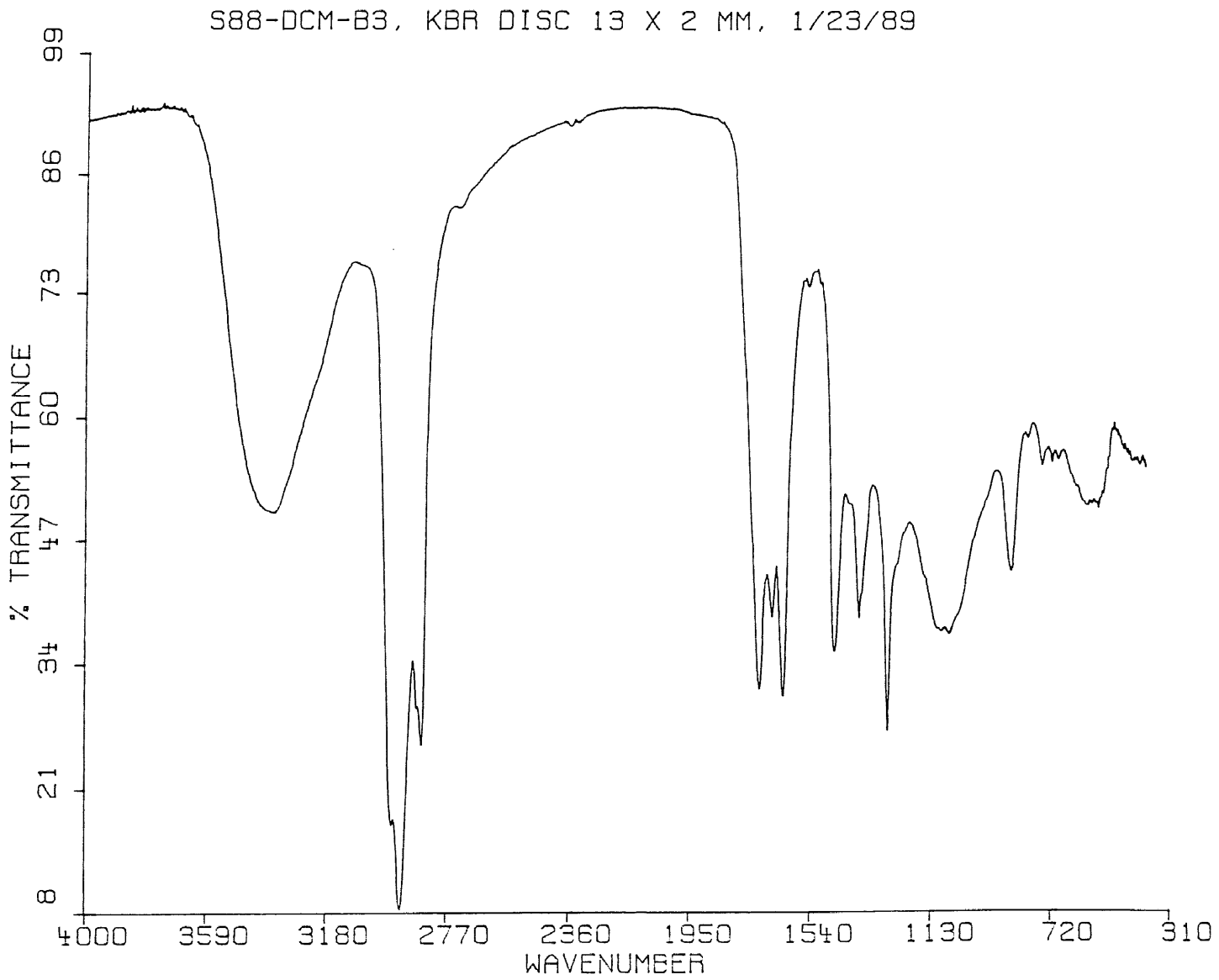

S88-DCM-B3, KBR DISC 13 X 2 MM, 1/23/89
% TRANSMITTANCE
99
86
73
60
47
34
21
8
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

Figure 4-7

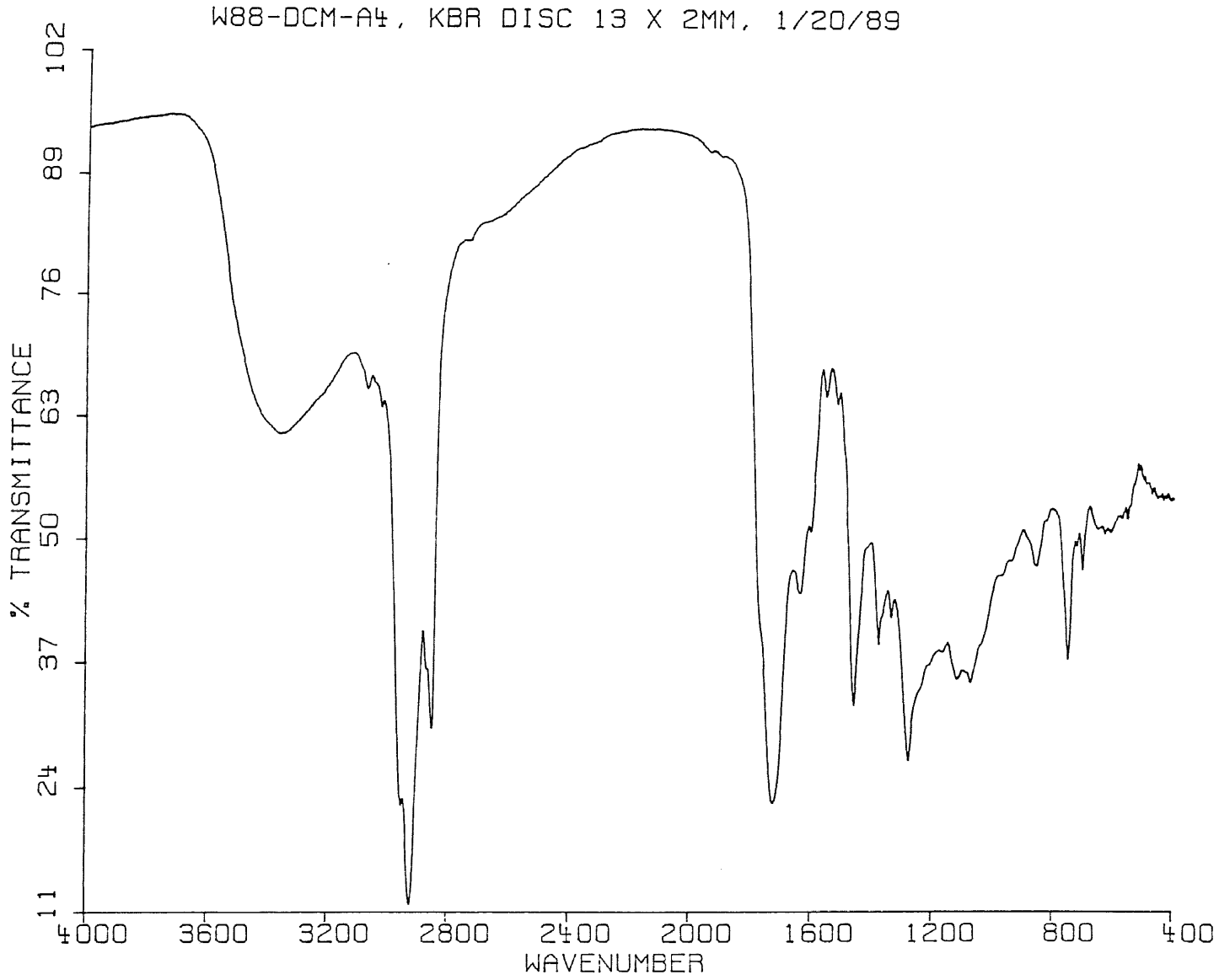

W88-DCM-A4, KBR DISC 13 X 2MM, 1/20/89
% TRANSMITTANCE
102
89
76
63
50
37
24
11
4000
3600
3200
2800
2400
2000
1600
1200
800
400
WAVENUMBER

Figure 4-8

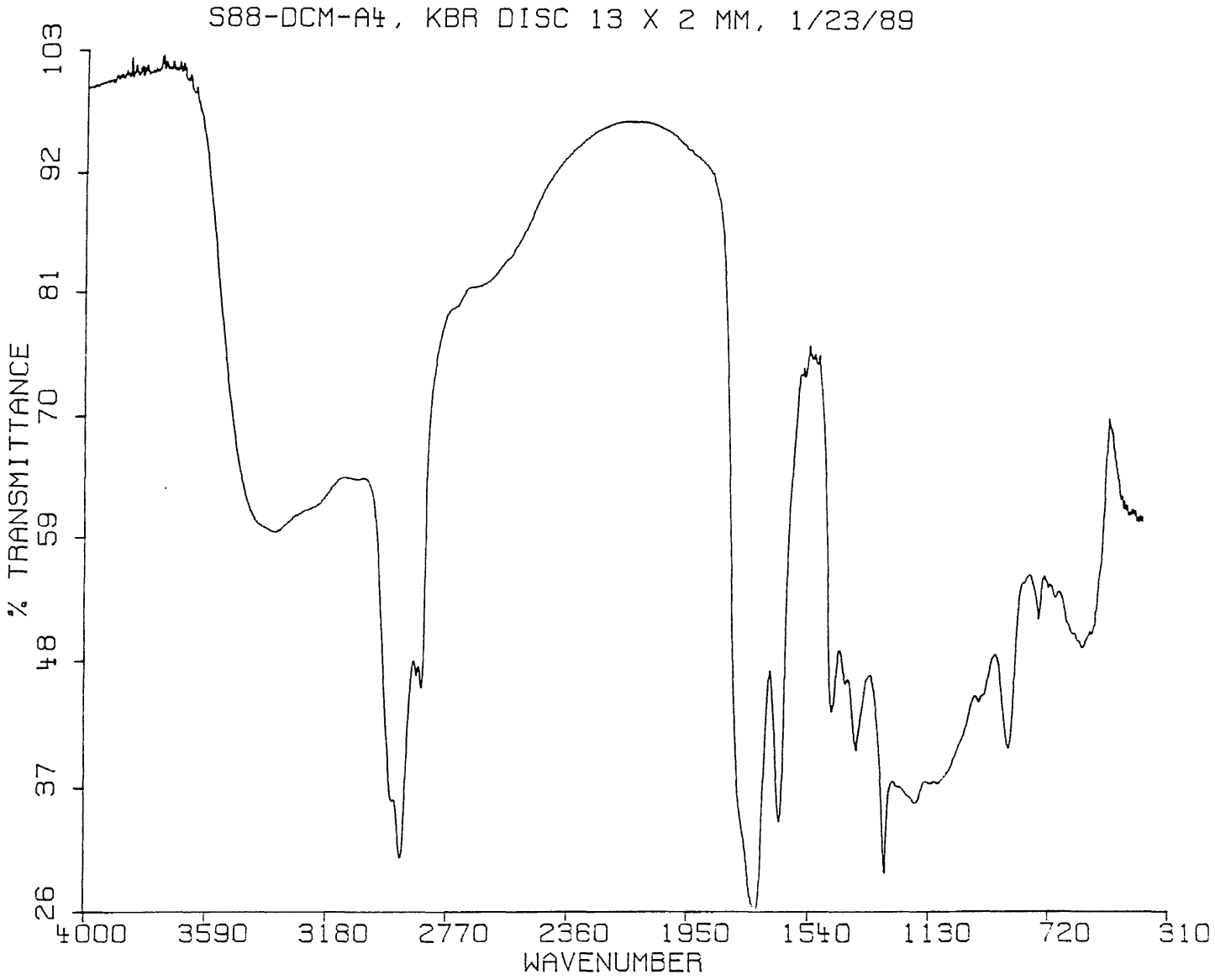

S88-DCM-A4, KBR DISC 13 X 2 MM, 1/23/89
% TRANSMITTANCE
103
92
81
70
59
48
37
26
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

Figure 4-9

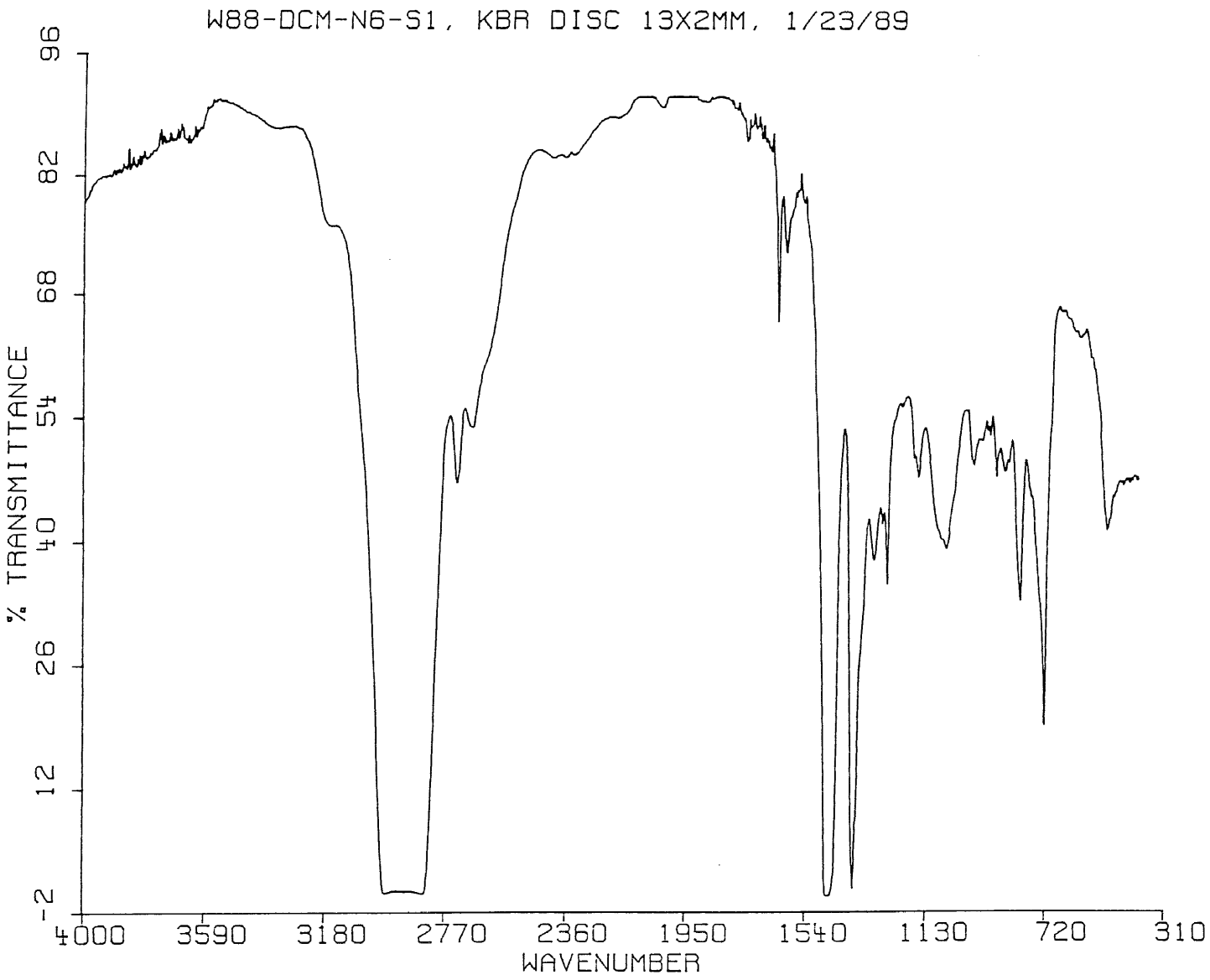

W88-DCM-N6-S1, KBR DISC 13X2MM, 1/23/89
% TRANSMITTANCE
96
82
68
54
40
26
12
-2
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

Figure 4-10

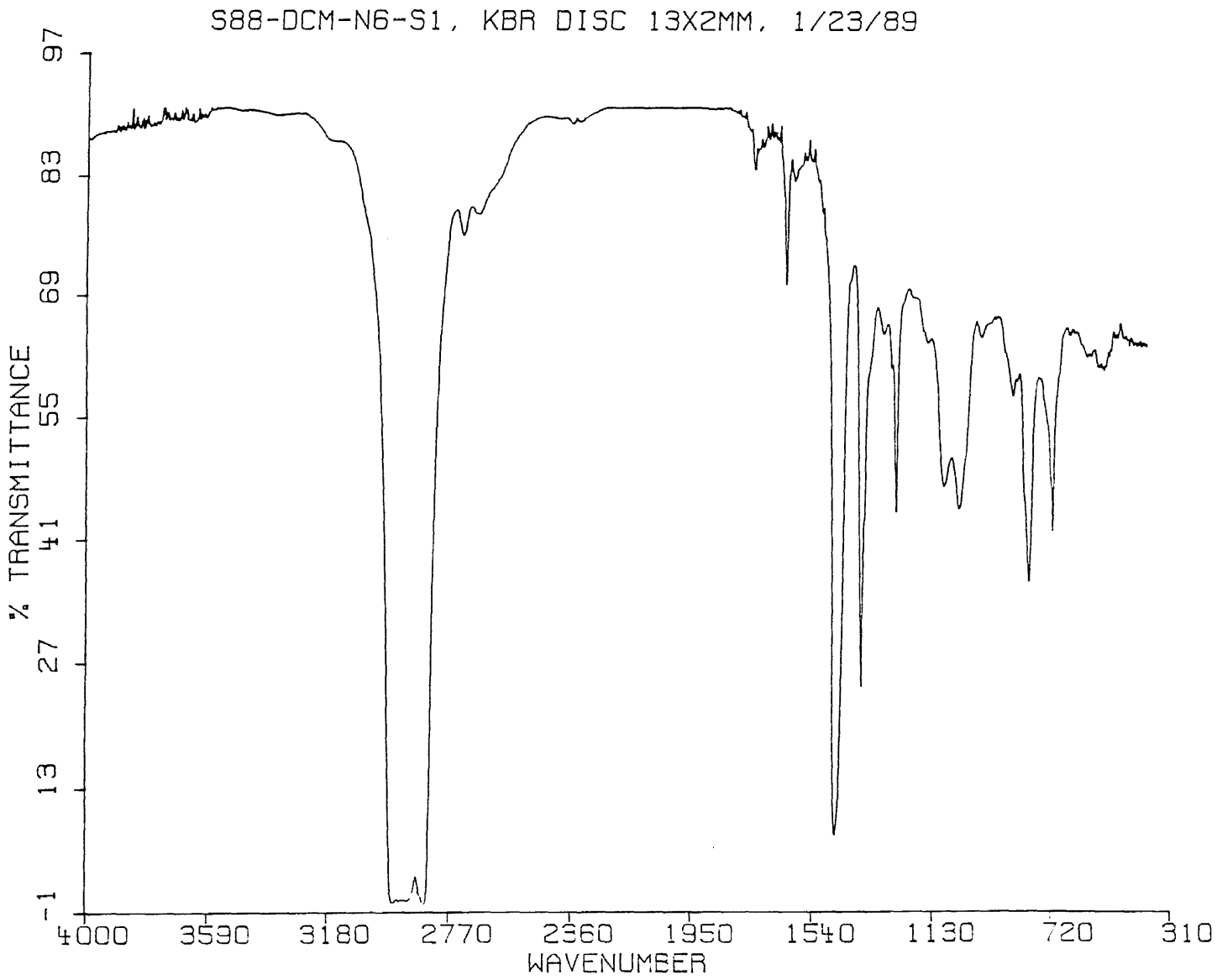

S88-DCM-N6-S1, KBR DISC 13X2MM, 1/23/89
% TRANSMITTANCE
97
83
69
55
41
27
13
-1
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

