# Halo acids and DMSO: Electrophilic aromatic substitution at room temperature conditions

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# HALO ACIDS AND DMSO: ELECTROPHILIC AROMATIC SUBSTITUTION AT ROOM TEMPERATURE CONDITIONS

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THESIS

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# ACKNOWLEDGEMENTS

# ABSTRACT

## HALO ACIDS AND DMSO: ELECTROPHILIC AROMATIC SUBSTITUTION AT ROOM TEMPERATURE CONDITIONS

### By Pankaj Kulshrestha under the guidance of Dr. James J. Worman

A simple, safe and cost effective experiment to demonstrate electrophilic aromatic substitution in the undergraduate laboratory can be accomplished by placing a small amount of N,N Dimethyl Aniline or a few crystals of Phenol in an NMR tube containing an appropriate level of deutero DMSO. After observation of the expanded proton NMR spectrum of the aromatic region, a few drops of concentrated HBr and HCl aqueous acids are added and the NMR spectrum recorded after one hour.

Observation of an A,A' B,B' typical four line pattern in the aromatic region of NMR spectrum is direct evidence for the halogenation on the aromatic ring at para position. Addition of gaseous hydrogen chloride provides a faster and cleaner reaction. A mechanism for the reaction, GC/MS data to support the NMR results, along with other examples are presented.

Application of the reaction for halogenation of complex biomolecules in an attempt to enhance their activity is plausible because room temperature experimental conditions should prevent structural degradation of sensitive molecules and produce fewer non-toxic byproducts.

# INTRODUCTION

The objective of my research was to synthesize halogenated biomolecules in an attempt to enhance the activity against antibiotic resistant strains of bacteria and to reduce toxic byproducts of halogenation reactions in an undergraduate laboratory, thereby minimizing environmental pollution.

Current methods of aromatic halogenation involve the use of reagents and catalysts under experimental conditions which present a safety hazard when performed in an undergraduate teaching laboratory. In addition, the disposal of wastes are costly and definitely not benign to the environment.

Existing methods of halogenation involve the use of liquid bromine, chlorine gas and other hazardous halogenating reagents in the presence of catalysts (1,2). The methods employ drastic conditions which would destroy the structure of the biomolecules and perhaps decrease the pharmacological activity of the biomolecules, even causing them to be acutely toxic. The reaction conditions presently employed may also produce byproducts, many of which are hazardous to the environment (3,4).

Experimental conditions which on one hand involve halogenation of biomolecules safely with the formation of fewer toxic byproducts and on the other hand stereospecifically and

stereoselectively allow for the synthesis of biomolecules targeted to resistant microbial strains, would minimize environmental pollution and add knowledge to the field of medicinal chemistry.

Biomolecular target specificity can reduce the toxic ill effects and side effects on human or animal species and also prevent environmental pollution, which in turn may provide a safe living environment for other organisms in air, soil and water.

Safe disposal of the toxic byproducts of chemical reactions, in particular reactions involving halogenation, have been a matter of deep concern because byproducts have the potential to act as environmental pollutants. A part of this research focuses on the issue of trying to design safe and environmentally benign synthetic reactions for the research labs and for industry.

The design of such reactions promises the generation of stereoselective and stereospecific products and the safe disposal of unwanted byproducts, primarily by the use of haloacids in dimethyl sulfoxide under room temperature conditions. This same experimental design can be used in undergraduate laboratories to demonstrate halogenation reactions which can be accomplished with minimum waste and improved safety, thereby decreasing the risk of severe laboratory accidents.

# EARLY INVESTIGATIONS

Before and during the 1960's and the 1970's electrophilic aromatic halogenation using liquid bromine/ferric bromide and chlorine gas/ferric chloride or other hazardous halogenating reagents and catalysts was not only studied in theory (1,2,5,6) but also included in laboratory courswork (7).

This important chemical reaction and its mechanism is taught in undergraduate coursework (8,9,10,11,12) and graduate coursework (13,14), but due to the use of expensive and hazardous reagents and unsafe experimental conditions, these experiments were removed from the undergraduate laboratory experimental curriculum (15,16).

As early as 1970's (17,18) during the ongoing research (<u>scheme 1 below)</u> by Dr. James J.Worman, it was found that the dihydrobromide salt of 3,7 dimethoxy-1,5 diphenyloctahydro-1,5 diazocine, underwent halogenation in the presence of dimethyl sulfoxide in the NMR tube when kept for a few hours.

**1**  R = H

**5**  R = C-CH$_3$
      O
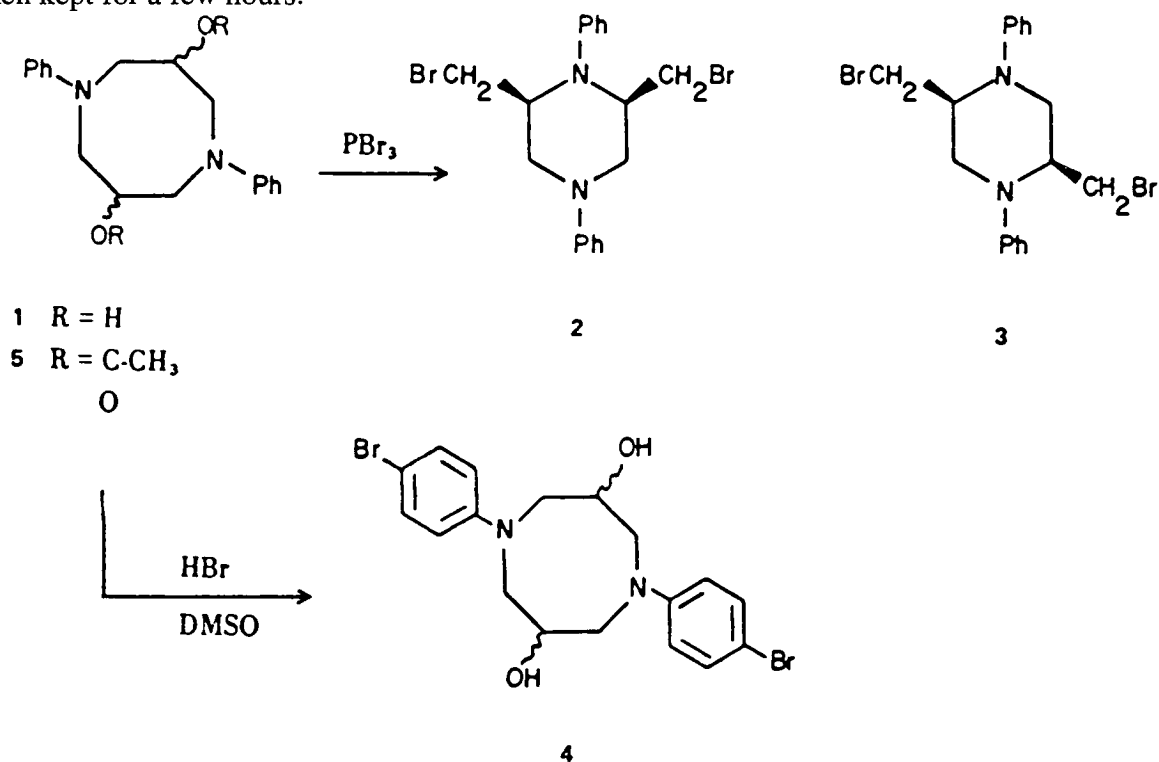
**Figure 1**. below shows a typical four line pattern of the aromatic region of a para substituted aromatic system(1,5-Bis-(p-bromo phenyl)octahydro-3,7- dimethoxy-1,5-diazocine) – the compound which was formed during the ongoing researchwork done by Dr.Worman's research group, when dimethyl sulfoxide was added to the dihydrobromide salt of octahydro 3,7- dimethoxy- 1,5- diphenyl- 1,5- diazocine in an NMR tube.