Figure 4-11

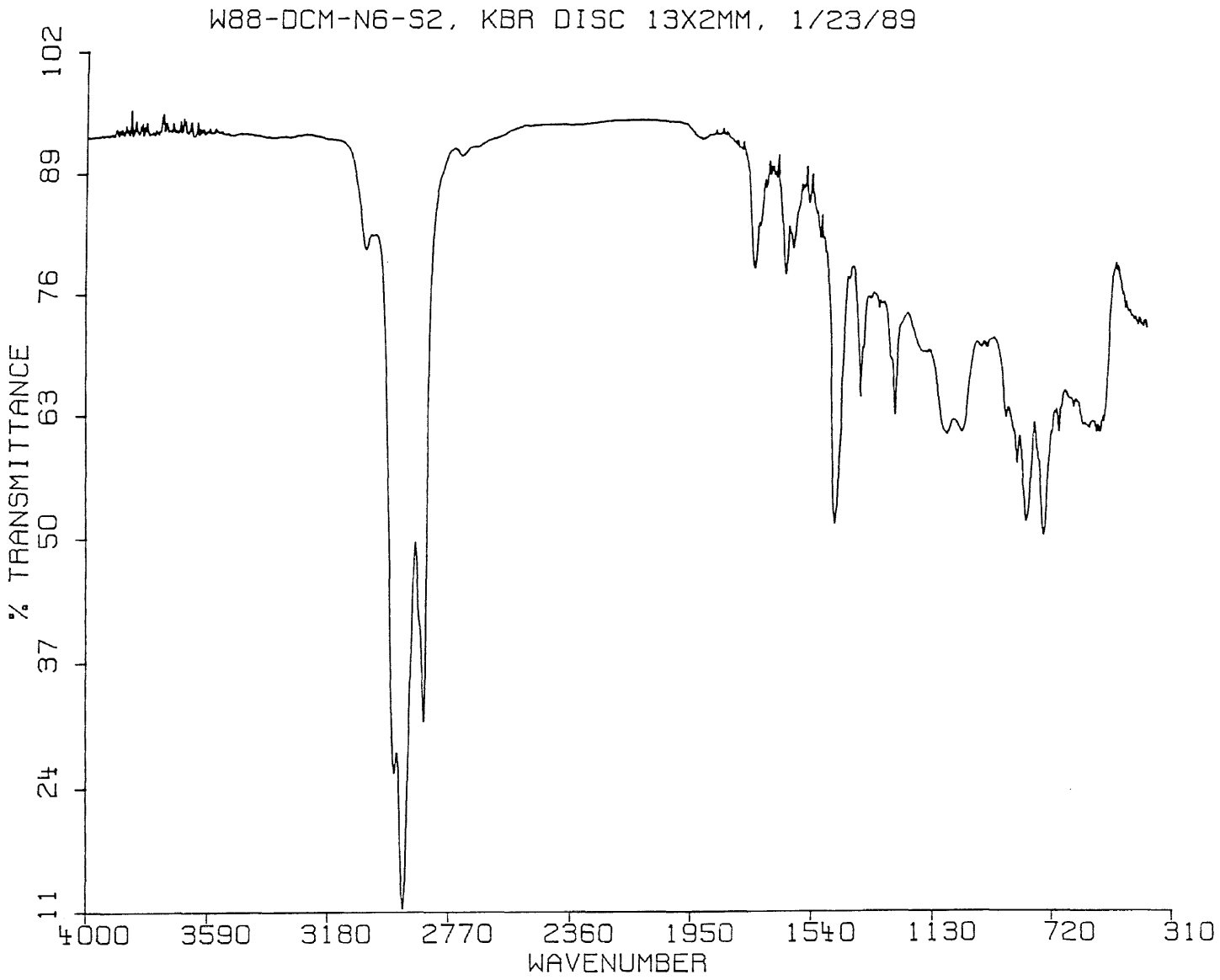

W88-DCM-N6-S2, KBR DISC 13X2MM, 1/23/89
% TRANSMITTANCE
102
89
76
63
50
37
24
11
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

Figure 4-12

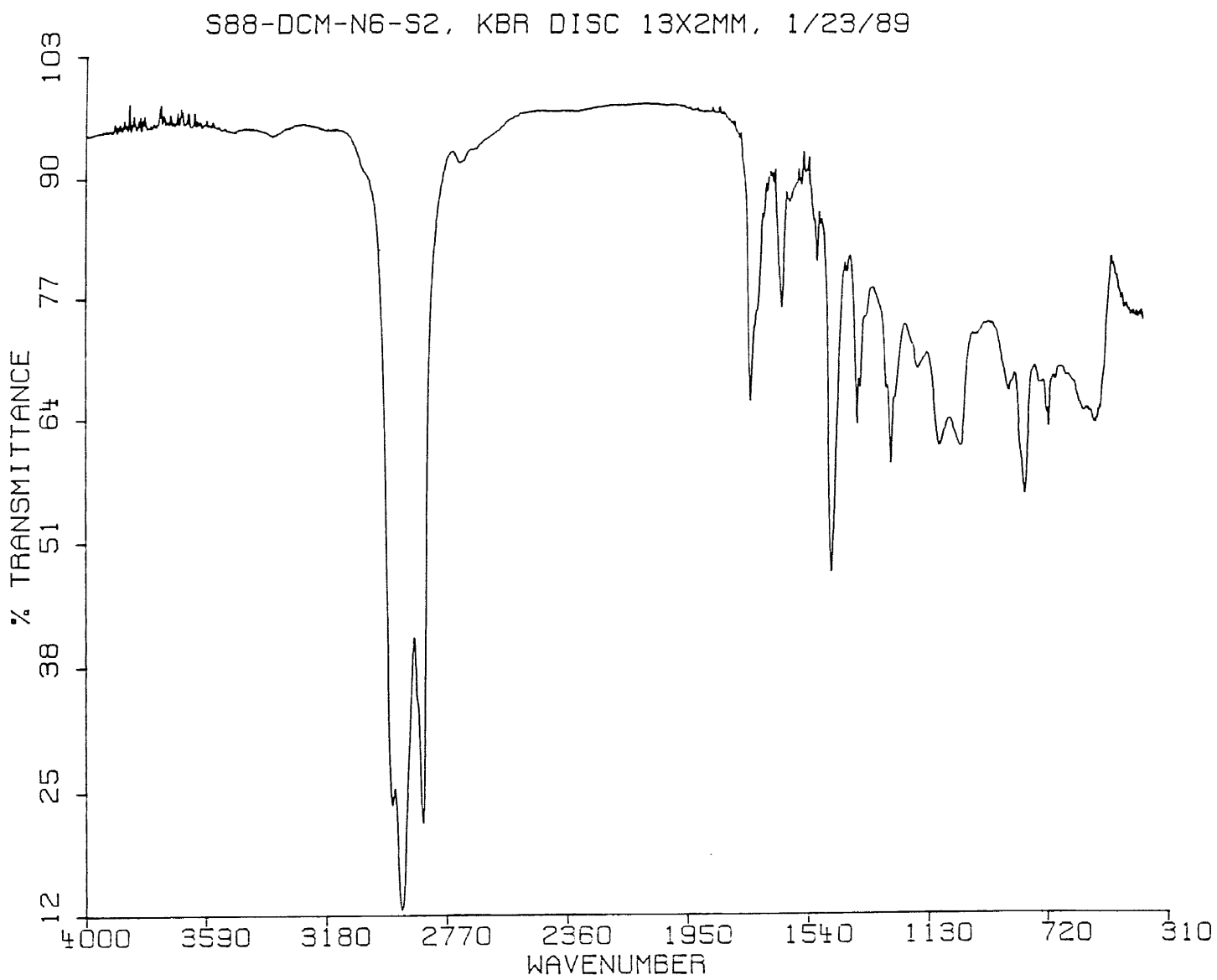

S88-DCM-N6-S2, KBR DISC 13X2MM, 1/23/89
% TRANSMITTANCE
103
90
77
64
51
38
25
12
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

Figure 4-13

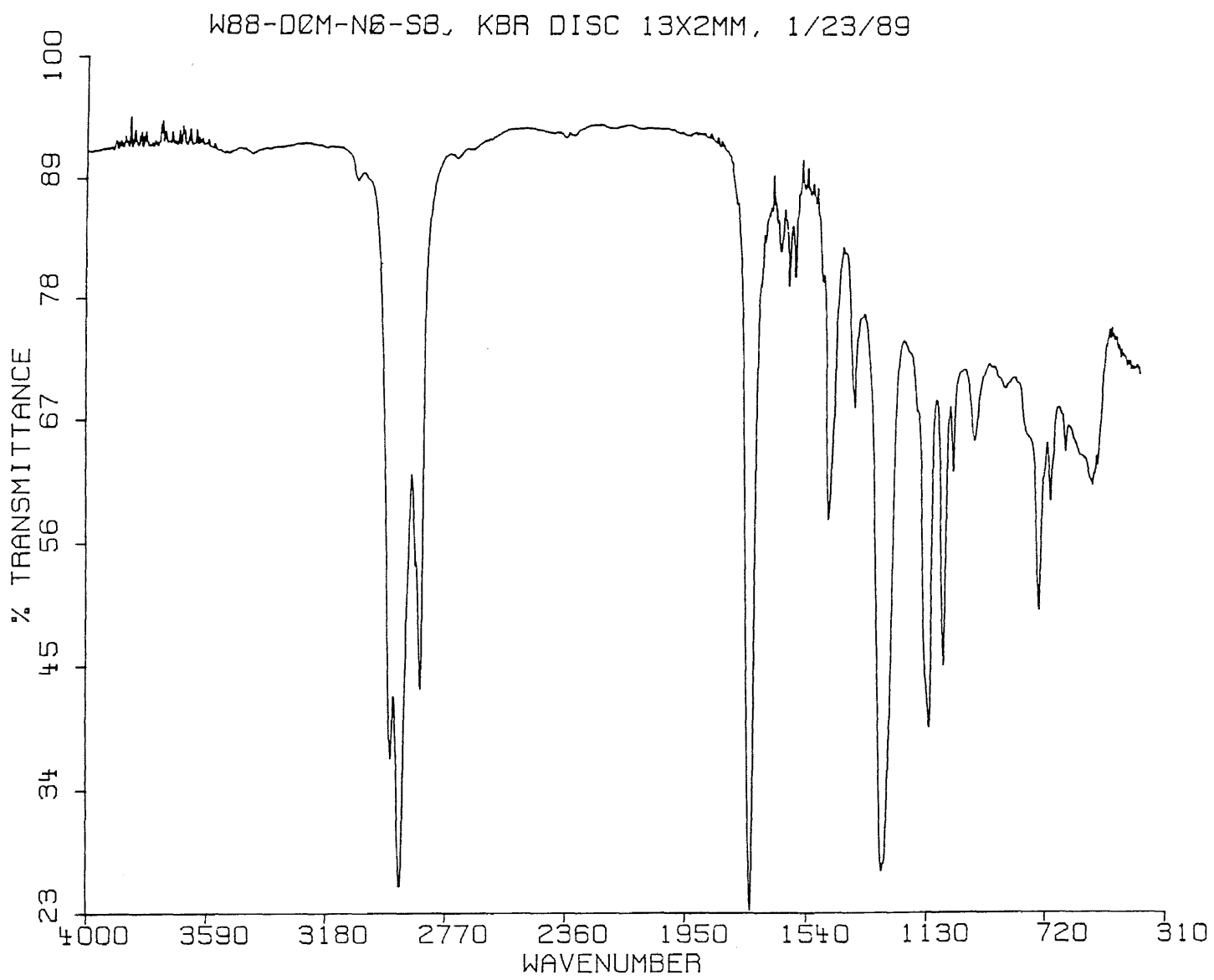

W88-DØM-NØ-S8, KBR DISC 13X2MM, 1/23/89
% TRANSMITTANCE
100
89
78
67
56
45
34
23
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

Figure 4-14

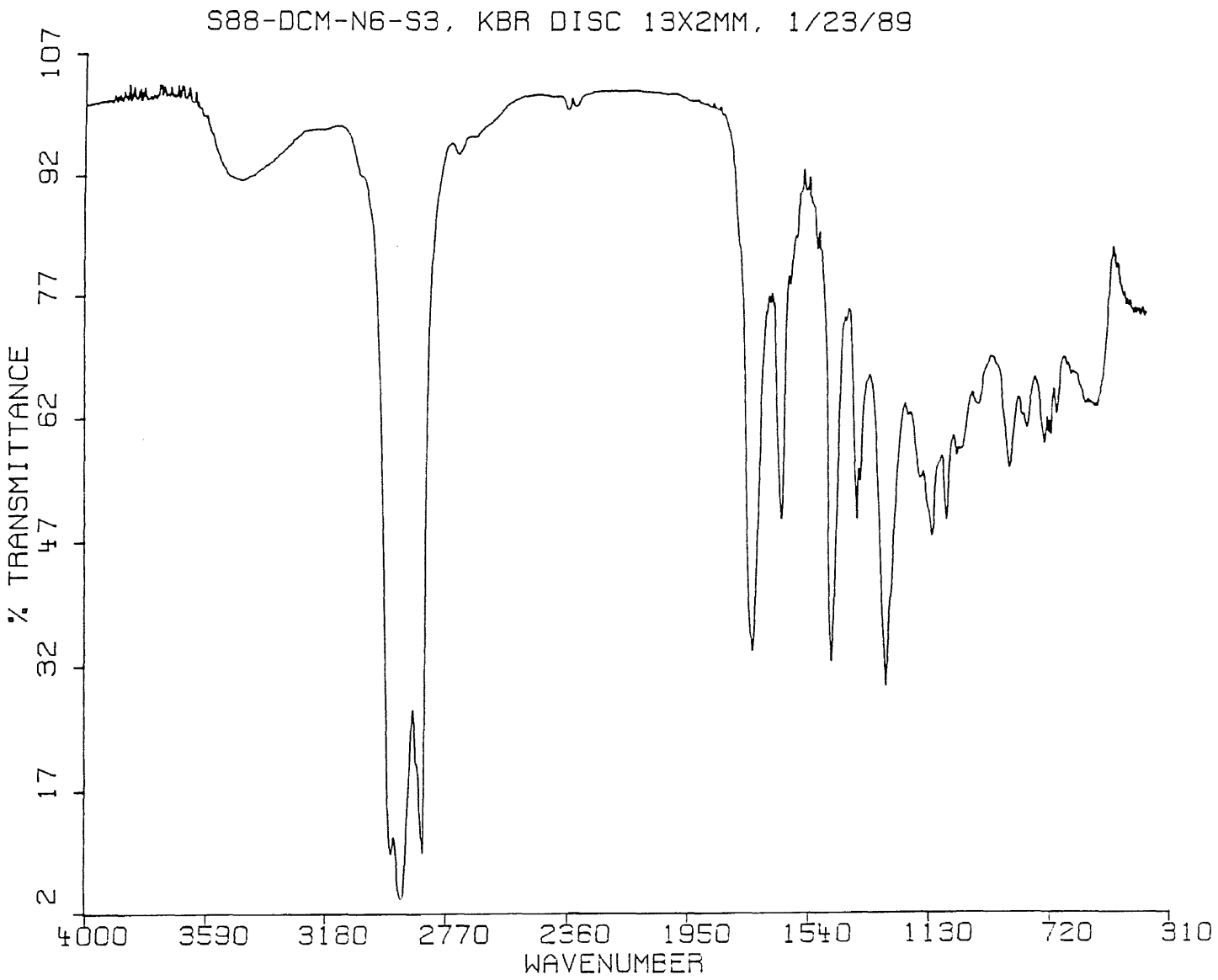

S88-DCM-N6-S3, KBR DISC 13X2MM, 1/23/89
% TRANSMITTANCE
107
92
77
62
47
32
17
2
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