## Figure 1.



(I)  R = Ph

(II)  R = $p$-BrC$_6$H$_4$
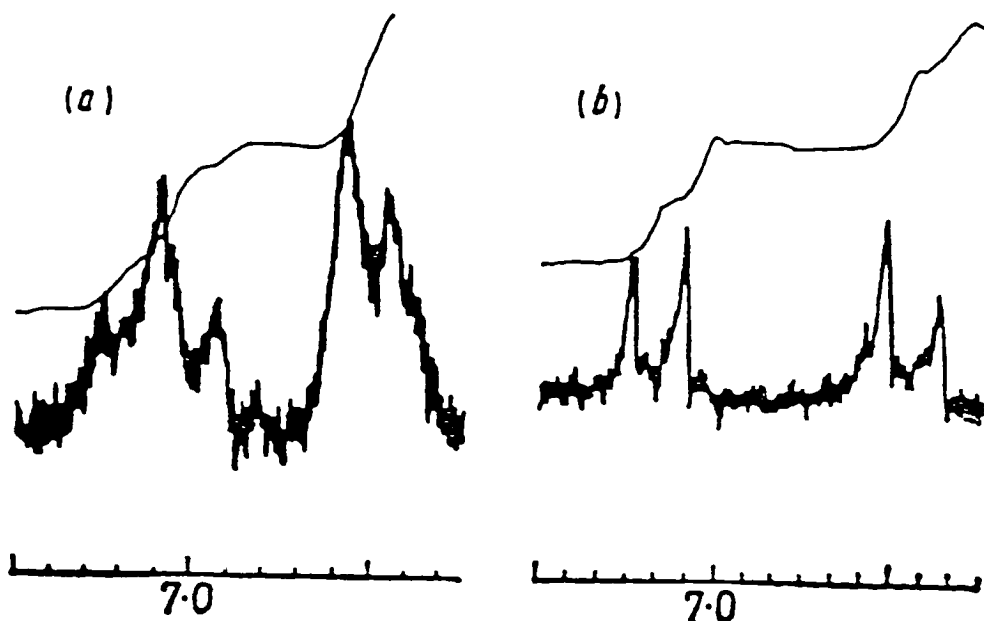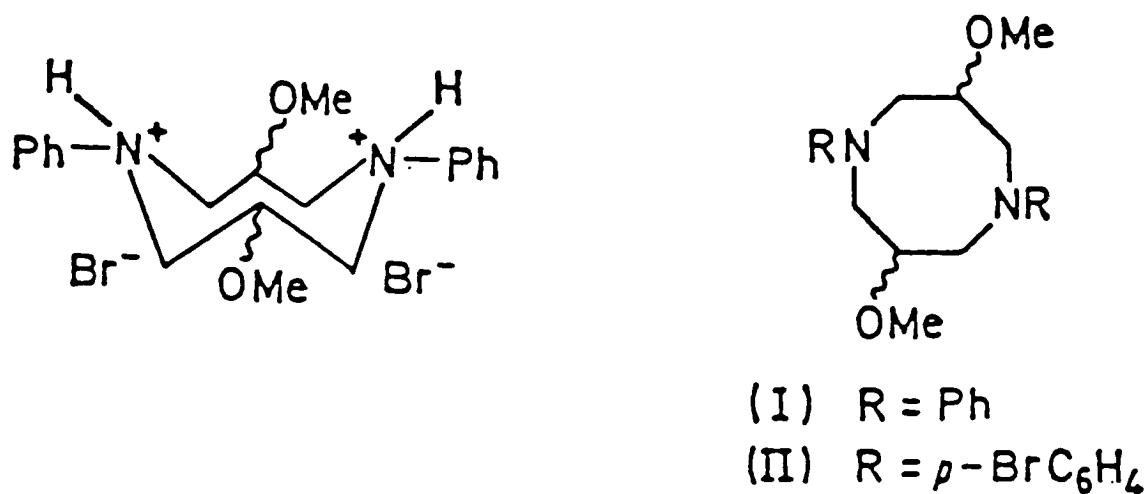
FIGURE 1 N.m.r. spectra of the dihydrobromide of octahydro-3,7-dimethoxy-1,5-diphenyl-1,5-diazocine (I) in dimethyl sulphoxide at room temperature; (a) aromatic-proton absorption immediately after dissolution of dihydrobromide in solvent; (b) same absorption after 8 h

Very little work was done in this area until 1989 when Dr.Megyeri Gabor and Dr. Tibor Keve of Hungary halogenated indole alkaloids using halodimethylsulfonium halogenids (compound no.2 in **scheme 2 below**) and halodimethylsulfoxonium halogenids (compound no.6 in **scheme 2 below**)(19).
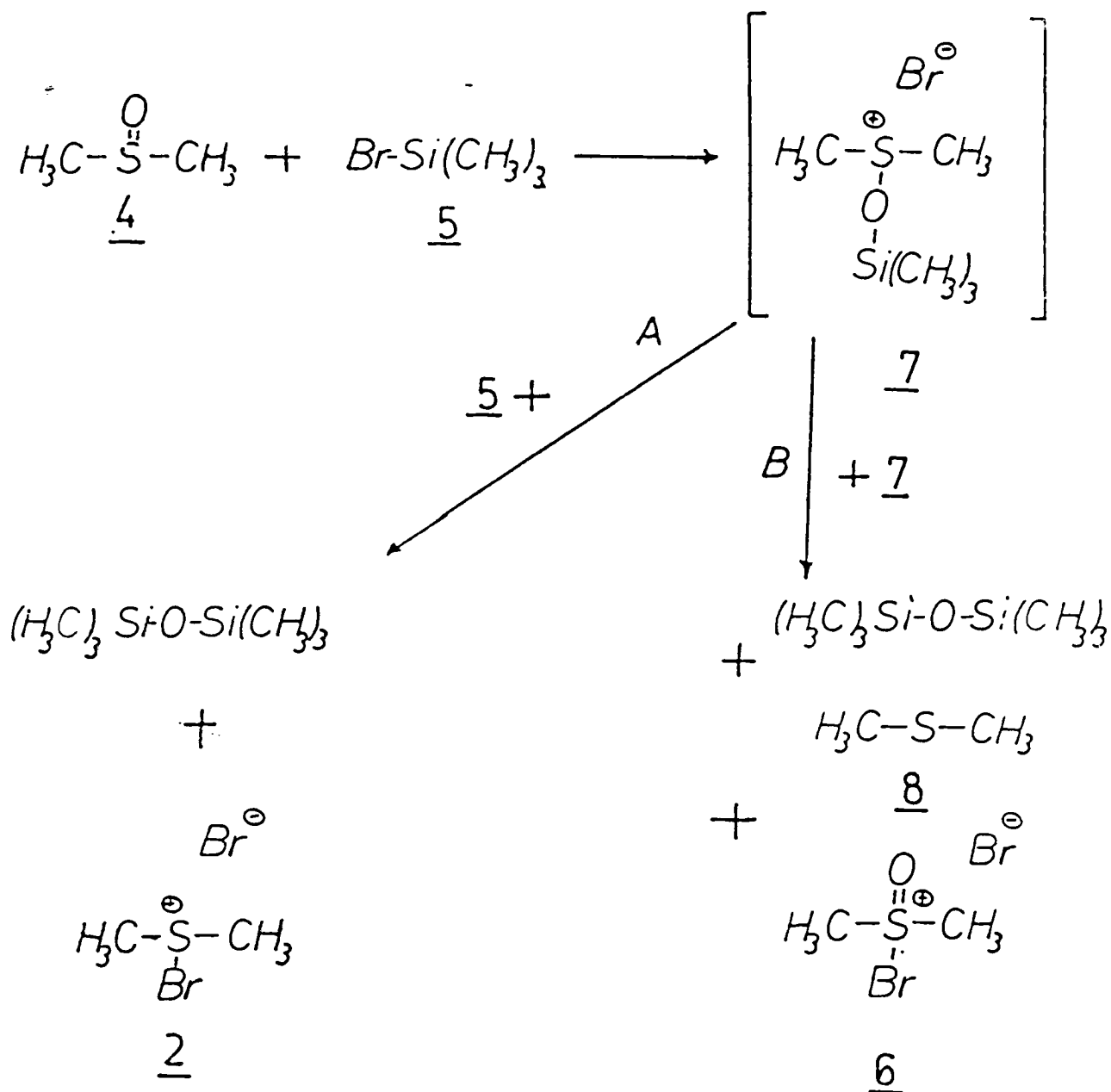
These are only a few examples out of many reactions carried out by Dr.Gabor's research group which show that halogenation can take place not only at the vinyl position but also on the aromatic ring using previously stated conditions (**scheme 3 below**).



$$X = Cl, Br$$
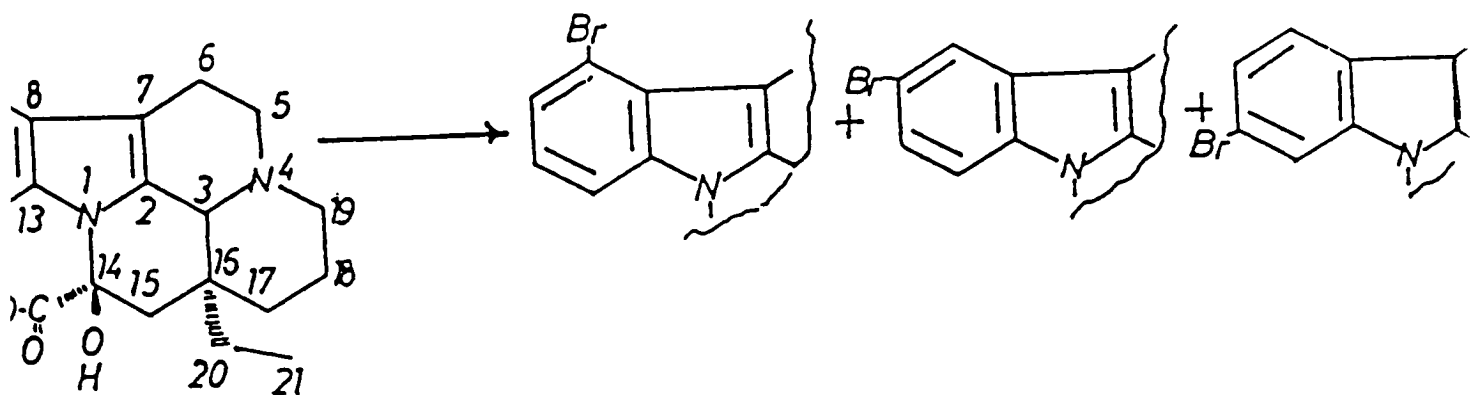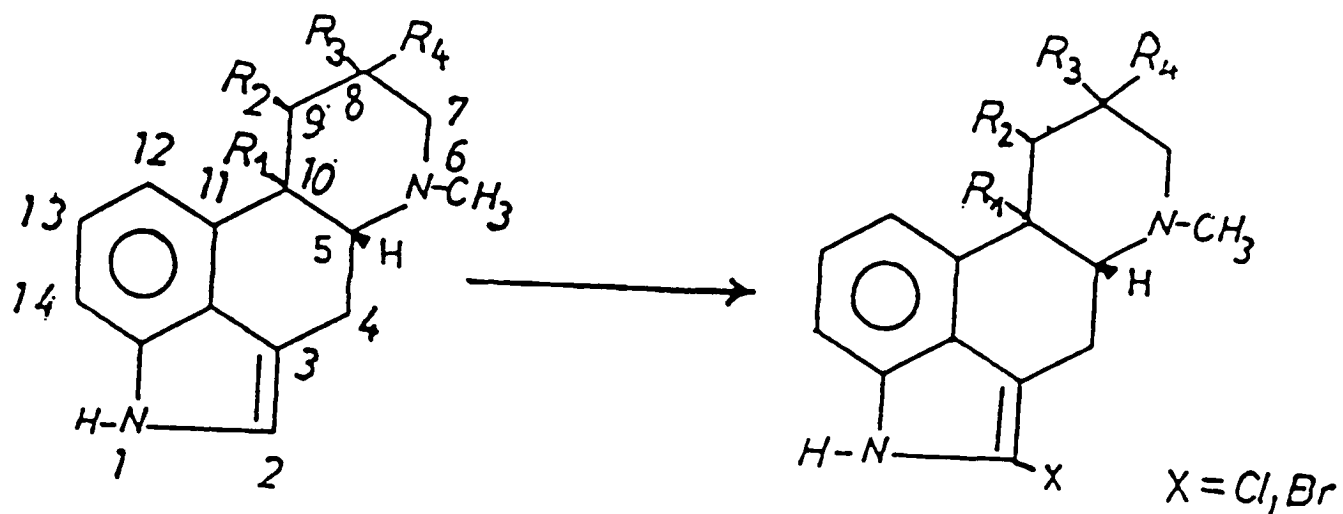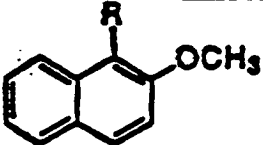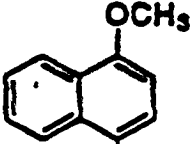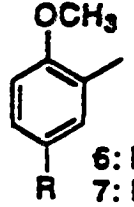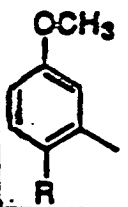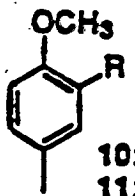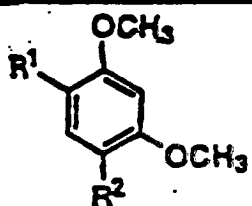
In 1997, pioneer work was done by Dr.George Majetich's research group when they presented a myriad of reaction conditions (**table1,2 and 3 below**)and procedures to specifically brominate various aromatic and heterocyclic compounds (20).

It is a distinct possibility that these conditions can be used to brominate aromatic macromolecules to give useful chemical compounds and produce few non-toxic byproducts, the reaction conditions are easy to follow and have the potential for reaction specificity, which could form targeted biomolecules. The utility of such halogenation conditions and reaction methodologies, which were not attempted earlier, were investigated.

## Table 1
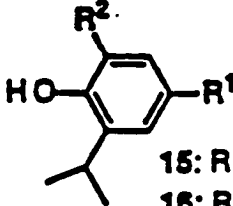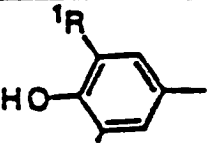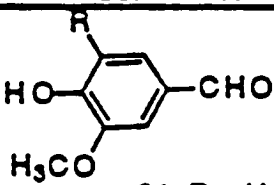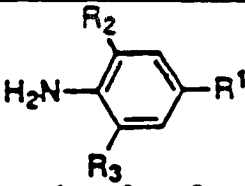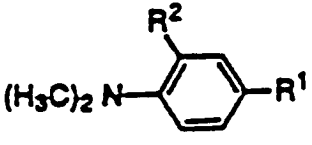
| solvent | reaction condns | yield of 3 (%) | reaction completion |
|---|---|---|---|
| DMSO | 2 h at rt | 96 | complete |
| acetonitrile | 32 h at rt | 84 | trace unreacted 2 |
| THF | 1 h at reflux | trace | only unreacted 2 |
| THF | 24 h at reflux | 99 | complete |
| THF/AcOH (3:1) | 2 h at rt | 50 | ~50% complete |
| AcOH/DMSO (2:1) | <5 min | 96 | complete |

## Table 2

| Substrate / Product | Percent Yield and Reaction Time Using Standard Conditions | | | |
| --- | --- | --- | --- | --- |
| | HBr, DMSO "A" | HBr, AcOH, DMSO "B" | $Br_2$, $CCl_4$ "C" | $Br_2$, AcOH "D" |
| (naphthalene, $OCH_3$ at 2, R at 1) 2: R = H 3: R = Br | 96% of 3 after 2-h | 96% of 3 after 5-min | 90% of 3 after 5-min | 89% of 3 after 5-min |
| (naphthalene, $OCH_3$ at 1, R at 4) 4: R = H 5: R = Br | 77% of 5 after 72-h | 97% of 5 after 24-h | 90% of 5 after 72-h | 85%[a] of 5 after 72-h |
| ($OCH_3$, methyl ortho, R para) 6: R = H 7: R = Br | 82% of 7 after 12-h | 86% of 7 after 12-h | 75% of 7 after 5-min | 75% of 7 after 2-h |
| ($OCH_3$, methyl meta, R ortho) 8: R = H 9: R = Br | 91% of 9 after 12-h | 90% of 9 after 12-h | 68%[a] of 9 after 20-min | 89%[a] of 9 after 20-min |
| ($OCH_3$, R ortho) 10: R = H 11: R = Br | 77% of 11 after 72-h | 75% of 11 after 72-h | 74% of 11 after 20-min | 91% of 11 after 5-min |
| ($R^1$, $OCH_3$, $OCH_3$, $R^2$) 12: $R^1$ = $R^2$ = H 13: $R^1$ = H; $R^2$ = Br 14: $R^1$ = $R^2$ = Br | 34% of 13 after 5-min / 89% of 14 after 20-min | 73% of 14 after 5-min / 87% of 14 after 20-min | 80%[a] of 14 after 5-min | 77% of 14 after 5-min |

[a] Product contained inseparable impurities. The yield shown is based on analysis of the ^{1}H NMR spectrum.

# Table 3

| Substrate / Product | Percent Yield and Reaction Time Using Standard Conditions | | | |
|---|---|---|---|---|
| | HBr, DMSO "A" | HBr, AcOH, DMSO "B" | Br$_2$, CCl$_4$ "C" | Br$_2$, AcOH "D" |
| <br>15: R$^1$ = R$^2$ = H<br>16: R$^1$ = Br; R$^2$ = H<br>17: R$^1$ = R$^2$ = Br | 90% of 16 after 24-h | 91% of 16 after 5-min | 23% of 16 and 64% of 17 after 5-min | 63% of 16 and 31% of 17 after 5-min |
| <br>18: R$^1$ = R$^2$ = H<br>19: R$^1$ = Br; R$^2$ = H<br>20: R$^1$ = R$^2$ = Br | 88% of 19 after 48-h | 87% of 19 after 2-h | 87% of 19 after 30-min | 83% of 19 after 30-min; trace 20 |
| <br>21: R = H<br>22: R = Br | 72% of 22 after 12-h | 57% of 22 after 12-h | 48% of 22 after 24-h | 80% of 22 after 20-min |
| <br>23: R$^1$ = R$^2$ = R$^3$ = H<br>24: R$^1$ = Br; R$^2$ = R$^3$ = H<br>25: R$^1$ = R$^2$ = Br; R$^3$ = H<br>26: R$^1$ = R$^2$ = R$^3$ = Br | 76% of 24 after 6-h | 74% of 24 after 24-h | 19% of 24; 33% of 25 and 30% of 26 after 5-min | 21% of 24; 41% of 25 and 31% of 26 after 5-min |
| <br>27: R$^1$ = R$^2$ = H<br>28: R$^1$ = Br; R$^2$ = H<br>29: R$^1$ = R$^2$ = Br | 43% of 28 after 24-h | 42% of 28 after 24-h | 60% of 28 after 5-min | 68% of 28 and 29% of 29 after 5-min |

$^a$ Inseparable byproducts were observed. The yield shown is based on analysis of the $^1$H NMR data.