Figure 4-15

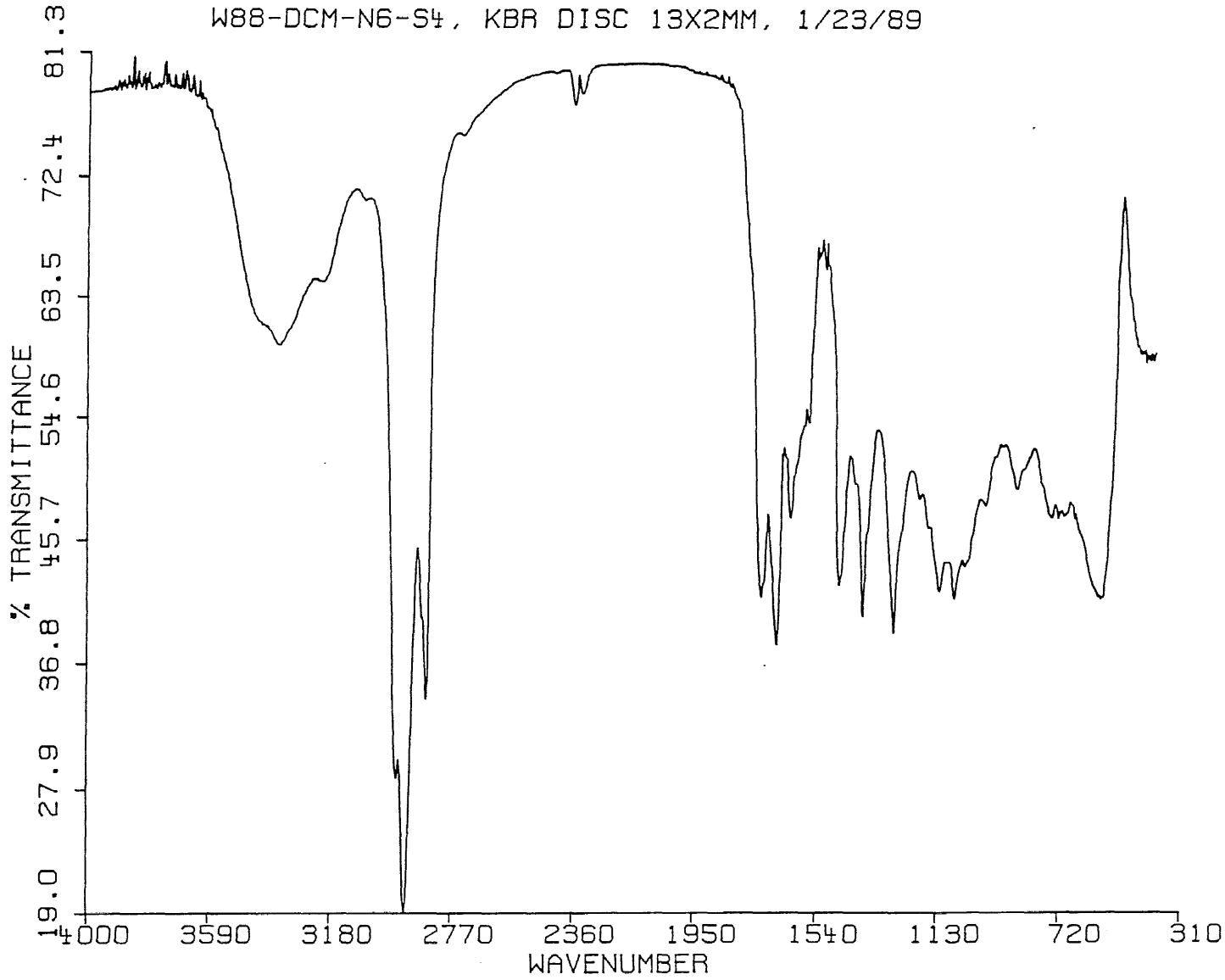

W88-DCM-N6-S4, KBR DISC 13X2MM, 1/23/89
% TRANSMITTANCE
81.3
72.4
63.5
54.6
45.7
36.8
27.9
19.0
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

Figure 4-16

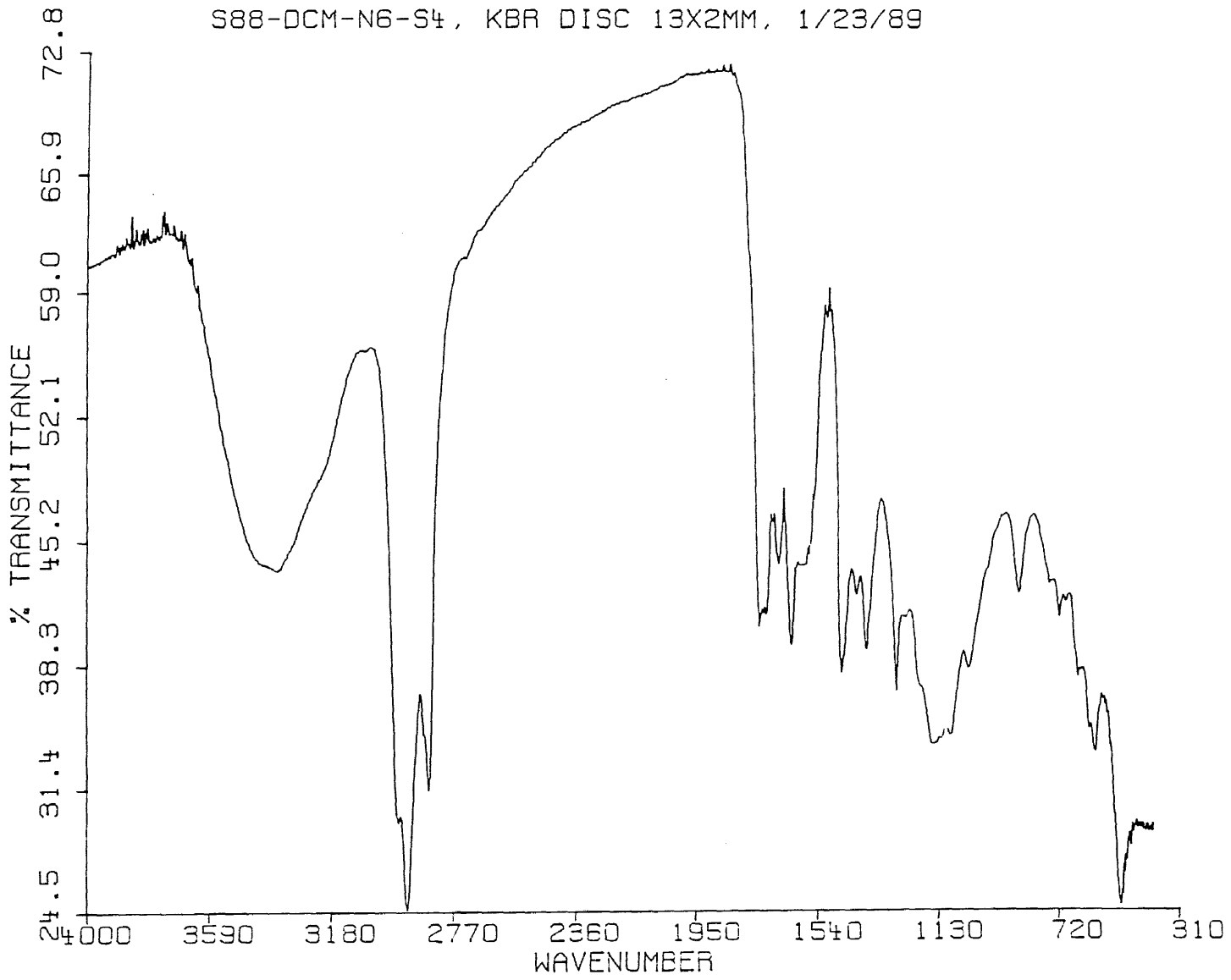

S88-DCM-N6-S4, KBR DISC 13X2MM, 1/23/89
% TRANSMITTANCE
72.8
65.9
59.0
52.1
45.2
38.3
31.4
24.5
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

Figure 4-17

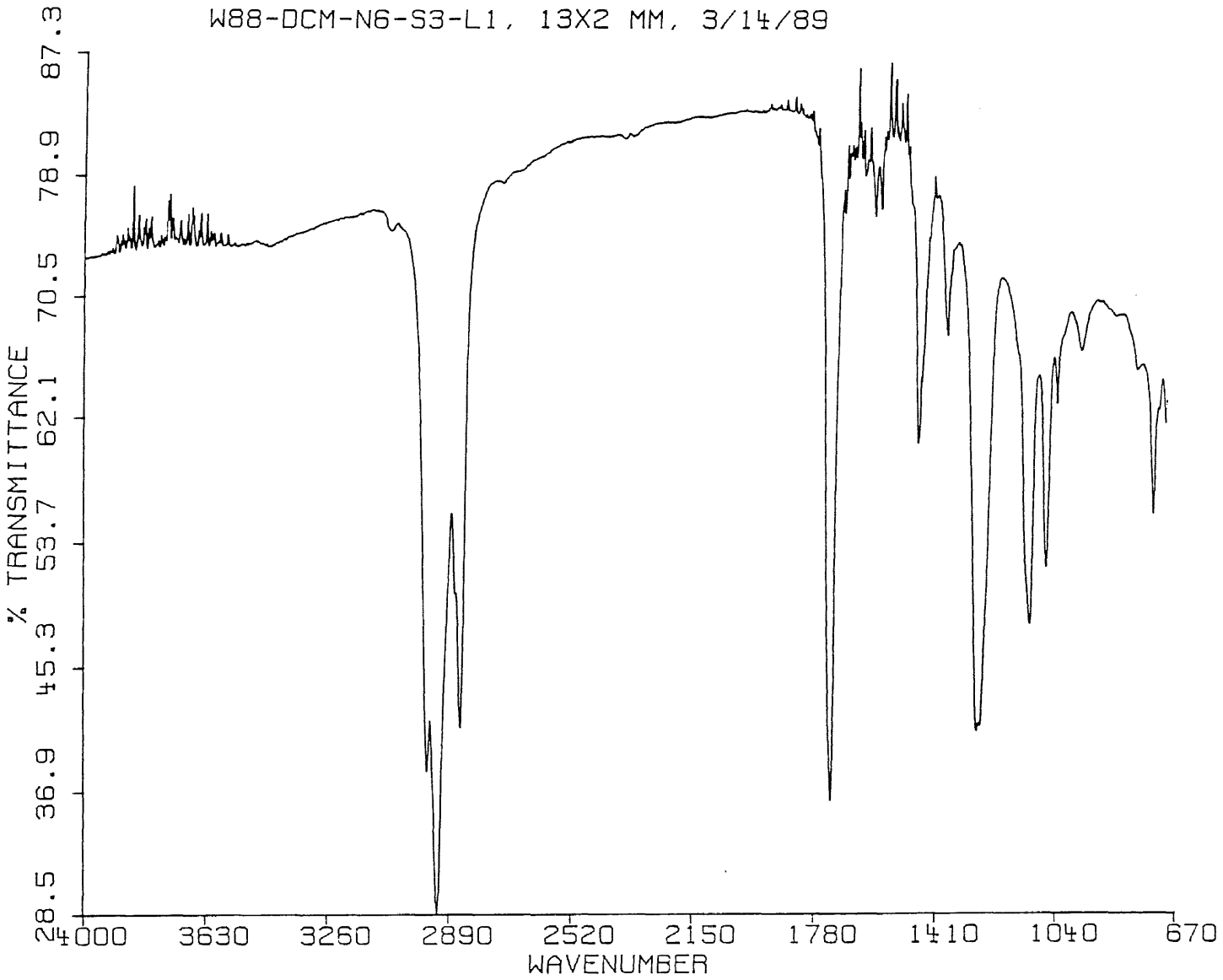

W88-DCM-N6-S3-L1, 13X2 MM, 3/14/89
% TRANSMITTANCE
87.3
78.9
70.5
62.1
53.7
45.3
36.9
28.5
4000
3630
3260
2890
2520
2150
1780
1410
1040
670
WAVENUMBER

Figure 4-18

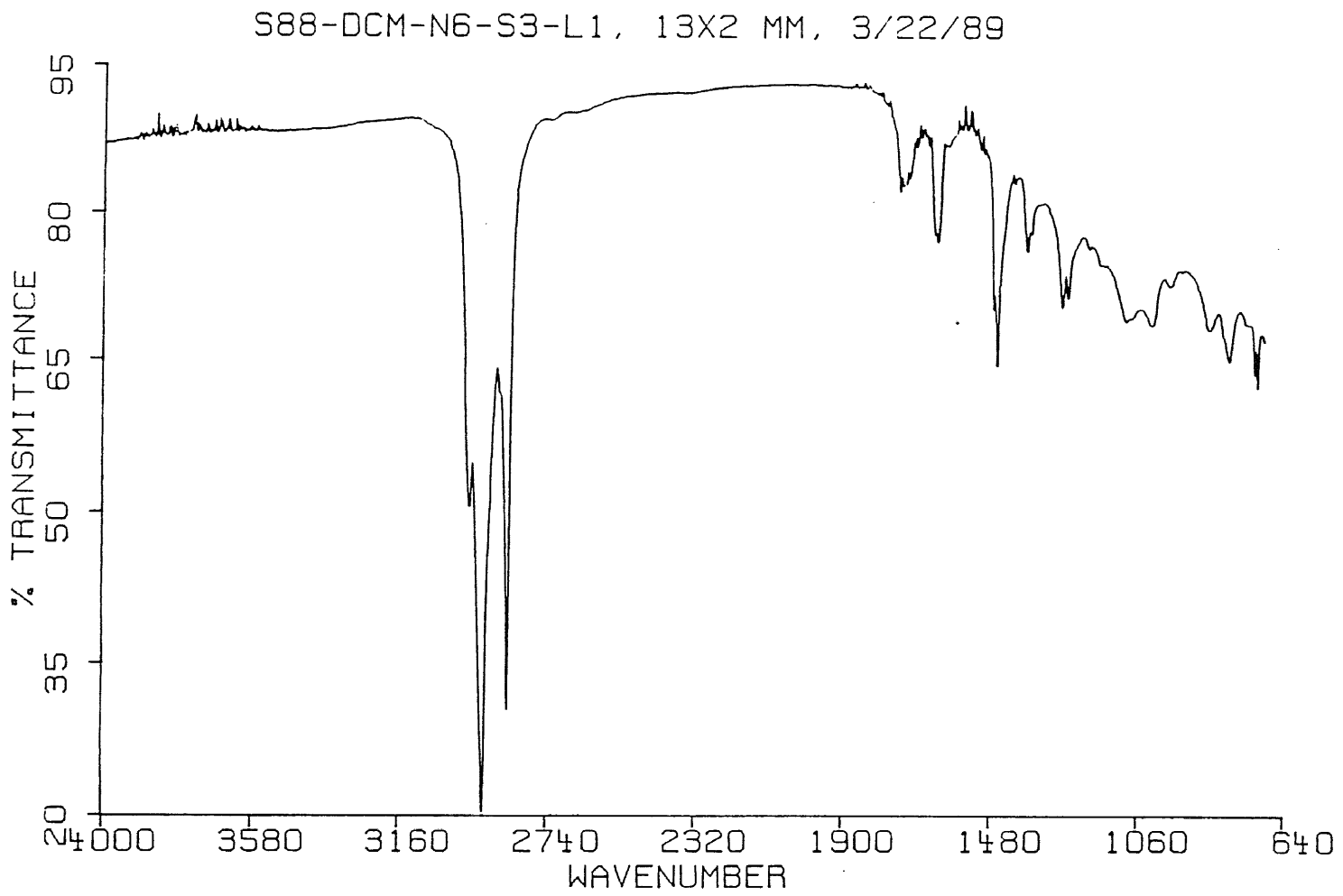

S88-DCM-N6-S3-L1, 13X2 MM, 3/22/89
% TRANSMITTANCE
95
80
65
50
35
20
4000
3580
3160
2740
2320
1900
1480
1060
640
WAVENUMBER

Figure 4-19

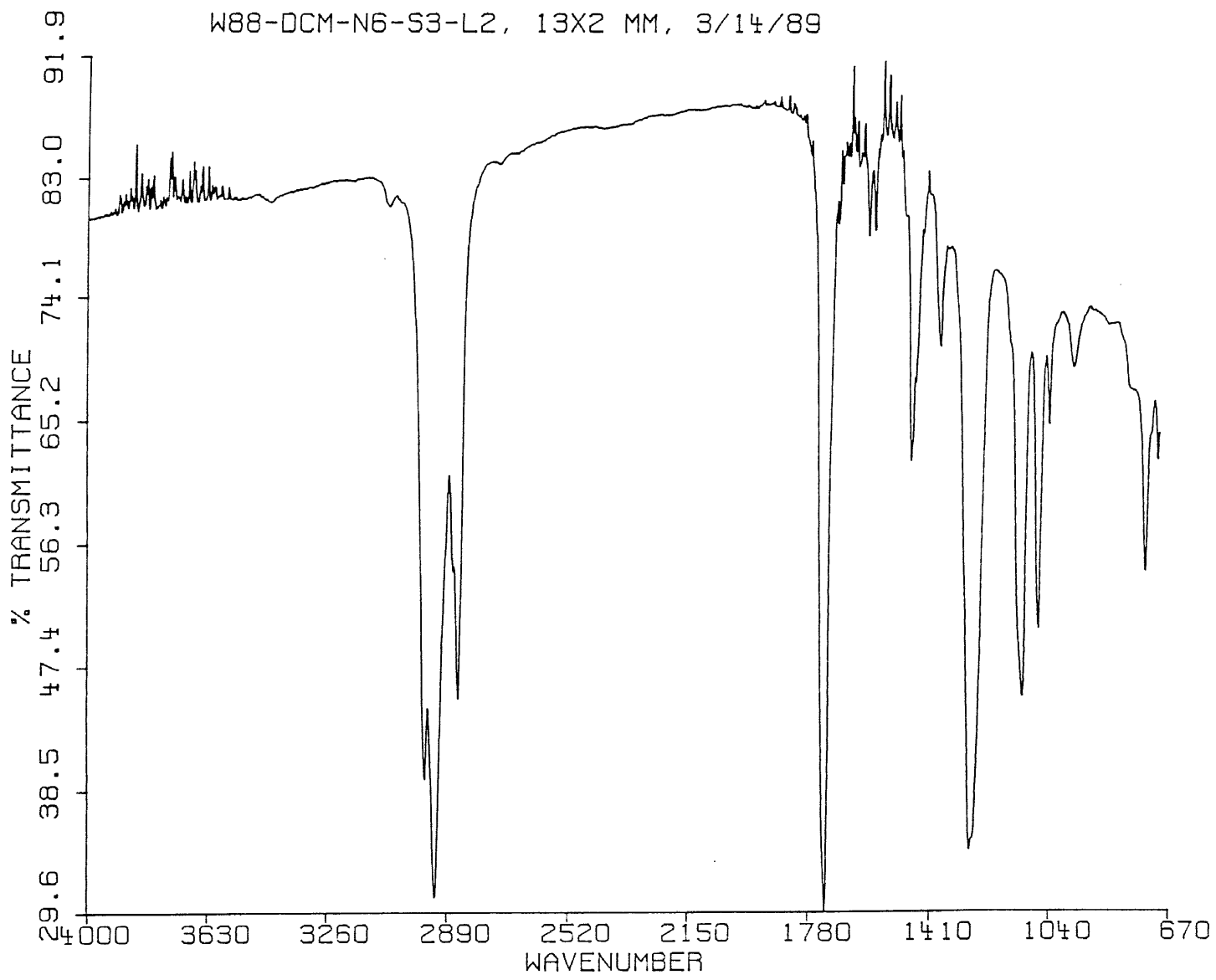

W88-DCM-N6-S3-L2, 13X2 MM, 3/14/89
% TRANSMITTANCE
91.9
83.0
74.1
65.2
56.3
47.4
38.5
29.6
4000
3630
3260
2890
2520
2150
1780
1410
1040
670
WAVENUMBER