**Scheme 4 and 5** show a few examples of many reactions carried out by Dr.Majetich's research group. Scheme 5 shows that under room temperature halogenation conditions one position on the aromatic ring of Veratrole (compound 30 in **scheme 5**) is halogenated and under severe reflux halogenation conditions more than one position on the aromatic ring of the same compound is halogenated.
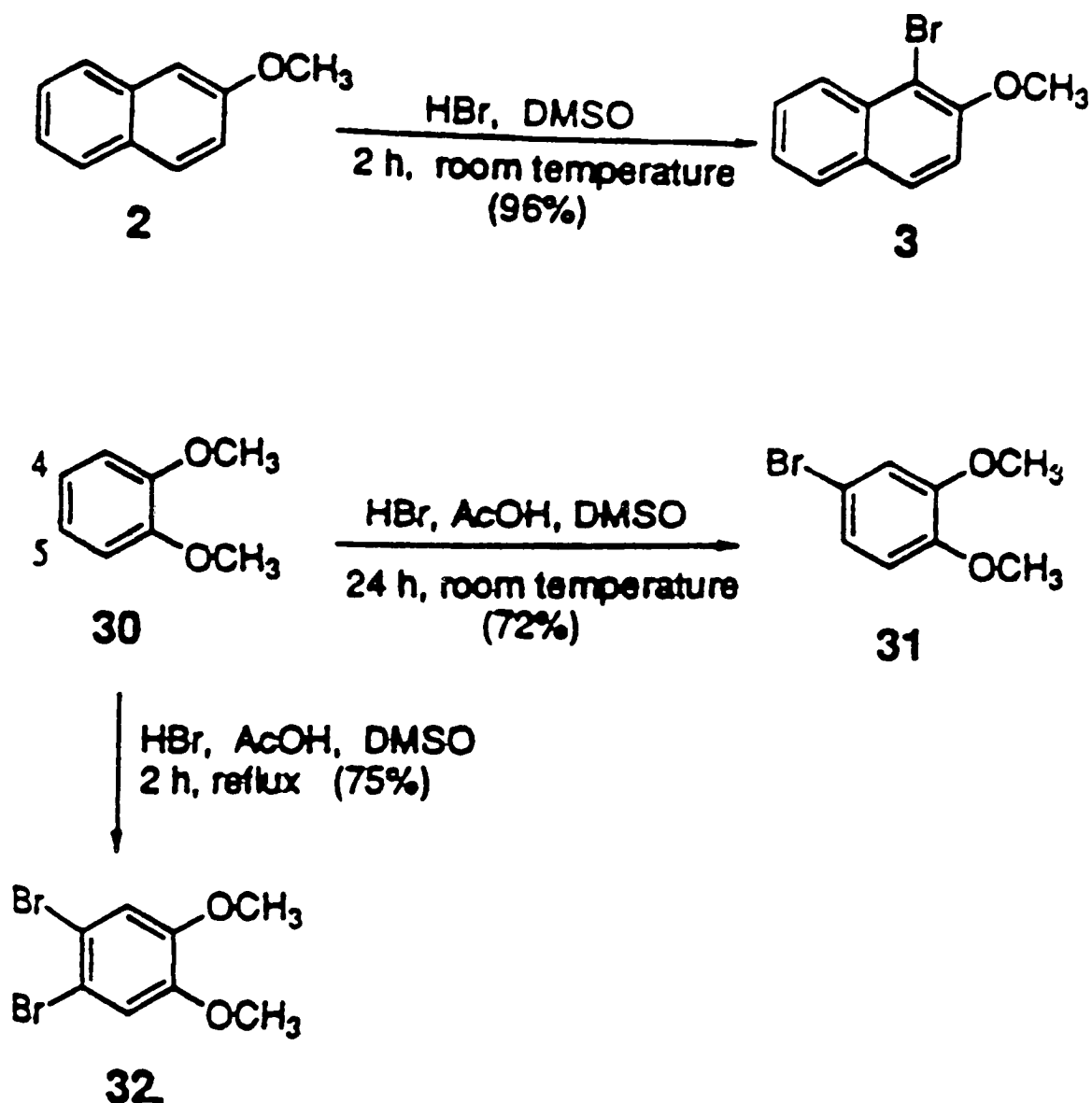
# EXPERIMENTAL WORK

The following experimental work was done in the Department of Chemistry at the Rochester Institute of Technology, Rochester, NY under the supervision of Dr.James J. Worman.

All nuclear magnetic resonance data were obtained on a 7 Tesla Bruker DRX – 300 300 MHz spectrometer. Deuterated dimethyl sulfoxide was used as the solvent for all samples.

All GC/MS data were obtained on a Hewlett Packard model HP190915S-936. The column used for the entire analysis was a capillary column (60.0mts * 250.0m * 0.25m in dimensions) tightly packed with HP-1MS-crosslinked Methyl Siloxane. Conditions employed were kept uniform during the entire analysis of samples. Uniform conditions used were constant flow of gas at the rate of 1ml/min and total flow being 33.4 ml/min under a total pressure of 35.23 psi, the temperature of the heater/injector was set at 250°C. The column was operated at a temperature range of 50°C-280°C. The temperature of the column was held at 50°C for one minute, then ramped at 20°C/min till 180°C then reached a final temperature of 280°C to which it was set at the rate 30°C/min and held for 2 minutes. Dimethyl Sulfoxide, Tetrahydrofuran and Methylene Chloride were used as diluting solvents during the GC/MS run of various samples.

Typical NMR and GC/MS spectra are shown in Appendix A and B respectively.

# THREE PHASES OF MY EXPERIMENTAL WORK INCLUDE

PHASE I :- involves the use of NMR to determine the formation of para substituted halogenated compounds of simple aromatic systems.

PHASE II :- involves the use of GC/MS analysis to verify the NMR results and to determine the isomeric distribution of halogenated species formed.

PHASE III :- involves the use of NMR and GC/MS analysis to determine the position of halogen substitution on the macro/biomolecules.

# TWO POINT APPROACH UTILIZED IN THE RESEARCH

1) Use of room temperature conditions for halogenation.

2) Ensure safe halogenation of aromatic systems by utilizing novel but simple laboratory techniques.

# REACTION MECHANISM

Two moles of a halo acid react with one mole of dimethyl sulfoxide. Halogen anion ($X^-$) of one mole of the halo acid attacks the sulfur of dimethyl sulfoxide and hydrogen cation H+ of the same halo acid attacks the oxygen of the dimethyl sulfoxide giving rise to a tetrahedral intermediate state. The hydrogen cation H+ of a second mole of the haloacid also attacks the oxygen of the hydroxy group to give a positive oxonium ion balanced by the halogen anion($X^-$). The halogen attached to the sulfur of the tetrahedral intermediate attacks the para position of the aromatic ring of an aromatic system with the simultaneous in-situ formation of the para substituted halogenated aromatic system, water and dimethyl sulfide.
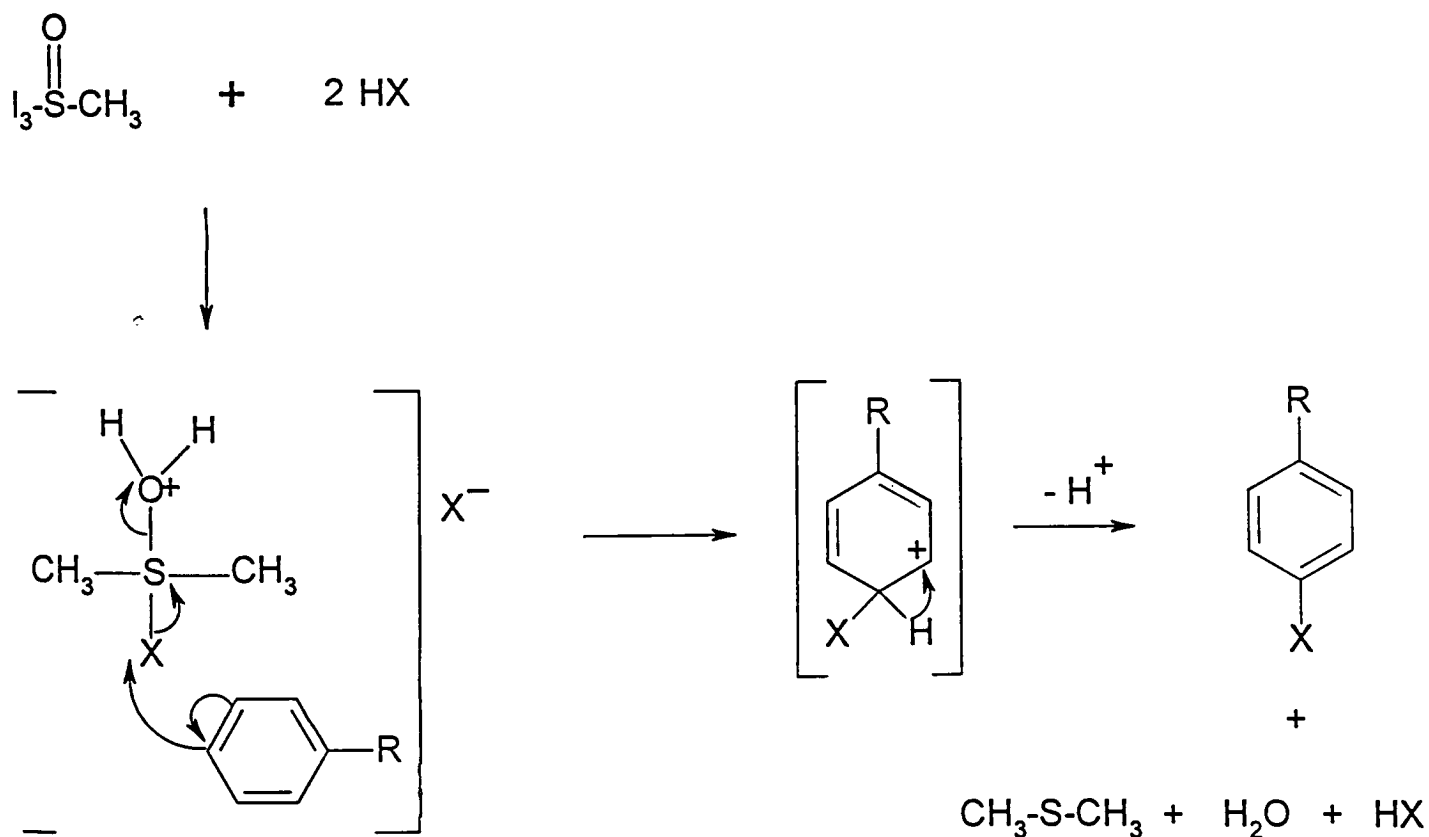
# EXPERIMENTAL PROCEDURE

**Aqueous Experimental Conditions:-** To two drops of N,N-Dimethylaniline in an NMR tube was added 1 ml of deuterated dimethyl sulfoxide followed by the addition of 2-4 drops of concentrated haloacid and the solution was left at room temperature for one to two hours. Thereafter, the NMR and GC/MS analysis was performed to determine the in-situ formation of para substituted halogenated aromatic species and other isomers (Typical NMR results are shown in **figure 2.** on next page.)

**Gaseous Experimental Conditions:-** To two drops Anisole in an NMR tube was added 1 ml of deutero DMSO followed by passing gaseous HCl at a controlled rate of one gas bubble/second for 30 seconds and then the solution was heated in an oil bath at the temperature of 100°C for one hour. The solution was left to stand at room temperature for one hour, afterwhich NMR and GC/MS analysis was performed to observe the in-situ formation of para substituted halogenated aromatic species (Typical NMR results are shown in **figure 2.** on next page.)

**Figure 2.** TYPICAL FOUR LINE PATTERN OF A PARA SUBSTITUTED

AROMATIC SYSTEM WITH TWO DIFFERENT SUBSTITUENTS
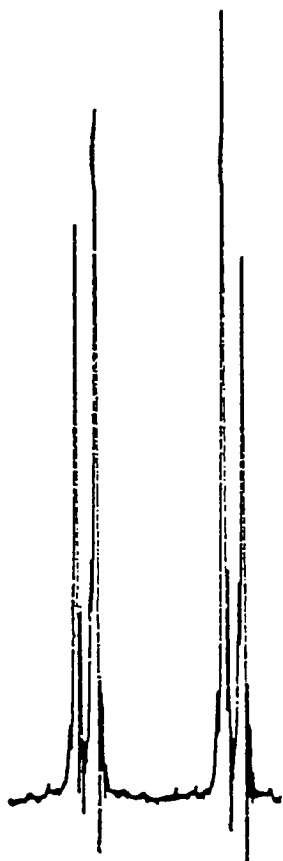


TYPICAL FOUR LINE PATTERN OF A PARA
SUBSTITUTED AROMATIC SYSTEM WITH
TWO DIFFERENT SUBSTITUENTS

# *EXPERIMENTAL PHASE I*

following aromatic systems were halogenated in an effort to establish environmentally benign room
iperature conditions for safe and simple halogenation experiments in an undergraduate laboratory.
he same time a search was made to develop the best halogenating reagents under room temperature
iditions which could be used for halogenation of biomolecules.
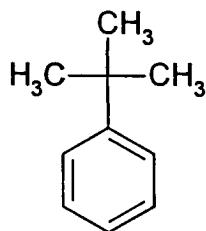
t-BUTYL BENZENE
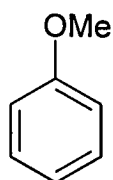
ANISOLE
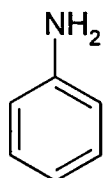
ANILINE
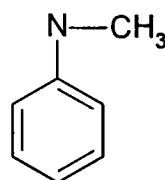
N METHYL ANILINE
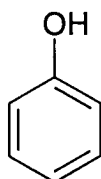
PHENOL
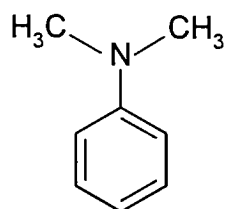
N,N DIMETHYL ANILINE

# Table 4 : LIST OF HALOGENATION REACTIONS AND NMR ANALYSIS RESULTS

| LIST OF REACTIONS | NMR RESULTS |
|---|---|
| TERT.BUTYLBENZENE +CONC.HF +DMSO | - ve |
| TERT.BUTYLBENZENE +CONC.HCl +DMSO | - ve |
| TERT.BUTYLBENZENE +CONC.HBr +DMSO | - ve |
| TERT.BUTYLBENZENE +CONC.HI +DMSO | - ve |
| TERT.BUTYLBENZENE +GASEOUS HCl + DMSO | - ve |
| ANISOLE + CONC.HF + DMSO | - ve |
| ANISOLE + CONC.HCl + DMSO | - ve |
| ANISOLE + CONC.HBr + DMSO | - ve |
| ANISOLE + CONC.HI + DMSO | - ve |
| ANISOLE + GASEOUS HCl + DMSO (HEATED IN OIL BATH FOR 1 Hr.) | + ve |
| ANILINE + CONC.HF + DMSO | - ve |
| ANILINE + CONC.HCl + DMSO | + ve |
| ANILINE + CONC.HBr + DMSO | + ve |
| ANILINE + CONC.HI + DMSO | - ve |
| ANILINE + GASEOUS HCl + DMSO | + ve |
| N-METHYLANILINE + CONC. HF + DMSO | ve |
| N-METHYLANILINE + CONC.HCl + DMSO | + ve |
| N-METHYLANILINE + CONC.HBr. + DMSO | + ve |
| N-METHYLANILINE + CONC.HI + DMSO | - ve |
| N-METHYLANILINE + GASEOUS HCl + DMSO | + ve |
| N,N-DIMETHYLANILINE + CONC. HF + DMSO | - ve |
| N,N-DIMETHYLANILINE + CONC. HCl + DMSO | + ve |
| N,N-DIMETHYLANILINE + CONC. HBr + DMSO | + ve |

| N,N-DIMETHYLANILINE + CONC. HI + DMSO | - ve |
|---|---|
| N,N-DIMETHYLANILINE + GASEOUS HCl + DMSO | + ve |
| PHENOL + CONC.HF + DMSO | ve |
| PHENOL + CONC.HCl + DMSO | + ve |
| PHENOL + CONC.HBr + DMSO | + ve |
| PHENOL + CONC.HI + DMSO | - ve |
| PHENOL + GASEOUS HCl + DMSO | + ve |

+ ve means that typical four line pattern of a para sustituted aromatic system was seen during NMR analysis

- ve means that typical four line pattern of a para substituted aromatic system was not seen during NMR analysis

# *EXPERIMENTAL PHASE II*

## Table 5 : NMR RESULTS(PARA, META, ORTHO SUBSTIUTED HALOGENATED AROMATIC SYSTEMS)SUPPORTED BY GC/MS ANALYSIS

| S.N. | LIST OF REACTIONS | PARA PRODUCT | META PRODUCT | ORTHO PRODUCT | % FORMED OUT OF ALL THE COMPOUNDS ELUTED |
|------|-------------------|--------------|--------------|---------------|------------------------------------------|
| 1. | TERT.BUTYL - BENZENE + CONC. HF + DMSO | - ve | -ve | -ve | -ve |
| 2. | TERT.BUTYL - BENZENE + CONC. HCl + DMSO | - ve | - ve | - ve | - ve |
| 3. | TERT.BUTYL - BENZENE + CONC. HBr + DMSO | - ve | - ve | - ve | - ve |
| 4. | TERT.BUTYL - BENZENE + CONC. HI + DMSO | - ve | - ve | - ve | - ve |
| 5. | TERT.BUTYL - BENZENE + GAS. HCl + DMSO | - ve | - ve | - ve | - ve |
| 6. | ANISOLE + CONC. HF + DMSO | - ve | - ve | - ve | - ve |
| 7. | ANISOLE + CONC. HCl + DMSO | - ve | - ve | - ve | - ve |
| 8. | ANISOLE + CONC. HBr + DMSO | - ve | - ve | - ve | - ve |
| 9. | ANISOLE + CONC. HI + DMSO | - ve | - ve | - ve | - ve |
| 10. | ANISOLE + GAS. HCl + DMSO (HEATED IN OIL BATH FOR 2 Hrs.) | + ve | - ve | + ve | 29.15% p-Chloroanisole 8.77% o-Chloroanisole |
| 11. | ANILINE + CONC. HF + DMSO | - ve | - ve | - ve | - ve |
| 12. | ANILINE + CONC. HCl + DMSO | + ve | - ve | + ve | 26.36% p-Chloroaniline 4.85% o-Chloroaniline 2.13% 2,4-Dichloroaniline |
| 13. | ANILINE + CONC. HBr + DMSO | + ve | + ve | + ve | 20.46% p-Bromoaniline 4.10% m-Bromoaniline 2.01% 2,4-Dibromoaniline |