Figure 4-20

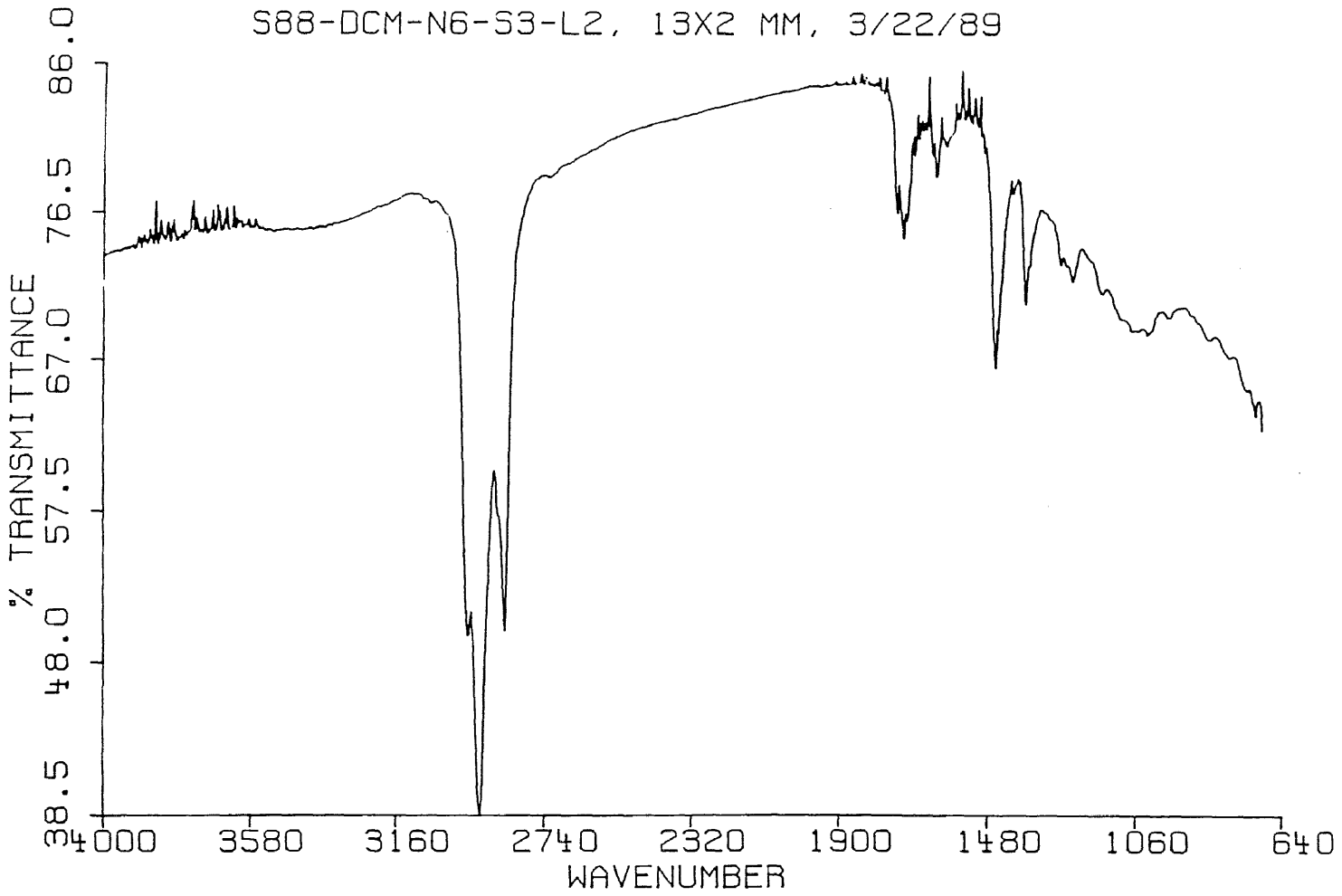

S88-DCM-N6-S3-L2, 13X2 MM, 3/22/89
% TRANSMITTANCE
86.0
76.5
67.0
57.5
48.0
38.5
4000
3580
3160
2740
2320
1900
1480
1060
640
WAVENUMBER

Figure 4-21

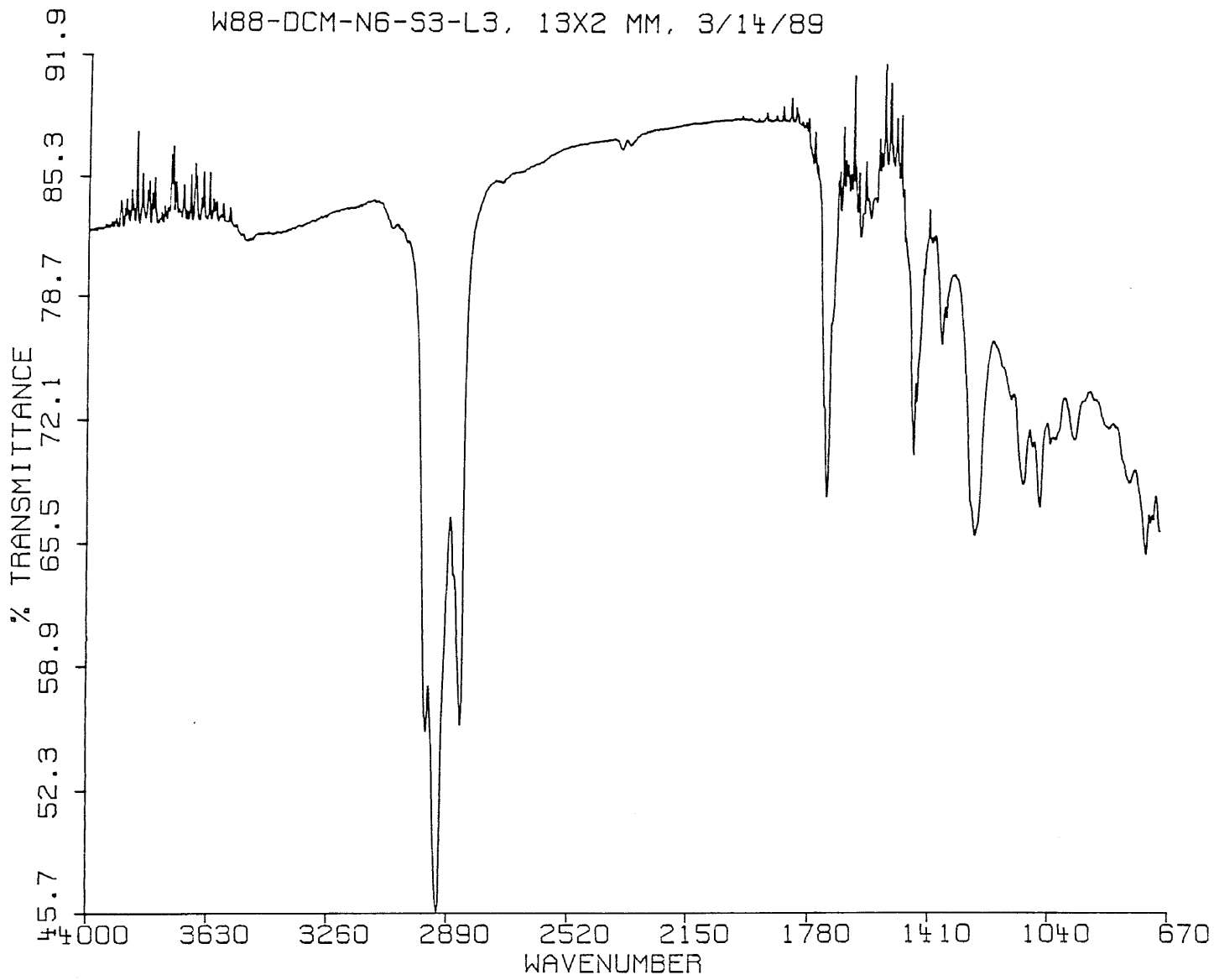

W88-DCM-N6-S3-L3, 13X2 MM, 3/14/89
% TRANSMITTANCE
91.9
85.3
78.7
72.1
65.5
58.9
52.3
45.7
4000
3630
3260
2890
2520
2150
1780
1410
1040
670
WAVENUMBER

Figure 4-22

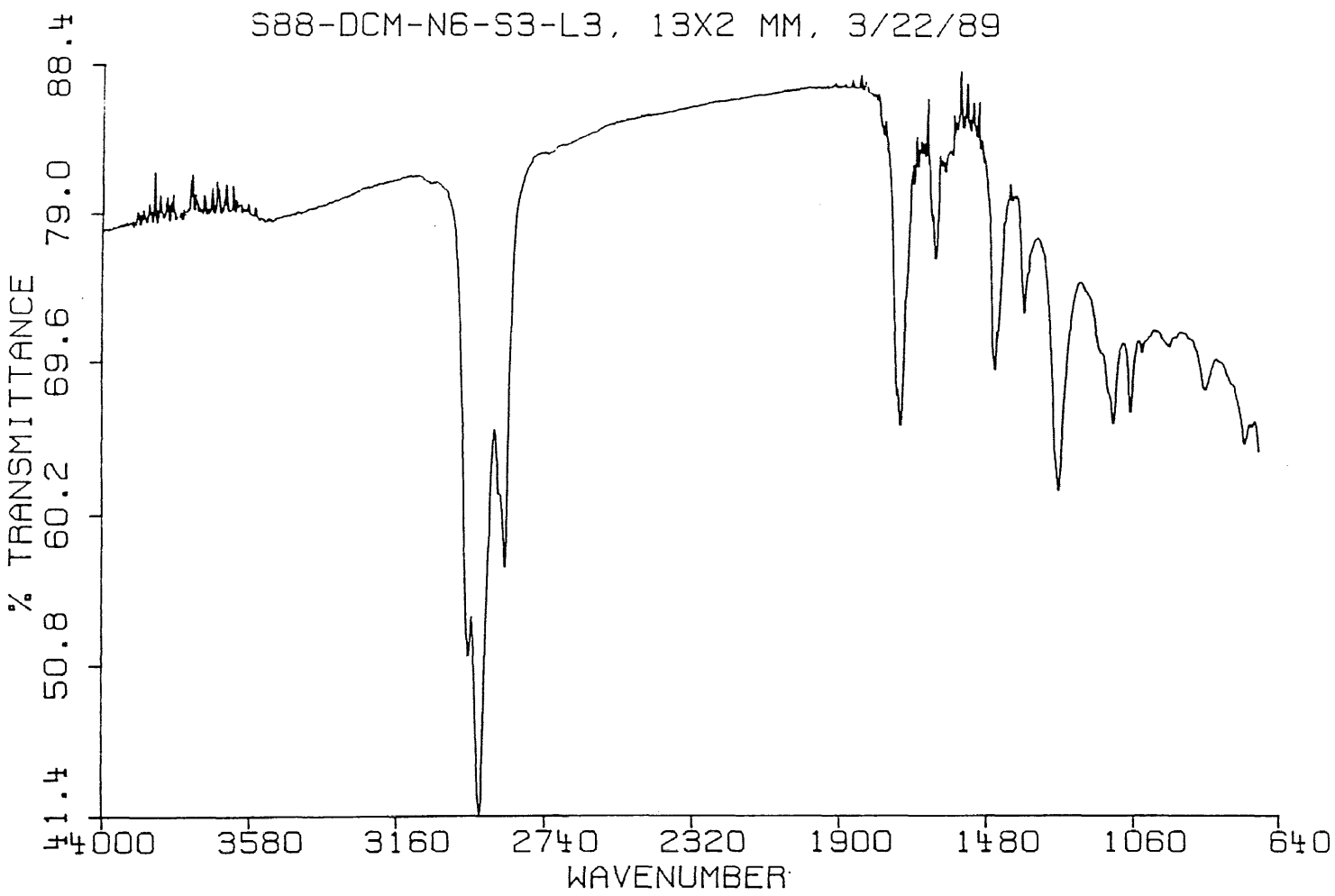
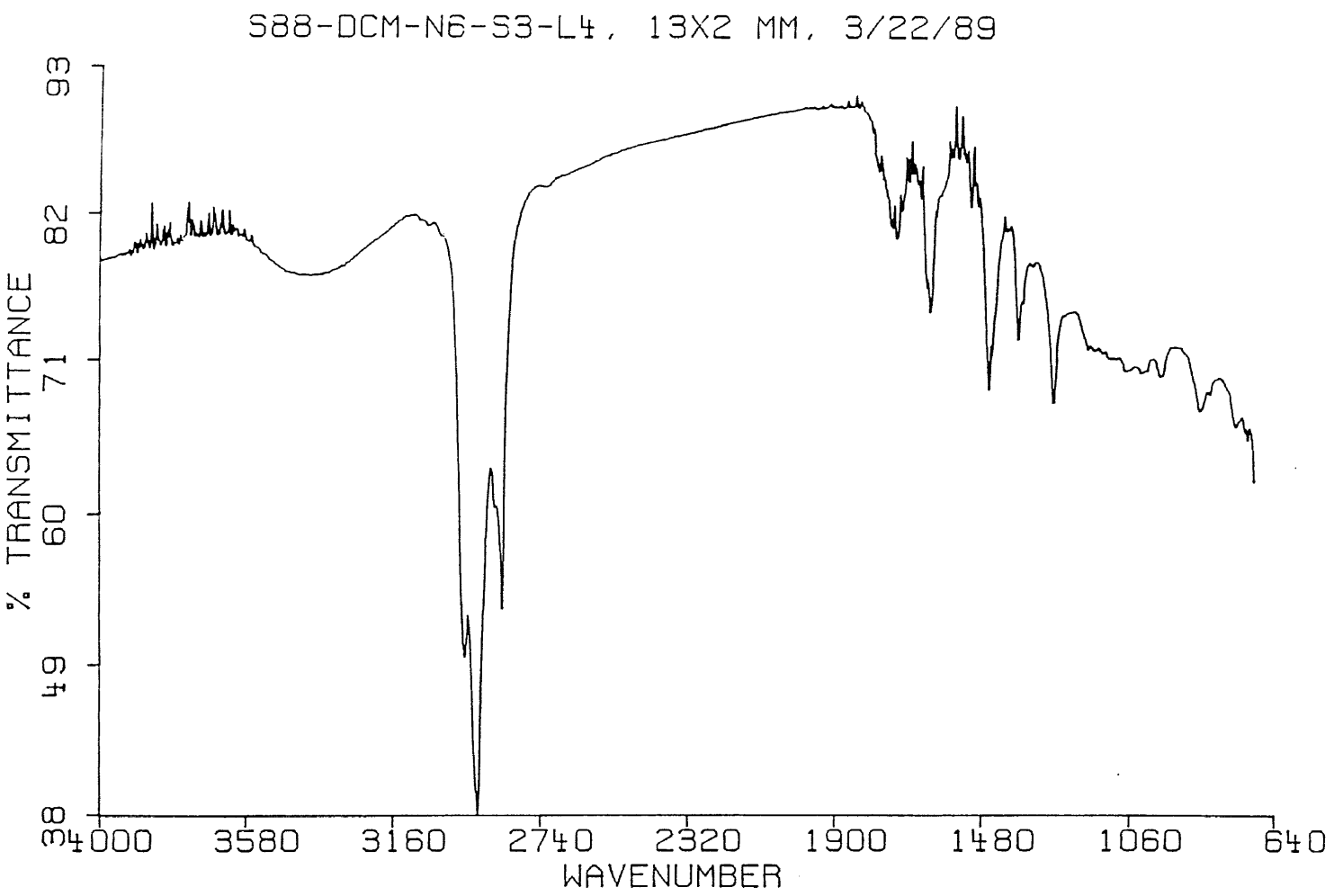

S88-DCM-N6-S3-L3, 13X2 MM, 3/22/89
% TRANSMITTANCE
88.4
79.0
69.6
60.2
50.8
41.4
4000
3580
3160
2740
2320
1900
1480
1060
640
WAVENUMBER

Figure 4-23


S88-DCM-N6-S3-L4, 13X2 MM, 3/22/89
% TRANSMITTANCE
93
82
71
60
49
38
4000
3580
3160
2740
2320
1900
1480
1060
640
WAVENUMBER

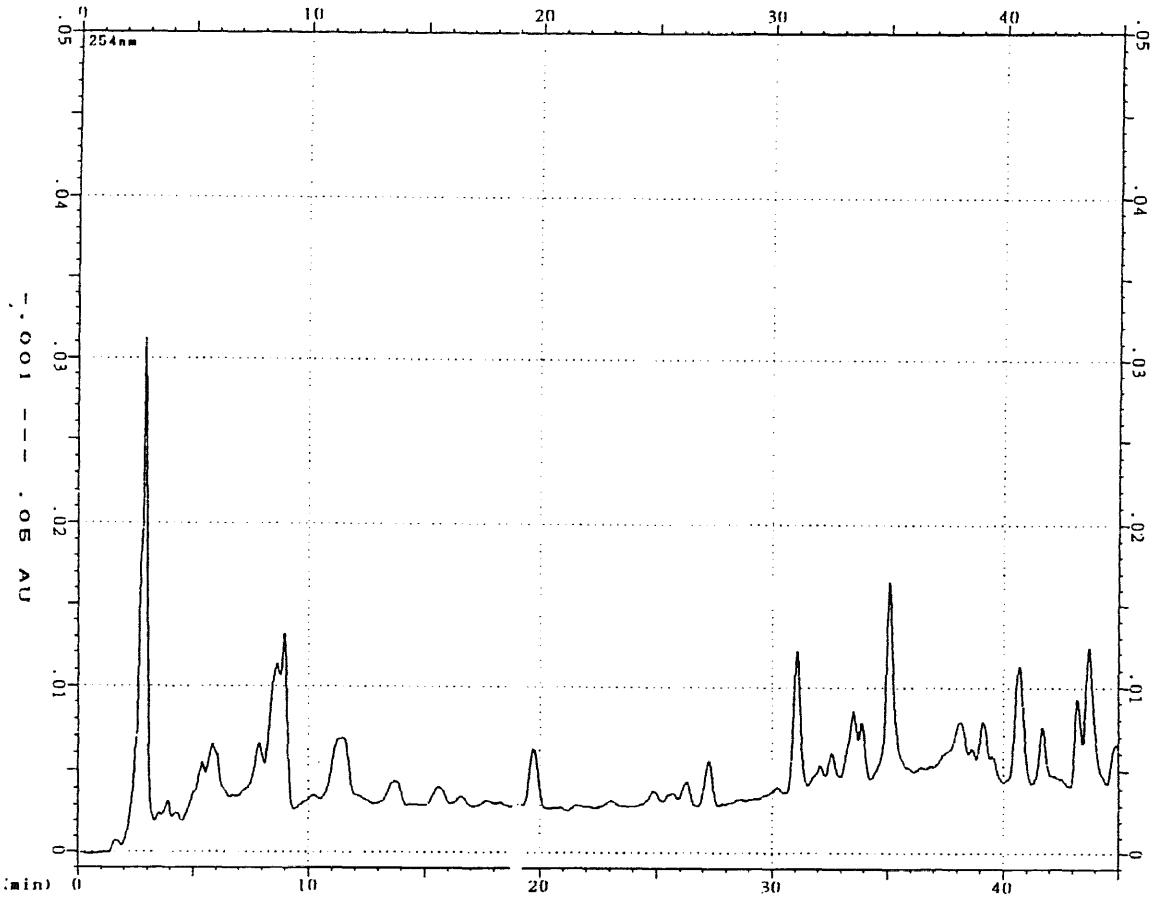


Figure 4-24 The HPLC Chromatogram of W88-DCM-N6-S3-L1 at 254 nm wavelength

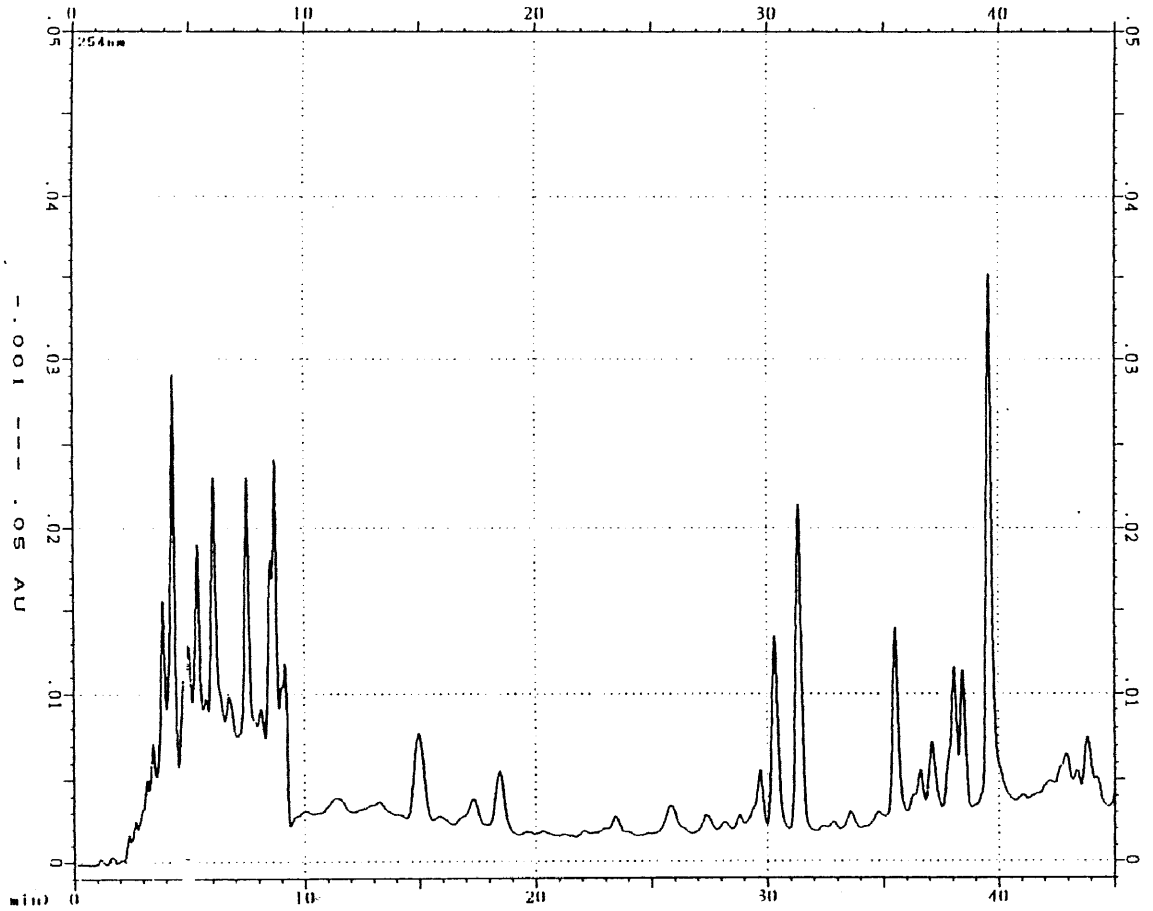


Figure 4-25 The HPLC Chromatogram of W88-DCM-N6-S3-L2 at 254 nm wavelength

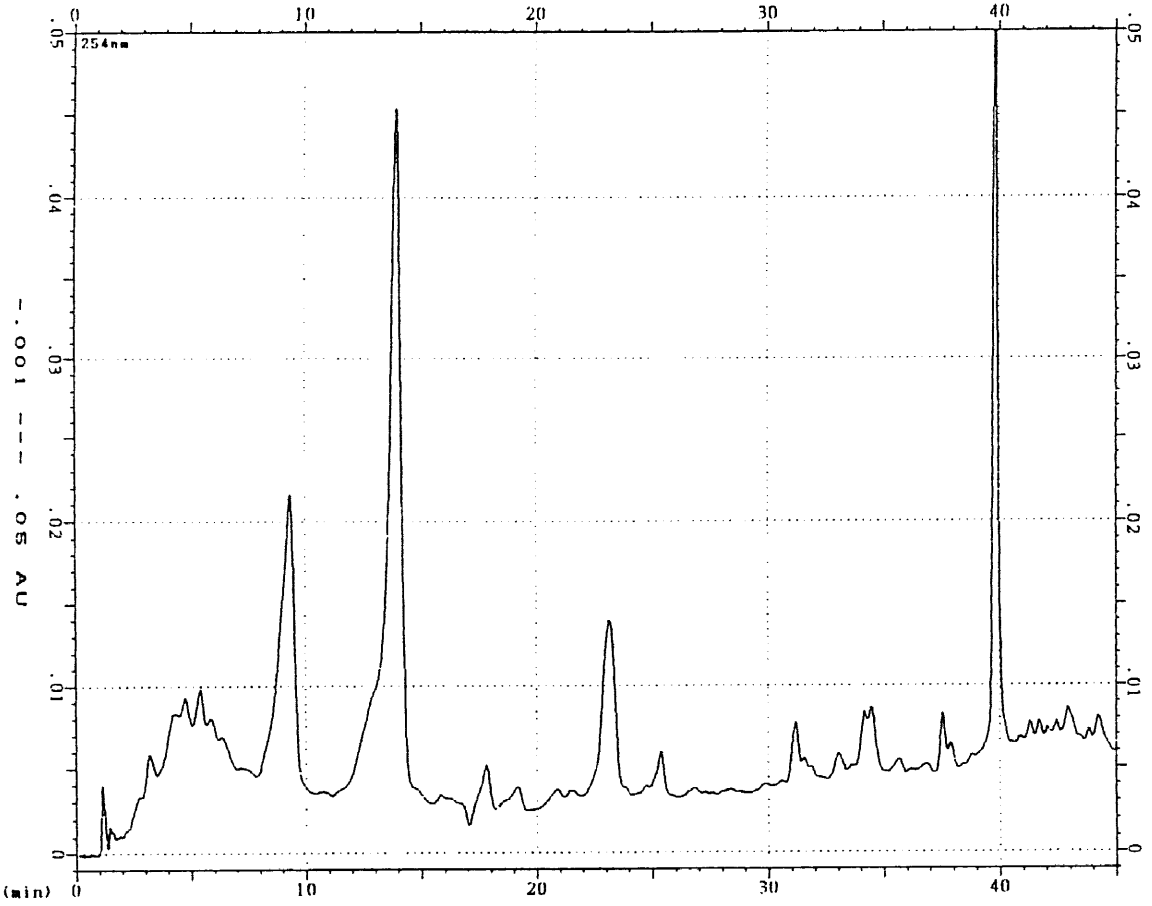


Figure 4-26 The HPLC Chromatogram of W88-DCM-N6-S3-L3 at 254 nm wavelength

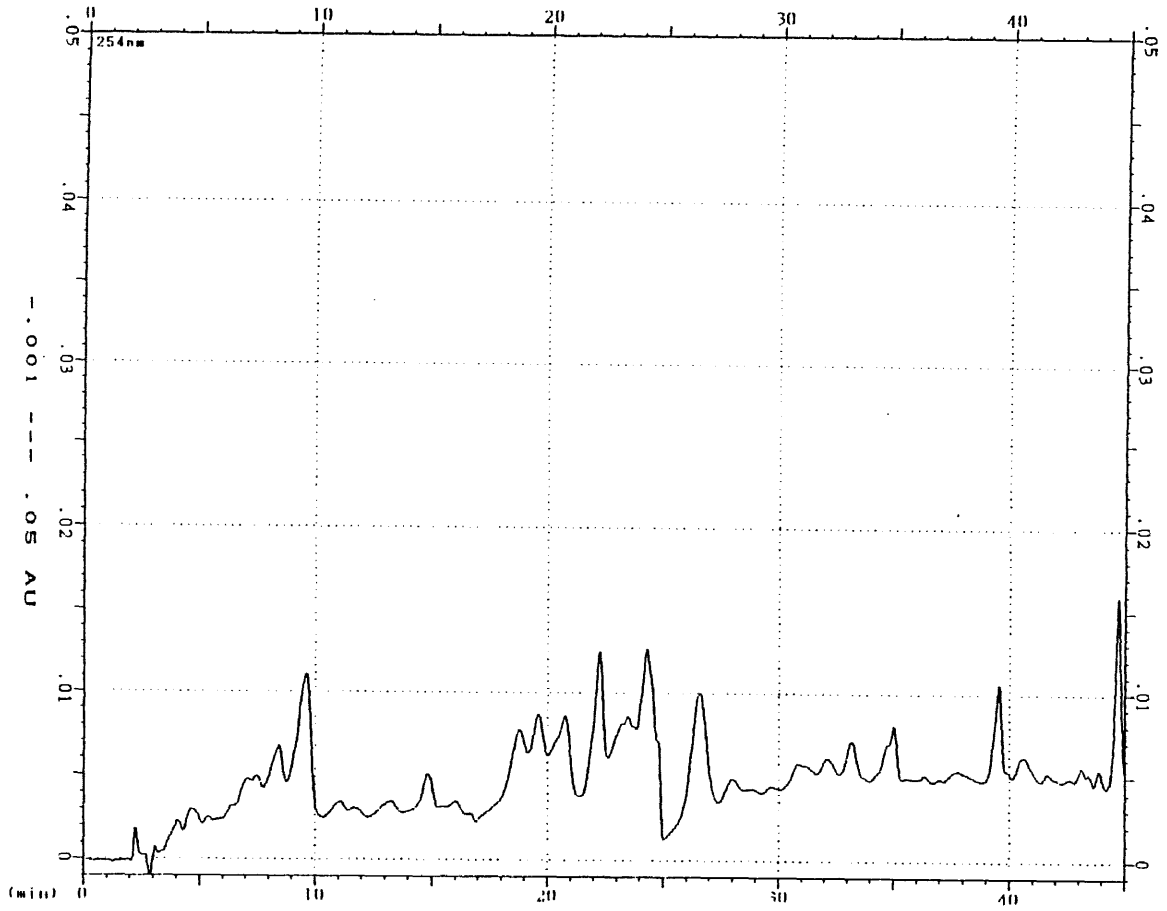


Figure 4-27 The HPLC Chromatogram of S88-DCM-N6-S3-L3 at 254 nm wavelength

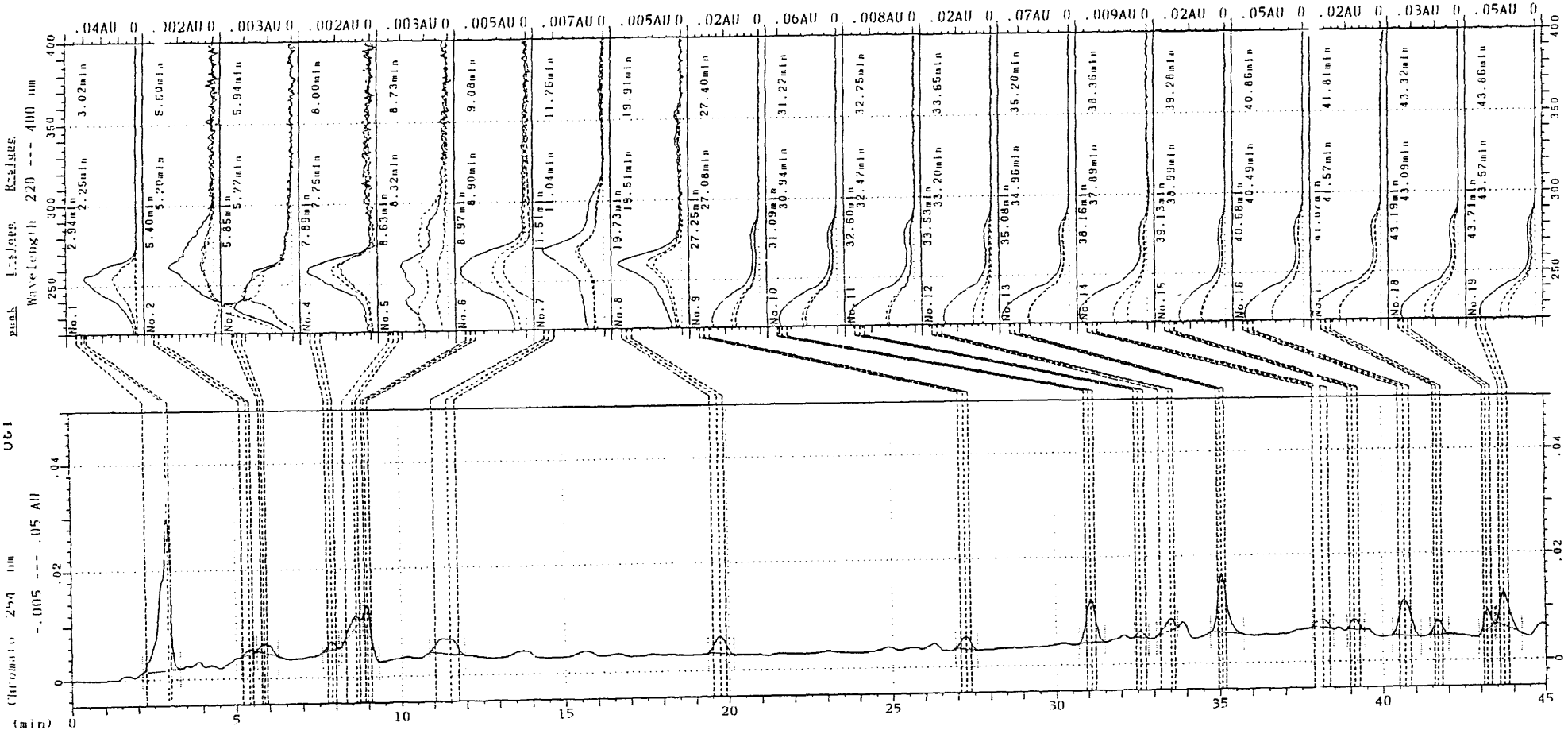


Figure 4-28 The HPLC Chromatogram with UV Spectra at Three Different Positions of Each Peak for W88-DCM-N6-S3-L1 Subsubfraction