| 14. | ANILINE + CONC. HI + DMSO | - ve | - ve | - ve | - ve |
|---|---|---|---|---|---|
| 15. | ANILINE + GAS. HCl + DMSO | + ve | - ve | - ve | 22.56% p-Chloroaniline<br>5.06% o-Chloroaniline<br>3.21% 2,4-Dichoroaniline |
| 16. | N-METHYL - ANILINE + CONC. HF + DMSO | - ve | - ve | -ve | -ve |
| 17. | N-METHYL - ANILINE + CONC. HCl + DMSO | + ve | + ve | + ve | 38.75% p-Chloro-N-methyl Aniline<br>9.25% m-Chloro-N-methyl Aniline<br>0.46% o-Chloro-N-methyl Aniline<br>1.19% 2,4-Dichloro-N-methyl Aniline |
| 18. | N-METHYL - ANILINE + CONC. HBr + DMSO | + ve | - ve | - ve | 58.57% p-Bromo-N-methyl Aniline<br>5.01% 2,4-Dibromo-N-methyl Aniline |
| 19. | N-METHYL - ANILINE + CONC. HI + DMSO | - ve | - ve | - ve | - ve |
| 20. | N-METHYL - ANILINE + GAS. HCl + DMSO | + ve | + ve | + ve | 35.05% p-Chloro-N-methyl Aniline<br>6.23% m-Chloro-N-methyl Aniline<br>1.08% o-Chloro-N-methyl Aniline<br>0.77% 2,4-Dichloro-N-methyl Aniline |
| 21. | N,N-DIMETHYL - ANILINE + CONC. HF + DMSO | - ve | - ve | - ve | - ve |
| 22. | N,N-DIMETHYL - ANILINE + CONC. HCl +DMSO | + ve | - ve | - ve | 15.03% p-Chloro-N,N-dimethyl Aniline |
| 23. | N,N-DIMETHYL - ANILINE + CONC. HBr +DMSO | + ve | ve | - ve | 60.32% p-Bromo-N,N-dimethyl Aniline |
| 24. | N,N-DIMETHYL - ANILINE + CONC. HI + DMSO | - ve | - ve | - ve | - ve |

| 25. | N,N DIMETHYL - ANILINE + GAS. HCl + DMSO | + ve | - ve | - ve | 17.78% p-Chloro-N,N-dimethyl Aniline |
|---|---|---|---|---|---|
| 26. | PHENOL + CONC. HF + DMSO | - ve | - ve | - ve | - ve |
| 27. | PHENOL + CONC. HCl + DMSO | + ve | - ve | - ve | 4.57% p-Chloro phenol |
| 28. | PHENOL + CONC. HBr + DMSO (2 DIFFERENT GC/MS RESULTS) | A) + ve<br><br>B) + ve | + ve<br><br>+ ve | - ve<br><br>+ ve | 12.86% p- & m-Bromo phenol in equal quantities<br><br>15.39% p-Bromo phenol<br>11.37% m-Bromo phenol<br>7.01% o-Bromo phenol |
| 29. | PHENOL + CONC. HI + DMSO | - ve | - ve | - ve | - ve |
| 30. | PHENOL + GAS. HCl. + DMSO | + ve | - ve | - ve | 5.03% p-Chloro phenol |

# *EXPERIMENTAL PHASE III*

The following macro/biomolecules were selected for halogenation after thorough study of the Merck Index (21) and other organic medicinal chemistry books (22, 23, 24). They were halogenated with a possibility of enhancing their activity and forming a minimal quantity of toxic byproducts.
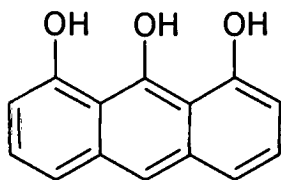


DITHRANOL

1,8,9 ANTHRACENETRIOL

USED IN DERMATOLOGIC PREPARATIONS AND ANTIFUNGAL PREPARATIONS
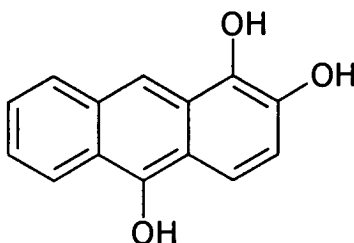


ANTHRAROBIN

1,2,10 ANTHRACENETRIOL

USED AS PARASITICIDE
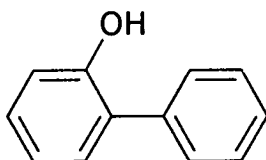


O- PHENYL PHENOL (DOWICIDE I)

[1,1'-BIPHENYL] 2 - OL

USED IN GERMICIDE AND FUNGICIDE PREPARATIONS
AND IN COMMERCIAL RUBBER INDUSTRY
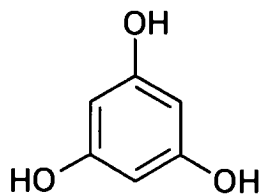
PHLOROGLUCINOL (1,3,5 BENZENETRIOL)

USED AS AN ANTISPASMODIC
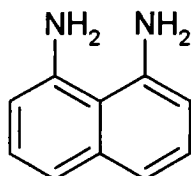


1,8 DIAMINO NAPTHALENE

USED AS AN ANTIOXIDANT FOR LUBRICATING OILS AND FOR THE DETECTION OF SELENIUM AND NITRITES
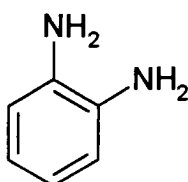


O- PHENYLENEDIAMINE(1,2- BENZENEDIAMINE)

USED IN THE MANUFACTURE OF DYES
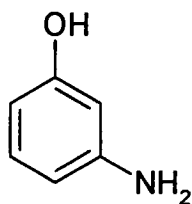
3 AMINO PHENOL

USED IN THE MANUFACTURE OF ANTITUBERCLAR AGENT P- AMINO SALICYLIC ACID(PAS)
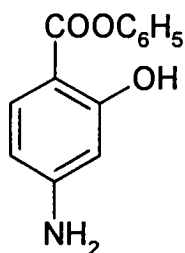


PHENYL AMINO SALICYLATE (PHENYL PAS)

USED AS AN ANTIBACTERIAL (TUBERCULOSTATIC)
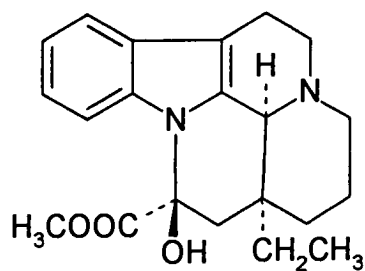


VINCAMINE

USED AS VASODILATOR
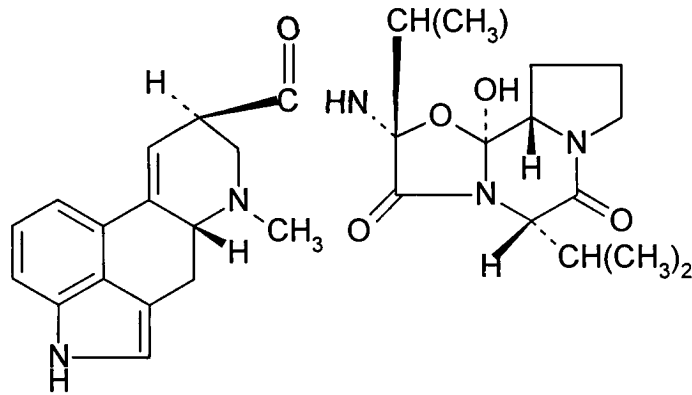
**ERGOCORNINE**

USED AS VASOCONSTRICTOR (SPECIFIC IN MIGRAINE).

IT HAS BEEN USED AS AN OXYTOCIC IN VETERINARY MEDICINE
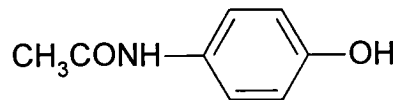


**4 ACETAMIDOPHENOL**

USED AS AN ANTIPYRETIC AND ANALGESIC

IT HAS BEEN USED COMMERCIALLY FOR THE MANUFACTURE OF AZO DYES
AND PHOTOGRAPHIC CHEMICALS
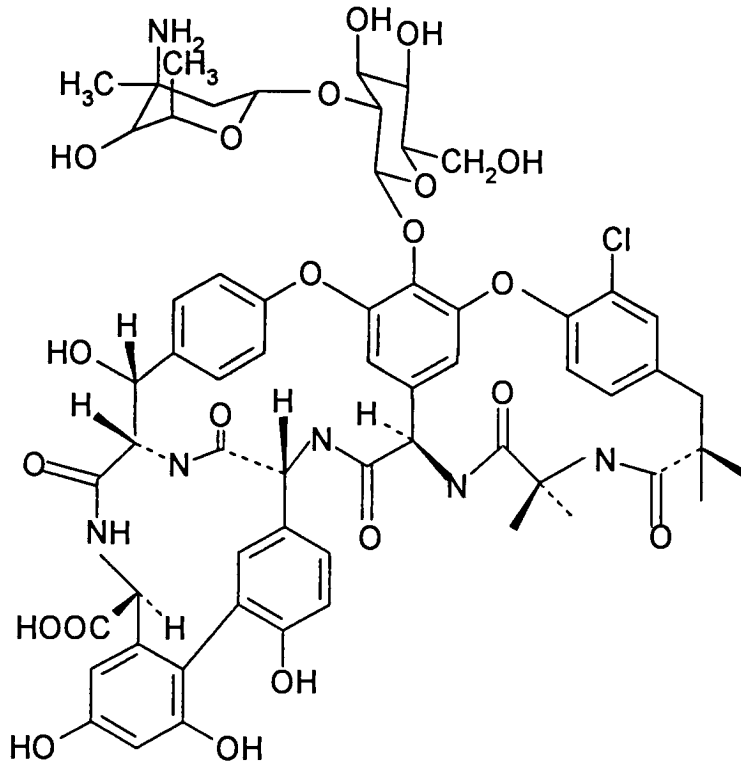
VANCOMYCIN (VANCOCIN)

NTIBIOTIC SUBSTANCE PRODUCED BY STREPTOMYCES ORIENTALIS FROM INDONESIAN AND INDIAN SOIL.