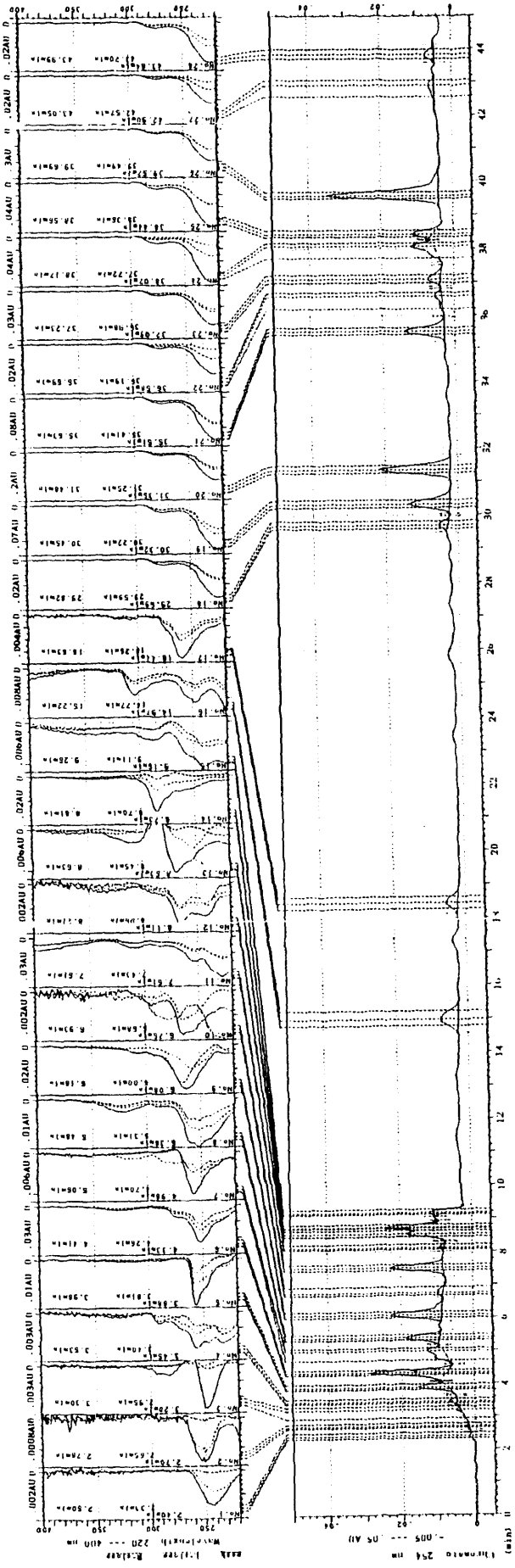


Figure 4-29 The HPLC Chromatogram with UV Spectra at Three Different Positions of Each Peak for W88-DCM-N6-S3-L2 Subsubfraction

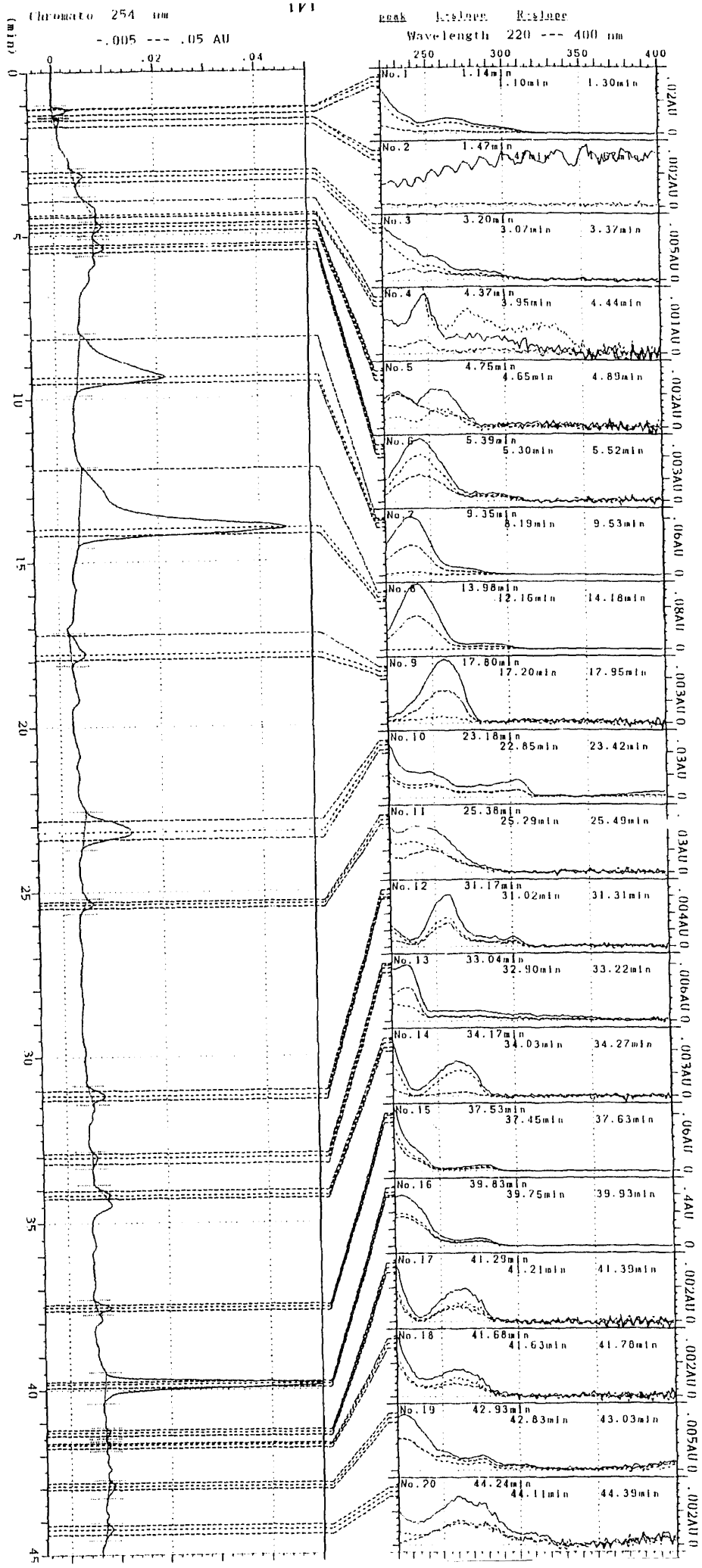


Figure 4-30 The HPLC Chromatogram with UV Spectra at Three Different Positions of Each Peak for W88-DCM-N6-S3-L3 Subsubfraction

Figure 4-31 The HPLC Chromatogram with UV Spectra at Three Different Positions of Each Peak for S88-DCM-N6-S3-L3 Subsubfraction

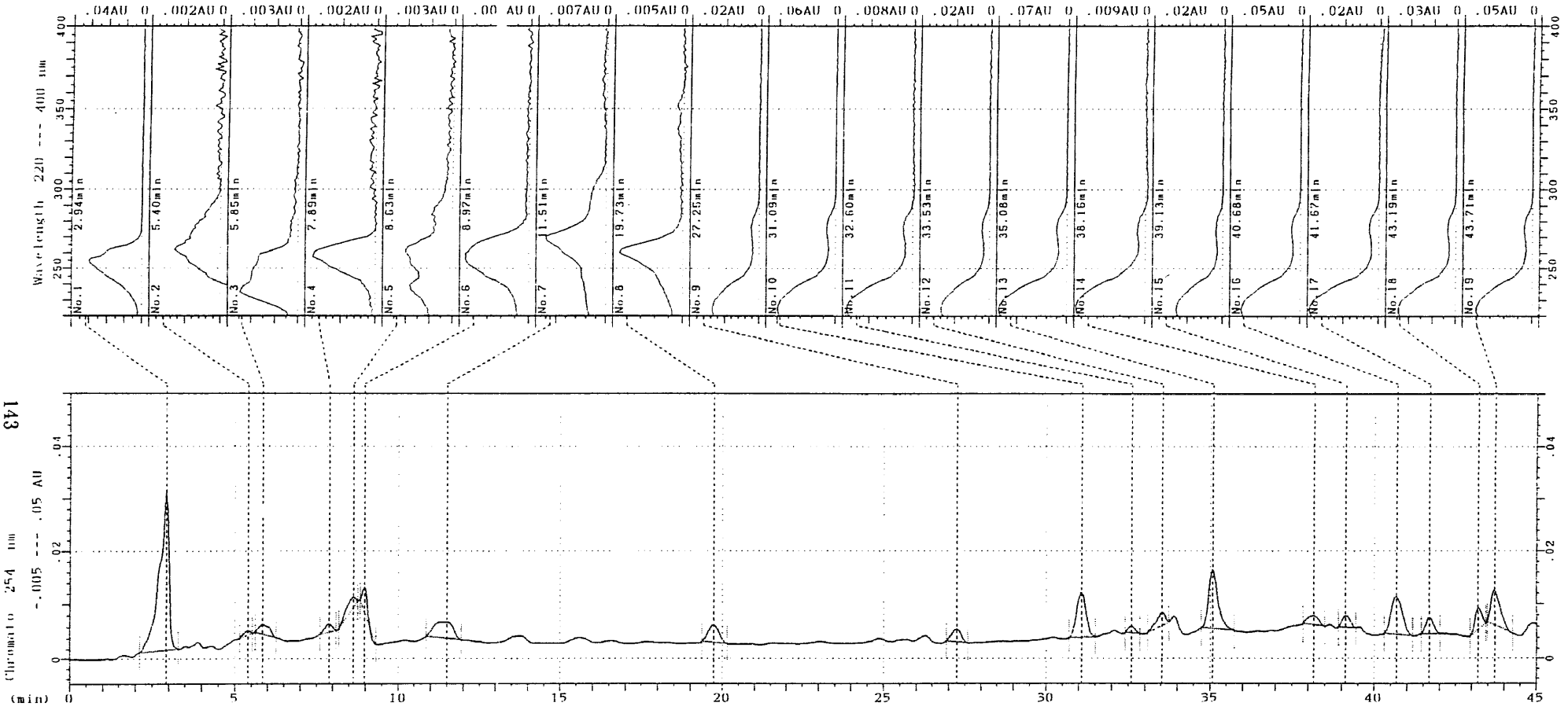


Figure 4-32 HPLC Chromatogram with Complete Diode Array UV Spectra for W88-DCM-N6-S3-L1

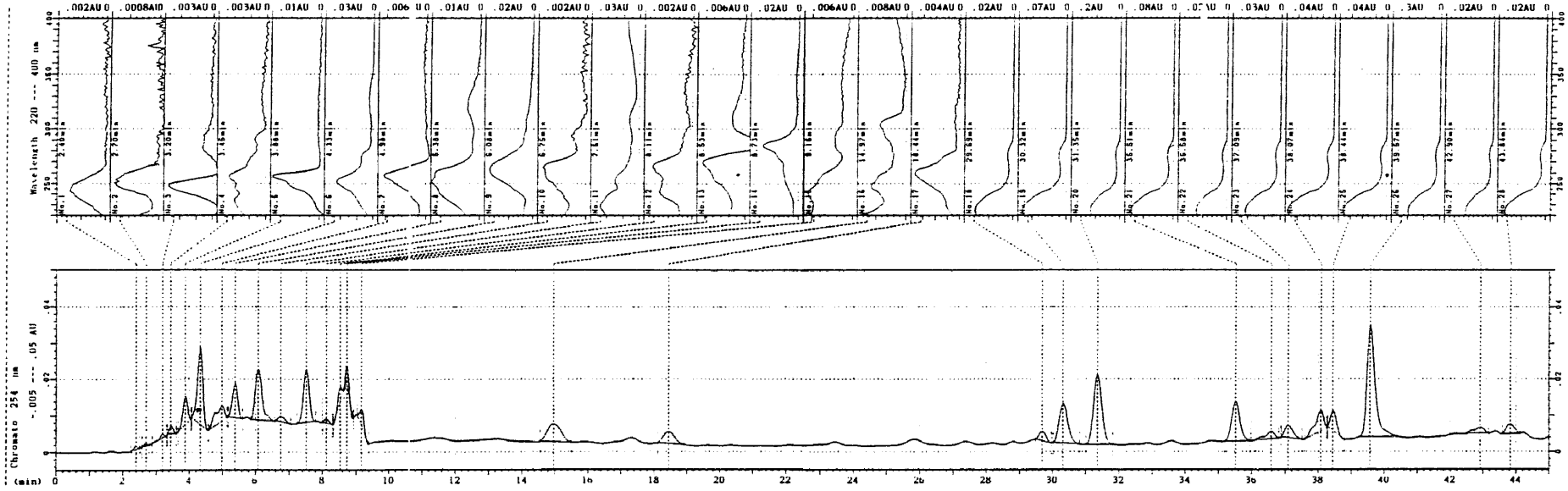


Figure 4-33 HPLC Chromatogram with Complete Diode Array UV Spectra for W88-DCM-N6-S3-L2

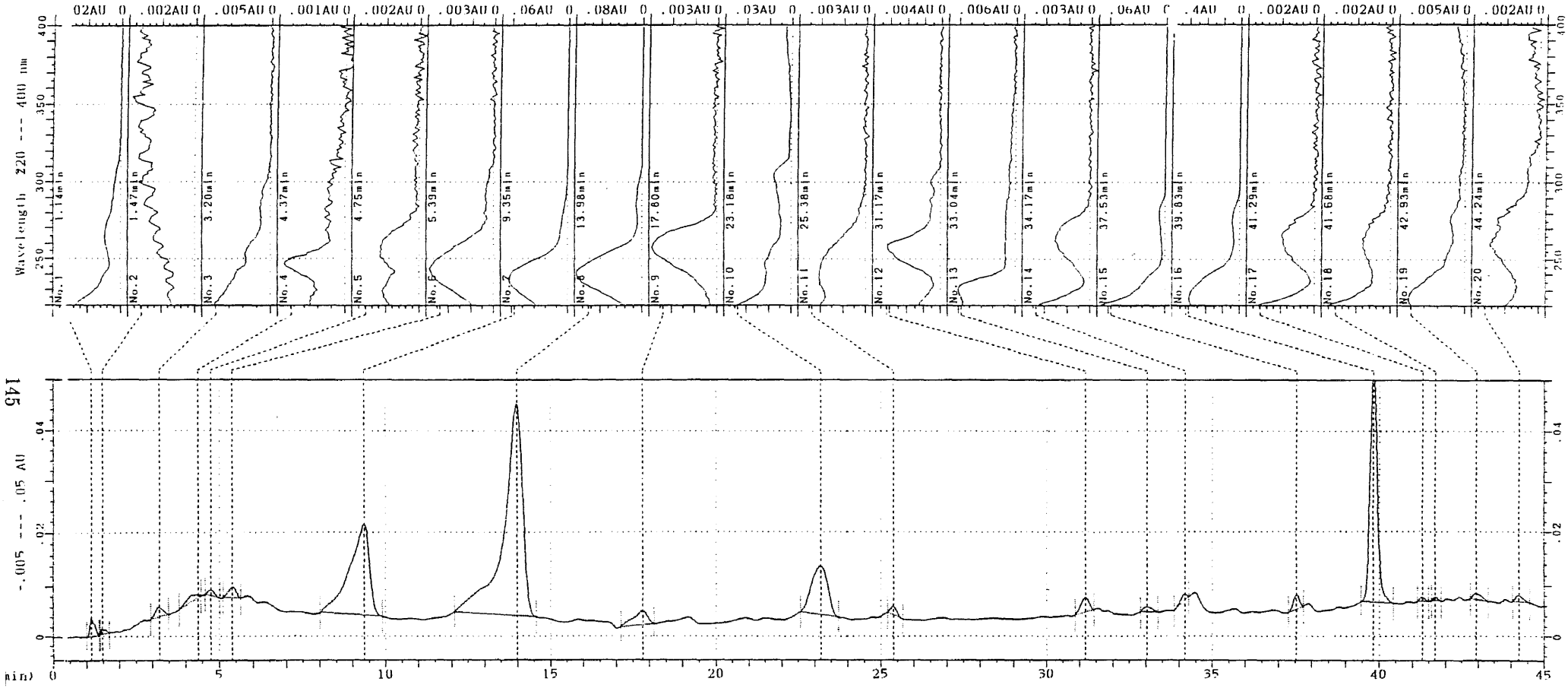


Figure 4-34 HPLC Chromatogram with Complete Diode Array UV Spectra for W88-DCM-N6-S3-L3

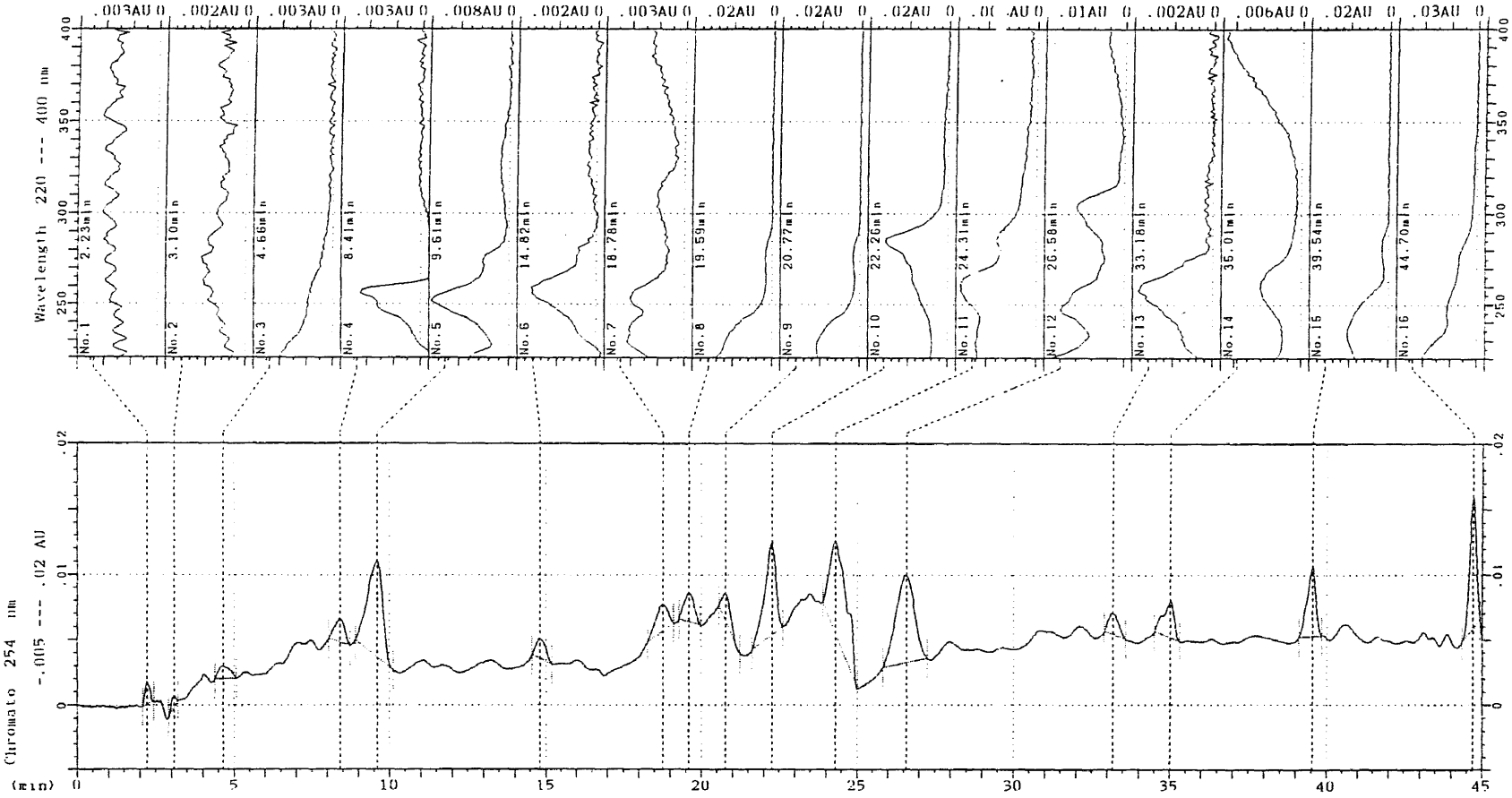
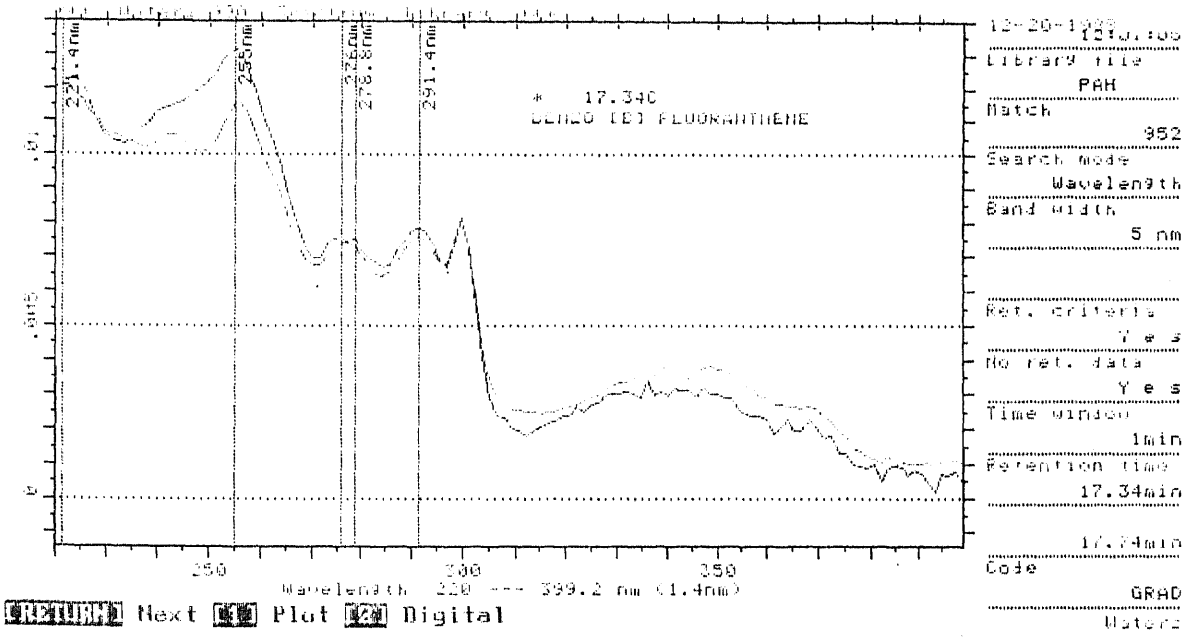


Figure 4-35 HPLC Chromatogram with Complete Diode Array UV Spectra for S88-DCM-N6-S3-L3



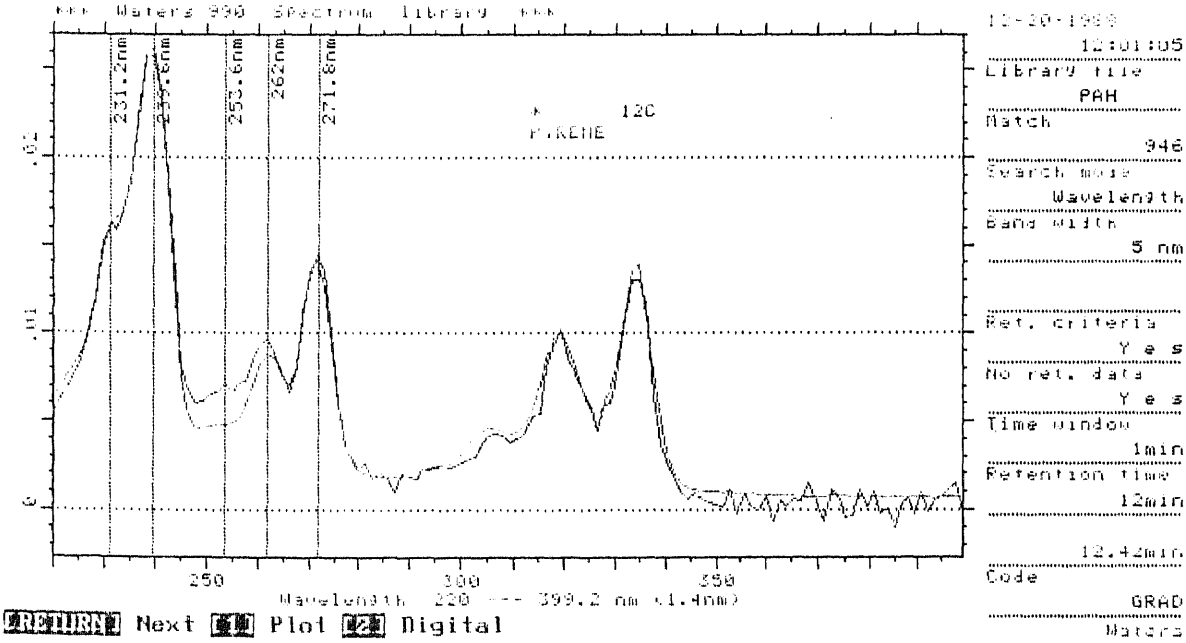
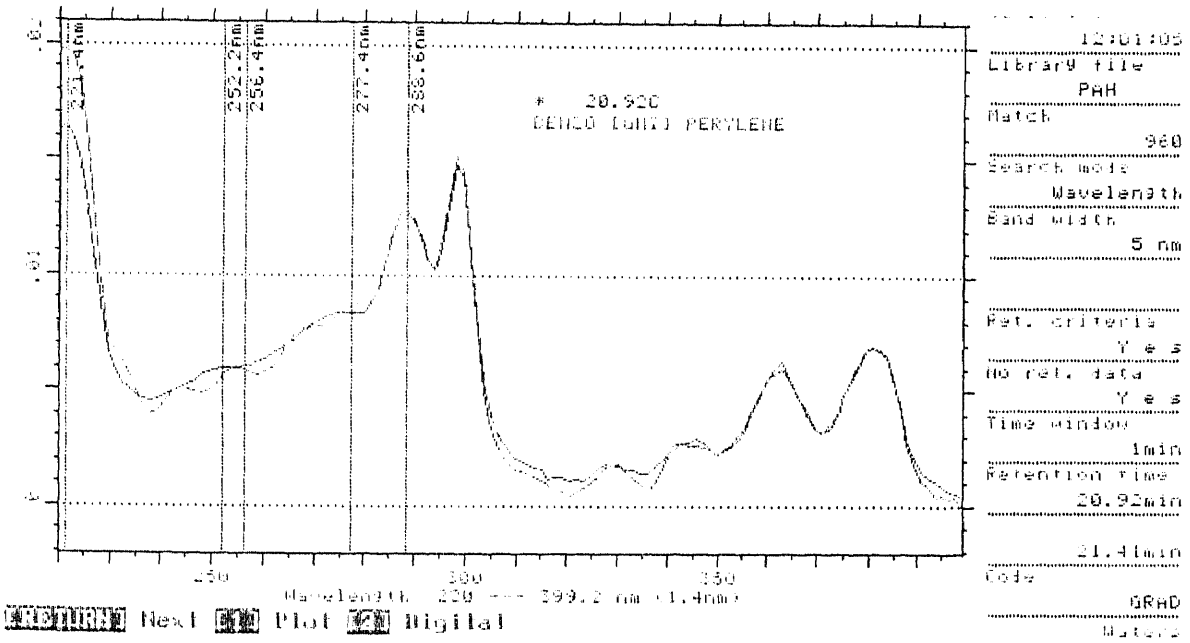


Figure 4-36a and Figure 4-36b Matching of Benzo(b)Fluoranthene and Pyrene in W88-DCM-N6-S2 with Library Spectra of Knowns



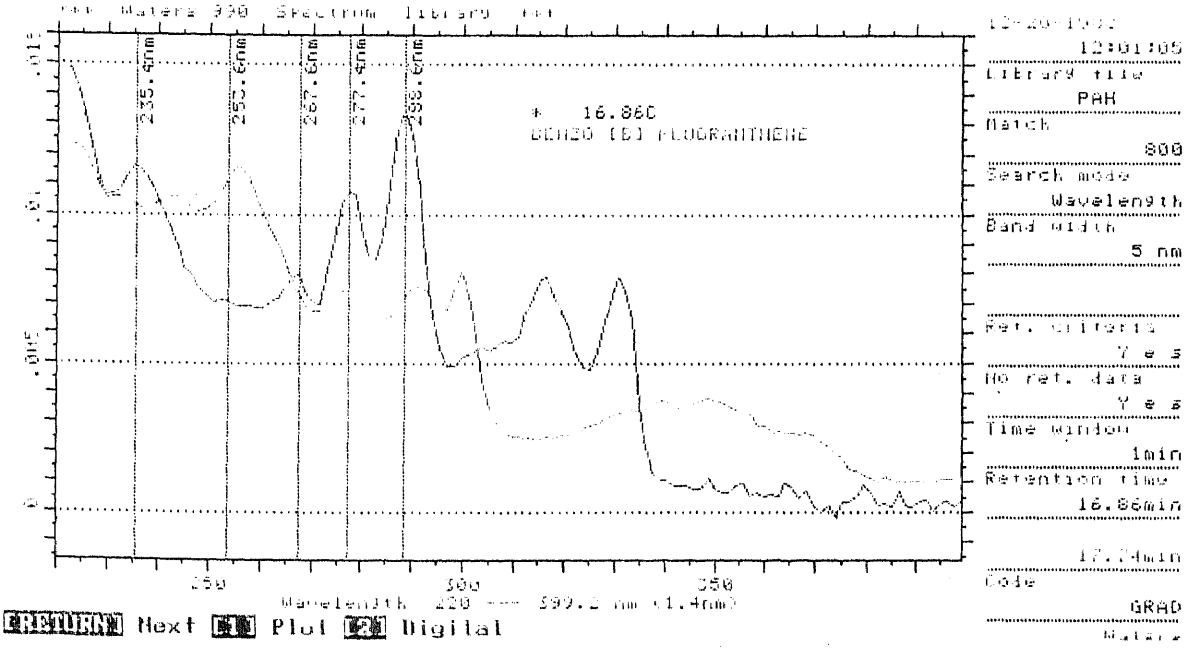


Figure 4-36c and Figure 4-36d Matching of Benzo(ghi)Perylene and Mismatching of Benzo(b)Fluoranthene with Library Spectra of Knowns

CHAPTER FIVE

Gas Chromatography/Mass Spectrometric Results

5.1 Introduction

A major research goal of this present study is to obtain data on classes of organic compounds, presently unknown, which contribute to mutagenicity of airborne particulates. Additionally, as mentioned earlier in this study, a large fraction of the mutagenic activity of the extracts is associated with more polar materials. There is literature evidence implicating nitro- substituted compounds and, more specifically, hydroxynitro-PAH. (1,2) Therefore, all major mutagenic fractions, subfractions and subsubfractions of DCM extracts were analyzed by GC/MS technique at the Center for Advanced Food Technology, Cook College of Rutgers University where samples were run by Drs. Robert Rosen and Thomas Hartman.

5.2 Experimental

All analysis were conducted using a Varian 3400 gas chromatograph directly interfaced to a Finnigan Mat model 8230 mass spectrometer. Data was acquired and processed using the SS-300 data system. Chromatography was performed using on-column injection techniques. Samples were injected on a 15 m x 0.32 mm i.d. DB-5 capillary column containing a 0.25 micron film thickness. The injector temperature

was 260^0C. Various column programs were used. The GC-MS interface lines were maintained at 320^0C. The mass spectrometer was scanned at a rate of 1 second per decade from mass 35 to 550 and mass spectra were produced using standard electron ionization (70eV).

Since the presence of plasticizers obscured GC/MS results, some of the acidic fractions were taken using acid/base extraction to further fractionate into strong acids (e.g. carboxylic acids) and weak acids (e.g. phenols). The original sample was evaporated under nitrogen, then a solution of $NaHCO_3$ was added to reach a pH of 8.4. The sample was partitioned with dichloromethane. The DCM extract was collected and designated weak acid fraction. The aqueous portion was acidified with HCl to a pH<2.0 and again partitioned with dichloromethane. This sample was collected and designated strong acid fraction. Samples were then concentrated and analyzed as was done with previous samples. Other fractions of samples were given similar treatment.

5.3 Results and Discussion

GC/MS results for selected fractions, subfractions and subsubfractions of winter and summer samples are listed in Table 5-1 to Table 5-10. It is clear that the fractionation into acids and bases was not a neat one and many water-soluble compounds are present that are neither acid nor base. Furthermore, the presence of a

lot of phthalates contaminated our samples and obscured GC/MS results, especially in nitro group compound identification. This is probably due to the ubiquitous nature of these plastics additives and perhaps due to plastics processing activity in the area. Even so, some interesting compounds were still found in the selected fractions, subfractions and subsubfractions, and will be discussed in turn.

At the first level of separation, nitrosomorpholine found in acid fractions (W88-DCM-A4 and S88-DCM-A2) and the weak base fraction (S88-DCM-B1) is very interesting to us, since it is both mutagenic and carcinogenic. This substance had been listed as a carcinogen by the EPA in 1981. (3) In fact, this compound is used in the manufacture of rubber, so the source of this pollutant could be from industries in the area as well as tires. Additionally, abietic acids are also seen in these samples. These compounds are employed in the manufacture of ester gums, lacquers, varnishes, soaps, plastics and paper sizing. There is automobile body stripping and painting activity at more than one location near the Newark site.

The DCM-N6-S2 subfractions are known to contain normal PAH as we have determined using HPLC techniques. The best GC/MS results were also obtained for these two subfractions (W88-DCM-N6-S2 and S88-DCM-N6-S2) for which PAH were clearly detected. A tributylphosphate found in summer S4 subfraction as well as B1, A2 and B3 fractions is a plasticizer for cellulose esters, lacquers, plastics, and vinyl resins, and it irritates mucous membrances. Moreover, some quinones or ketones were seen in S3 subfraction and a compound of considerable interest to us

is benzanthracenedione since it is very closely related to 7H- benzanthracene-7-one (benzanthrone) which was reported to be the most abundant oxygenated non-volatile PAH. (4)

At the same time, several chloro compounds such as chlorostyrylquinoline and cholrobenzoylchloride are the findings of unusual chemicals. Nonylphenol, a chemical used in the preparation of lubricating oil additives, resins, plasticizers, surface active agents, was also found to be present in the S88- DCM-N6-S3 subfraction.

Of the subsubfractions, a lot of quinones, ketones, alcohols and phenols were found in W88-DCM-N6-S3-L1, L2 and L3. These three subsubfractions displayed the most direct-acting and nitro group mutagenic activity in the Ames assay results. Thus, the nitro group compounds were presumably involved in these samples. However, there is no evidence for hydroxynitro-PAH or any other nitro compounds in these polar subsubfractions, since our samples were too contaminated with phthalates and adipates. It is also possible that there is some thermal decomposition on the GC column of these polar, nonvolatile nitrated molecules. In addition, although we believe the nitro compounds are present, they are at low enough levels that they are obscured by the more abundant substances. How to clean up or prevent our samples from being contaminated is definitely important for the further identification using GC/MS techniques.