USED AS AN ANTIBACTERIAL

## TABLE 6 : LIST OF HALOGENATION REACTIONS WITH MACRO/BIOMOLECULES AND NMR ANALYSIS RESULTS

| LIST OF REACTIONS | NMR RESULTS |
|---|---|
| DITHRANOL + CONC. HCl + DMSO | UNINTERPRETABLE |
| DITHRANOL + CONC. HBr + DMSO | UNINTERPRETABLE |
| ANTHRAROBIN + CONC. HCl + DMSO | UNINTERPRETABLE |
| ANTHRAROBIN + CONC. HBr + DMSO | UNINTERPRETABLE |
| O- PHENYLPHENOL + CONC. HCl + DMSO | UNINTERPRETABLE |
| O- PHENYLPHENOL + CONC. HBr + DMSO | UNINTERPRETABLE |
| PHLOROGLUCINOL + CONC. HCl + DMSO | UNINTERPRETABLE |
| PHLOROGLUCINOL + CONC. HBr + DMSO | UNINTERPRETABLE |
| 1,8 DIAMINONAPTHALENE + CONC. HCl + DMSO | UNINTERPRETABLE |
| 1,8 DIAMINONAPTHALENE + CONC. HBr + DMSO | UNINTERPRETABLE |
| O- PHENYLENEDIAMINE + CONC. HCl + DMSO | UNINTERPRETABLE |
| O- PHENYLENEDIAMINE + CONC. HBr + DMSO | UNINTERPRETABLE |
| 3 AMINOPHENOL + CONC. HCl + DMSO | UNINTERPRETABLE |
| 3 AMINOPHENOL + CONC. HBr + DMSO | UNINTERPRETABLE |
| PHENYLAMINO SALICYLATE + CONC. HCl + DMSO | UNINTERPRETABLE |
| PHENYLAMINO SALICYLATE + CONC. HBr + DMSO | UNINTERPRETABLE |
| 4 ACETAMIDOPHENOL + CONC. HCl + DMSO | UNINTERPRETABLE |
| 4 ACETAMIDOPHENOL + CONC. HBr + DMSO | UNINTERPRETABLE |
| VINCAMINE + CONC.HCl + DMSO | UNINTERPRETABLE |
| VINCAMINE + CONC. HBr + DMSO | UNINTERPRETABLE |

| ERGOCORNINE + CONC. HCl + DMSO | UNINTERPRETABLE |
|---|---|
| ERGOCORNINE + CONC. HBr + DMSO | UNINTERPRETABLE |
| VANCOMYCIN + CONC. HCl +DMSO | UNINTERPRETABLE |
| VANCOMYCIN + CONC. HBr + DMSO | UNINTERPRETABLE |

## TABLE 7 : GC/MS ANALYSIS OF HALOGENATED PRODUCTS FORMED AS A RESULT OF HALOGENATION REACTIONS OF MACRO/BIOMOLECULES USING THE SAME CONDITIONS

| S.N | LIST OF REACTIONS | HALOGENATED PRODUCT FORMED | PERCENTAGE PRODUCT FORMED |
|---|---|---|---|
| 1. | DITHRANOL + CONC. HCl + DMSO | - ve | - ve |
| 2. | DITHRANOL + CONC. HBr + DMSO | - ve | - ve |
| 3. | ANTHRAROBIN + CONC. HCl + DMSO | - ve | - ve |
| 4. | ANTHRAROBIN + CONC HBr + DMSO | - ve | - ve |
| 5. | O- PHENYLPHENOL + CONC. HCl + DMSO | A) 5-Chloro-o-Phenyl phenol<br>B) 3-Chloro-o-Phenyl phenol<br>C) 2,2',3,4,5-pentaChloro-o-Phenyl phenol | 3.57%<br>2.08%<br>0.4% |
| 6. | O- PHENYLPHENOL + CONC. HBr + DMSO | A) 3,5-Dibromo-o-Phenyl phenol<br>B) Bromophenoxy-Benzene | 31.38%<br>3.78% |
| 7. | PHLOROGLUCINOL + CONC. HCl + DMSO | - ve | - ve |
| 8. | PHLOROGLUCINOL + CONC. HBr + DMSO | - ve | - ve |
| 9. | 1,8 DIAMINO - NAPTHALENE + CONC. HCl + DMSO | - ve | - ve |
| 10. | 1,8 DIAMINO - NAPTHALENE + CONC. HBr + DMSO | - ve | - ve |
| 11. | O-PHENYLENEDIAMINE + CONC.HCl + DMSO | - ve | - ve |
| 12. | O-PHENYLENEDIAMINE + CONC.HBr + DMSO | - ve | - ve |
| 13. | 3 AMINOPHENOL +CONC. HCl + DMSO | 2,4-Dichloro 3-amino phenol | 4.00% |
| 14. | 3 AMINOPHENOL + CONC. HBr + DMSO | 2,4-Dibromo 3-amino phenol | 18.35% |
| 15. | PHENYLAMINO - SALICYLATE + CONC. HCl + DMSO | - ve | - ve |

| 16. | PHENYLAMINO - SALICYLATE + CONC. HBr + DMSO | - ve | - ve |
|-----|----------------------------------------------|------|------|
| 17. | 4 ACETAMIDOPHENOL + CONC. HBr + DMSO | - ve | - ve |
| 18. | 4 ACETAMIDOPHENOL + CONC. HCl + DMSO | - ve | - ve |
| 19. | VINCAMINE + CONC. HCl + DMSO | ve | - ve |
| 20. | VINCAMINE + CONC. HBr + DMSO | - ve | - ve |
| 21. | ERGOCORNINE + CONC. HCl + DMSO | - ve | - ve |
| 22. | ERGOCORNINE + CONC. HBr + DMSO | - ve | - ve |
| 23. | VANCOMYCIN + CONC. HCl + DMSO | - ve | - ve |
| 24. | VANCOMYCIN + CONC. HBr + DMSO | - ve | - ve |

# RESULTS AND DISCUSSION

## SIGNIFICANCE OF THE RESULTS ACHIEVED:-

1) Environmentally benign conditions were established for safe and simple halogenation experiments in the undergraduate laboratory

2) NMR and GC/MS analysis were used to verify the formation of specific isomers

## POTENTIAL USE:-

1) Synthesis of halogenated biomolecules sensitive to pathogenic microbes in an attempt to enhance their bioactivity and reduce toxicity.

2) Minimizing environmental pollution, lessening hazards and ensuring safe disposal of waste products and toxic byproducts specifically to demonstrate electrophilic aromatic substitution in the undergraduate laboratory.

# CONCLUSION

Aqueous HBr and HCl in the presence of DMSO prove to be good halogenating reagents of aromatic systems in the reactions previously described. Aqueous HI shows very little or no reactivity at all even though it is a stronger acid and should protonate the DMSO more effectively. Aqueous HF was also used, but there was no evidence that a reaction took place. This would be expected because fluorine does not like to be even partially positive, which is a necessity for electrophilic aromatic substitution. Gaseous HCl has almost the same reactivity as aqueous HCl. The only advantage in using gaseous HCl over aqueous HCl is that water interferes during NMR or GC/MS analysis of the reaction samples. The disadvantage in using gaseous HCl is that it is hazardous to use gas cylinders in undergraduate laboratories which besides being expensive, can pose a great safety hazard, cause environmental pollution and lead to unsafe handling problems.

The NMR data were uninterpretable for the reactions of macro/biomolecules in the presence aqueous acids and DMSO, because it seemed that water present in haloacids and deuterated DMSO(which absorbs water readily) was interfering with the final results, and the expected NMR pattern was not showing up clearly in the final results. Gaseous HCl was used for some of the reactions instead of aqueous HCl acid, but again because of interference of water present in DMSO (which appeared as a dominant peak in the NMR spectrum) and also

because of the complex aromatic region of most of these macro/biomolecules, the NMR spectrum was difficult to interpret.

Since all macro/biomolecules selected had –OH and –NH$_2$ as activating groups, and conditions for halogenation were also set according to those used for halogenating simple aromatic systems, it was assumed that some kind of halogenation reaction is taking place in the NMR tube even though NMR patterns were uninterpretable. Most of the reactions with macro/biomolecules did not show any positive GC/MS results. In some cases it was concluded that since most of these macromolecles have complex chemical structures with polar and non-polar groups and high boiling points, they tend to stick to the methyl siloxane GC/MS column and do not elute. This can be supported by the fact that even the parent molecule of these macro/biomolecules did not elute from the column. In some cases, as with Vancomycin and Phenyl Amino Salicylate, fragments of the molecules which were halogenated eluted from the column. It was inferred that halogenation might be taking place on the complex aromatic structure of these molecules, but since they did not elute as whole molecules during GC/MS analysis the above statement cannot be supported by any kind of evidence. Molecules like O-Phenyl phenol and 3-Amino phenol showed GC/MS evidence of halogenation when their halogenated products were successfully eluted from the GC/MS column by using a different eluting solvent i.e. CH$_2$Cl$_2$ rather than regular DMSO or THF. From this, a conclusion can be drawn that infact some macro/biomolecules can be halogenated using the conditions established for halogenation. Slight modification of the

halogenation conditions used and the use of suitable diluting and eluting solvents for analysis could be useful.