Reference

1. J. Siak, T.L. Chan, T.L. Gibson and G.T. Wolff, "Contribution to Bacterial Mutagenicity from Nitro-PAH compounds in Ambient Aerosols", *Atmos. Environ., 19*, 369 (1985).
2. M.G. Nishioka, C.C. Howard and J. Lewtas, "Detection of Hydroxy-Nitro-PAHs in Ambient Air Particulate Extract Using Bioassay Directed Fractionation", in *Polynuclear Aromatic Hydrocarbons: Chemistry, Characterization and Carcinogenesis*, M. Cook and A.J. Dennis (eds), Battelle Press, Columbus, 701-715 (1986).
3. *EPA Second Annual Report on Carcinogens* (NTP 81- 43), 186-188 (Dec. 1981).
4. T. Ramdahl, "Polycyclic Aromatic Ketones in Environmental Samples", *Environ. Sci. Technol., 17*, 666-670 (1983).

Table 5-1 The GC/MS Results for W88-DCM-B1 Fraction

Assignment	MW
Nitrosomorpholine	116
Dimethylquinoline	157
Trimethylquinoline	171
Tributylphosphate	266
Diethylbiphenyl or isomer	210
Benzoquinoline or acridine or phenylethylpyridine	179
Caffein	194
Hexadecanamide	255
Octadecenamide	281

Sample too contaminated by phthalates for further identification.

Table 5-2a The GC/MS Results for W88-DCM-A2 Fraction

Assignment	MW
Dimethylnonenone	168
Propanoic acid, 2-methyl-, 1-(1,1-dimethyl-1,3 propanediol)	286
Phenol-, 2,6-bis(1,1-dimethylethyl)-4-ethyl-	234
Caffeine	194
Hexadecanoic acid	256
Octadecanoic acid	284
Butylphenylmethylphthalate	312
Octadecanamide	281
Abietic acid	300

Sample too contaminated with phthalates for further identification.

Table 5-2b The GC/MS Results for S88-DCM-A2 Fraction

Assignment	MW
Nitrosomorpholine	116
Tributylphosphate	266
Methylethylpropylpropandiyl propanoate	286
Aliphatic amide	337

Sample too contaminated with phthalates for further identification.

Table 5-3a The GC/MS Results for W88-DCM-B3 Fraction

Assignment	MW
Phosphorodithioic acid 0,0,s-trimethylester	172
Butenedioic acid, diethyl ester	172
Pentenedioic acid, diethyl ester	186

Sample too contaminated by phthalates for further identification.

Table 5-3b The GC/MS Results for S88-DCM-B3 Fraction

Assignment	MW
Butoxyethoxyethoxyethanol	206
Diethylethanediamine	116
Chlorotoluene isomer	126
Diethylaminobutanone	143
Bis(methylethyl)aminoethanol	145
Methylpyrrolidinyl pyridene	162
Tributylphosphate	266
Caffeine	194
Hexadecanamide	255
Azabicyclooctanecarboxylic acid benzyloxy-8-methylester	303
Tributylacetyloxylpropanecarboxylic acid	402
Octadecenamide	281

Sample too contaminated by phthalates for further identification.

Table 5-4a The GC/MS Results for W88-DCM-A4 Fraction

Assignment	MW
Nitrosomorpholine	116
Butoxyethoxyethanol	162
Benzenedicarboxylic acid	166

Sample too contaminated by phthalates for further identification.

Table 5-4b The GC/MS Results for S88-DCM-A4 Fraction

Assignment	MW
Methylethylpropylpropandiyl propanoate	286

Sample too contaminated by phthalates for further identification.

Table 5-5a The GC/MS Results for W88-DCM-N6-S2 Subfraction

Assignment	MW
Naphthalene	128
Phenanthrene	178
Methylanthracene	192
Phenylindene	192
Dihydrophenylnaphthalene	206
Fluoranthene	202
Pyrene	202
Abietic acid analog	324
Methylpyrene	216
Benzofluoranthene	226
Chrysene	228
Naphthacene	228
Methylchrysene	242
Methylbenzanthracene	242
Dihydroxypropylanthracendione	282
Binaphthalene	254
Benzopyrene	252
Benzofluoranthene	252
Methylbenzaceanthrylene	266
Methylbenzaceanthrylenol	284
Quaterphenyl	306
Benzochrysenopyrandione	322
Benzochrysene	276
Benzoperylene	276
Diethyldiphenylpyrazine	288
Hexdecylhexadecanoate	480
Dibenzochrysene	302
Coronene	300
Dibenzonaphthacene	302

Table 5-5b The GC/MS Results for S88-DCM-N6-S2 Subfraction

Assignment	MW
Isocyanonaphthalene	153
Dimethylethyldihydrodimethyl-1H-indenone	216
Ethoxydimethylcyclohexoenylidene methylfuran	232
Dimethylphenylmethylbenzene	196
Methylbutylidene-1H-indene-2H-dione	214
Hydroxyphenylbenzeneacetic acid	226
Methylphenylbenzylamine	211
Methylene-9H-fluorene	178
Methylanthracene (or isomer)	192
Fluoranthene or pyrene	202
Methoxystilbene	210
Abietic acid analog	314
Triphenylene	228
Chrysene	228
Pentaethylstyrene	244
Tetramethoxybenzobisbenzofurandione	408
Benzo(a)pyrene	252
Benzo(e)pyrene	252
Dibenzochrysene	276
Benzoperylene	276
Indenopyrene	276

Table 5-6 The GC/MS Results for S88-DCM-N6-S3 Subfraction

Assignment	MW
Diphenylmethanone	182
Methylethylpropylpropandiyl propanoate	286
Dimethylethylhydroxymethyl benzoate	264
Benzanthracenedione	258
Dihydroxyphenyl-4H-benzopyranone	254
Stigmastenol	414
Chlorobenzylchloride	160
Caffeine	194
Nonylphenol	220
Chlorostyrylquinoline	265
Naphthalenepropanol derivative	272

Table 5-7 The GC/MS Results for S88-DCM-N6-S4 Subfraction

Assignment	MW
Bis(dimethylpropyl)benzenediol	250
Dis(dimethylethyl)benzene	190
Dimethylethylhydroxymethyl benzoate	264
Tributylphosphate	266
Unknown subsituted phenol	286
Aminobenzamide	136
Dimethyloxohexylcyclohexenecarboxylic acid methyl ester	266
Tetramethylbutylphenoxyethoxyethanol	294
Octyldiphenylphosphate	362
Methylene bis(dimethylethyl)methylphenol	340
N-propylbenzamide	163
Stigmastenol	414
Cholesterol	386

Table 5-8 GC/MS Results of W88-DCM-N6-S3-L1 Subsubfraction

Assignment	MW
Bis(dimethylethyl)cyclohexadienedione	220
Octahydro(2H)cyclopropanaphthalenone	218
Diphenylmethanone	182
Phenanthrenol	194
Ethyldimethylpyridine	135
7H-benzanthracenone	230
Benzanthracenedione	258
Naphthalene	128

Aliphatic hydrocarbons, low molecular weight aromatic hydrocarbons (as toluene, xylene etc) fatty acid esters, phthalates and adipates not listed in this table.

Table 5-9 GC/MS Results of W88-DCM-N6-S3-L2 Subsubfraction

Assignment	MW
Methoxybenzenediol	140
9H-fluorenone	180
Bis(diethylamino)phenylmethanone	324
Anthracenecarboxaldehyde	206
Bis(dimethylethyl)isocyanophenol	231
Cyclopropyl octadecenamide	321
Friedooleananone	426
Methylethylpropylpropandiyl propanoate	286
9H-fluorenamine	181
Caffeine analogn	208
Trimethylpentadecanone	268
7H-indeno(2,1-A)anthracenone	280
7H-benzanthracenone	230
Dimethoxyanthracendione	268

Aliphatic hydrocarbons, low molecular weight aromatic hydrocarbons (as toluene, xylene etc) fatty acid esters, phthalates and adipates not listed in this table.

Table 5-10 GC/MS Results of W88-DCM-N6-S3-L3 Subsubfraction

Assignment	MW
Methylethenyl benzene	118
Propenylbenzene	118
Tetramethylbutyl phenol	206
Dimethylbutylidenebisbenzene	236
Trimethylphenyl-1H-indene	236
Dimethyltrimethylphenylester propanoic acid	220
Abietic acid analog	284
Bis(diethylamino)phenylmethanone	324
Ethenylbenzene ethanol	148
Abietic acid analog	318
Triphenyl phosphate	326
Abietic acid analog	300

Aliphatic hydrocarbons, low molecular weight aromatic hydrocarbons (as toluene, xylene etc) fatty acid esters, phthalates and adipates not listed in this table.

CHAPTER SIX

Conclusion

This study had as its goal the identification of levels and variations of known atmospheric mutagens as well as the identification of mutagenic fractions and investigation of unknown mutagens or classes of mutagens in these fractions. Thus, the strategy employed here is one of "biologically-driven" chemical analysis. A modification of the published fractionation scheme based on acid-base partitioning and silica gel column chromatography was developed for the separation of ambient air particulate extracts. Then, bioassays were run to isolate the most potent mutagenic fractions which were further fractionated using semi-preparative HPLC. Meanwhile, in order to identify the compounds in these major mutagenic fractions, FTIR, HPLC/Photodiode Array UV and GC/MS techniques were achieved. The previous studies summarized here lead to the following conclusions:

While the amounts of material present in the total winter and summer DCM composites (593.8 mg and 532.0 mg) respectively extracted from 70,000 m^3 of winter air particulates and 100,000 m^3 of summer air particulates are just about equal to that in the ACE extract composites (566.6 mg and 523.0 mg), the total mutagenicities of the former are greater, reflecting material more highly mutagenic on a revertants per mass basis: as shown in Table 6-1, for TA98-S9: DCM extract composites: 1.25 rev/ug or 742,300 total rev for winter sample and 0.77 rev/ug

or 409,600 total rev for summer sample; ACE extract composites: 0.51 rev/ug or 289,000 total rev for winter sample but the summer ACE sample was lost. Therefore, the winter DCM extract is responsible for 72% of direct TA98 mutagenicity. For TA98+S9: DCM extract composites: 0.87 rev/ug or 516,600 total rev for winter sample and 0.48 rev/ug or 255,400 total rev for summer sample; ACE extract composites: 0.26 rev/ug or 147,300 rev total for winter sample. Thus, the winter DCM extract is responsible for 78% of activated TA98 mutagenicity.

Comparison of mass and mutagenicity at three levels of separation for winter and summer samples is shown in Figure 6-1. From this figure, we note the great similarity in profiles between summer and winter samples. It is clear that the amount of mutagenic activity in the summer DCM extract is considerably smaller than for the winter extract. In addition, as in Nishioka's study, the greatest activity in our winter and summer DCM extracts is also in the N6 fractions. However, in the winter, total mutagenicity was fairly evenly divided between the B1, A2, B3 and A4 fractions. This differs from Nishioka's finding in which a specific class of mutagenic compounds, organic acids, collected over a period in excess of 12 months are the second major mutagenic fraction. For the summer, the two acidic fractions appear to have more than double the mutagenicity of the two basic fractions. It is the same result as in Nishioka's study.

The most mutagenic subfraction displayed in Figure 6-1 is DCM-N6- S4 for both winter and summer, but the DCM-N6-S3 subfraction has the most potent

mutagenicity per mass, as Nishioka/Lewtas found. That is the reason why we analyzed the S3 subfractions in greater detail. In fact, the Ames assay results of subsubfractions showed the L1, L2 and L3 are more important in winter S3 subfraction, and L3 represents the most mutagenicity in summer S3 subfraction. As to the winter and summer S4 subfractions, the L2 appears to contain the highest level of direct-acting mutagens.

A lot of PAH compounds identified using HPLC and GC/MS techniques were found in DCM-N6-S2 subfractions. The levels of polycyclic aromatic hydrocarbons (PAH) are significantly greater in winter compared to summer. Meanwhile, the constancy in PAH profiles (except for semivolatile tetracyclic species) indicates relatively little reactivity of PAH (or conceivably some total levelling reactivity of all exposed PAH immediately upon atmospheric exposure).

Fourier Transform Infrared (FTIR) analysis indicated that the massive DCM-N6-S1 subfractions are probably largely composed of aliphatic hydrocarbons thus explaining the nonmutagenicity of these subfractions. FTIR also indicates the presence of carbonyl compounds in the most mutagenic DCM fractions, subfractions and subsubfractions. However, nitro-PAH levels were too low to be observed by FTIR.

Furthermore, the GC/MS results indicate that the samples were highly contaminated with phthalates and adipates and, thus, no evidence for any nitro - substituted PAH or hydroxynitro-PAH was found in these samples. However, a carcino-

genic and mutagenic compound, 4-nitrosomorpholine, was found in W88-DCM-A4, S88-DCM- A2 and S88-DCM-B1 fractions, and three compounds likely to be hydroxynitropyrene isomers were also seen in W88-DCM-N6-S3-L3 subsubfraction from Photodiode Array UV results. Therefore, more extensive cleanup of the samples and contaminant reduction are steps clearly necessary for further identification.

Even though some unsatisfactory results are present in this study, the approach using a modification fractionation and silica column chromatography and semi-preparative HPLC to isolate and monitor the most mutagenic known or unknown compounds is definitely necessary for continuation of this study. The reason why this strategy is strongly recommended is that much greater detail in the analysis of fractions, subfractions and subsubfractions from a small number of sample composites is obtained than using the older strategy of analyzing many more samples superficially.

Table 6-1

Comparison of Mass and Mutagenicity for DCM and ACE Extracts

		TA98-S9		TA98+S9	
Name	*Mass(ug)*	*rev/ug*	*Total(rev)*	*rev/ug*	*Total(rev)*
W88-DCM	*593,800*	*1.25*	*742,250* (72%)	*0.87*	*516,600* (78%)
W88-ACE	*566,600*	*0.51*	*289,000* (28%)	*0.26*	*147,300* (22%)
S88-DCM	*532,000*	*0.77*	*409,600*	*0.48*	*255,400*
**S88-ACE*	*523,000*	-	-	-	-

*The S88-ACE extract was not run on TA98 strains since it was lost.

**The approximate total air volumes collected in Winter and Summer are 70,000 m^3 and 100,000 m^3.

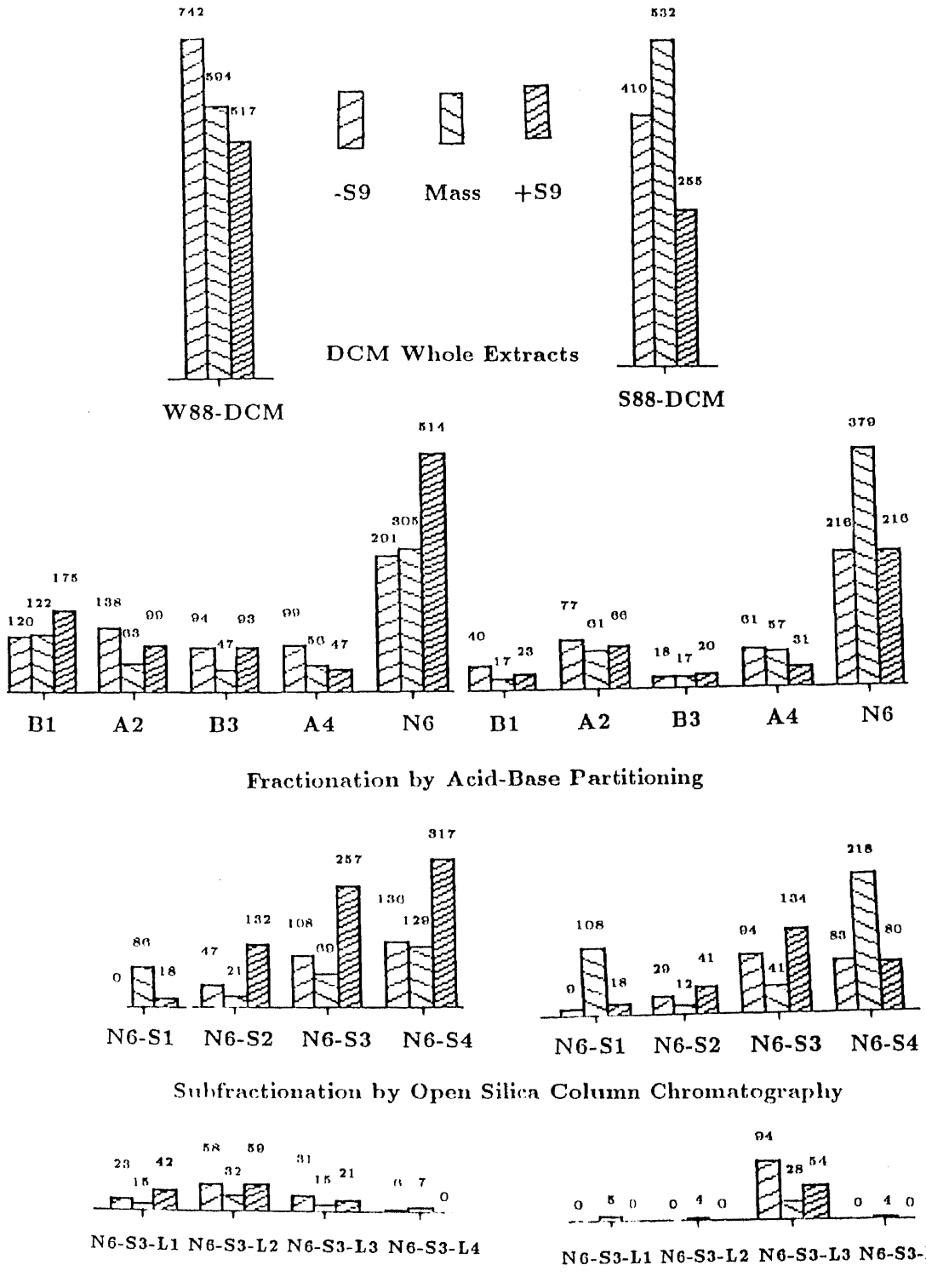


Figure 6-1 Tracking Mass and Mutagenicity Distribution for W88 and S88 DCM Extracts