During GC/MS analysis, the percentage of halogenated products formed from all the products eluted from the GC/MS column and reported in tables 5 and 7 are not in very high yields. Many of the products could have been obtained from previous analysis and eluted with the solvents used in our GC/MS method of analysis. Isomer percentages were based on the area of individual isomers divided by the total area observed for all the products eluting from the GC/MS column.

Whatever the limitations, it can be successfully concluded from extensive halogenation reactions with simple aromatic systems that the reactions of haloacids with DMSO can be used to demonstrate electrophilc aromatic substitution in an undergraduate laboratory.

Similar conditions with slight modifications can be used to halogenate some bio/macromolecules.

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# APPENDIX A

Page

# APPENDIX B

# APPENDIX C

# APPENDIX D

Page

**APPENDIX A**

## Spectrum 1.

Hz

2181.51
2175.20
2144.85
2112.99
2111.65
2104.81
2097.50
2053.22
2045.66
2034.55
2027.15
2017.93
2008 47
1976.89
1968.69
1958.12
1950.87
1943.65
1898.55
1890.11
1870.44

2339.81 Hz to 1824.40 Hz changed!
22.503 Hz/cm changed!

J1Hsch/Ju113-00/1/1
Y exp. factor:       1  1
Int. plot exp. factor:

Integral

66 276

ppm  7.7  7.6  7.5  7.4  7.3  7.2  7.1  7.0  6.9  6.8  6.7  6.6  6.5  6.4  6.3  6.2

## ANILINE (STANDARD) + DMSO

## Spectrum 2.

Hz

2130.13
2104.33
2098.49
2092.01
2089.81
2078.45
2076.35
2069.63
2061.50
2058.19
2056.23
2051.60
2049.49
2046.32
2016.80
2000.58
1997.79
1989.15
1976.88
1972.93
1969.00
1966.33
1960.63
1951.89
1940.36
1938.14
1931.59
1923.51
1920.22
1918.17
1913.56
1911.51
1908.27
1878.13
1839.12
1832.82
1829.86
1803.94
1798.43

2136.13 Hz to 1775.95 Hz changed!
15.002 Hz/cm changed!

J1Hsch/Ju113-00/3/1
Y exp. factor:       1  1
Int. plot exp. factor:

Integral

53.735

ppm  7.0  6.9  6.8  6.7  6.6  6.5  6.4  6.3  6.2  6.1  6.0

## ANILINE + AQUEOUS HCl + DMSO

## Spectrum 3.



**ANILINE + AQUEOUS HBr + DMSO**

## Spectrum 4.

Hz

3898.00
3890.82
3883.61
3861.02
3853.64
3845.22
3839.17
3821.23
3819.33
3813.84
3805.36
3767.70
3760.61
3754.12
3742.28
3734.88
3727.74
3721.00
3713.77
3708.31
3700.98
3688.49
3681.22
3674.75
3673.85
3660.65
3659.35
3646.77
3630.35
3621.80
3607.91
3600.40
3590.55
3569.73
3561.95
3512.44
3504.76
3502.33

3984.95 Hz to 3242.73 Hz
30.004 Hz/cm changed!

Y exp. factor    1
Int. plot exp. factor.    1

JJWSCH/JJ113-00//5/1

Integral

54 880

ppm  7.7    7.6    7.5    7.4    7    7.2    7.1    7.0    6.9    6.8    6.7    6.6    6.5

## N METHYL ANILINE (STANDARD) + DMSO

## Spectrum 5.

Hz
2371.83

2301.34
2292.67

2213.28
2210.74
2206.00
2204.17
2203.03
2201.68
2170.82
2161.97
2150.88
2141.98
2134.47
2131.11
2129.02
2124.41
2122.31
2118.98
2109.89
2107.75
2103.19
2101.14
2097.90
2088.53
2049.41
2048.19
2045.95
2041.68
2038.96
2031.13
2000.93
1992.06
1990.42
1977.04
1974.92
1970.47
1968.30
1964.57
1961.23
1959.12
1954.50
1952.40
1949.08
1932.51
1882.02
1880.03

2379.36 Hz to 1878.65 Hz
22.503 Hz/cm changed!

Y exp. factor    1
Int. plot exp. factor.    1

JJWSCH/JJ113-00///1

Integral

47 576

ppm  7.8    7.7    7.6    7.5    4    7.3    7.2    7.1    7.0    6.9    6.8    6.7    6.6    6.5    6.4    6.3

## N METHYL ANILINE + AQUEOUS HCl + DMSO

Spectrum 6.



N METHYL ANILINE + AQUEOUS HBr + DMSO

## Spectrum 7.
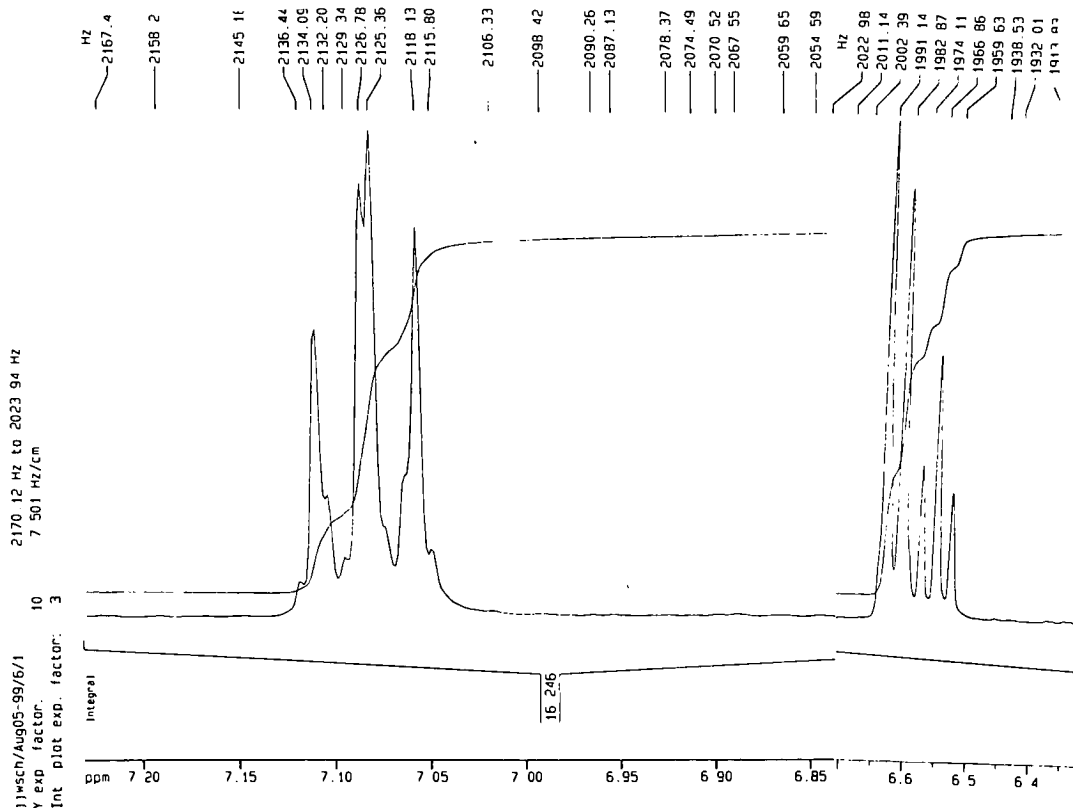


**N,N DIMETHYL ANILINE (STANDARD) + DMSO**

## Spectrum 8.



**N,N DIMETHYL ANILINE + AQUEOUS HCl + DMSO**

## Spectrum 9.



2388.03 Hz to 2133.72 Hz
15.002 Hz/cm changed

)1wscn/Aug11-99/2/1
Y exp factor
Int. plot exp. factor

2269.78
2260.59

2229.38
2225.72
2220.35
2216.92
2209.22

2193.00
2189.44
2187.29
2182.57
2180.40
2176.91

2037.56
2030.85
2028.65
2022.08
2017.26

2001.05
1997.56
1995.44
1990.58
1988.50
1984.98

Integral: 14.198    15.118

ppm 7.9   7.8   7.7   7.6   7.5   7.4   7.3   7.2   6.8   6.7   6.6   6.5

# N,N DIMETHYL ANILINE + AQUEOUS HBr + DMSO

## Spectrum 10.



2333.02 Hz to 1967.19 Hz
15.002 Hz/cm changed

)1wscn/Oct08-99/75/1
Y exp. factor.
Int. plot exp. factor.

2270.14
2262.85
2255.61

2232.02
2225.07
2218.06
2200.30
2194.34
2192.19
2190.20
2187.09
2184.89
2183.48
2181.32
2178.13
2176.18
2173.85
2168.38
2157.22
2142.65
2136.61
2128.67

2096.92
2088.83

2070.20
2062.92
2055.66

Integral: 25.148

ppm 7.7   7.6   7.5   7.4   7.3   7.2   7.1   7.0   6.9   6.8

# N,N DIMETHYL ANILINE +AQUEOUS HI + DMSO

**PHENOL (STANDARD) + DMSO**

Spectrum 12.
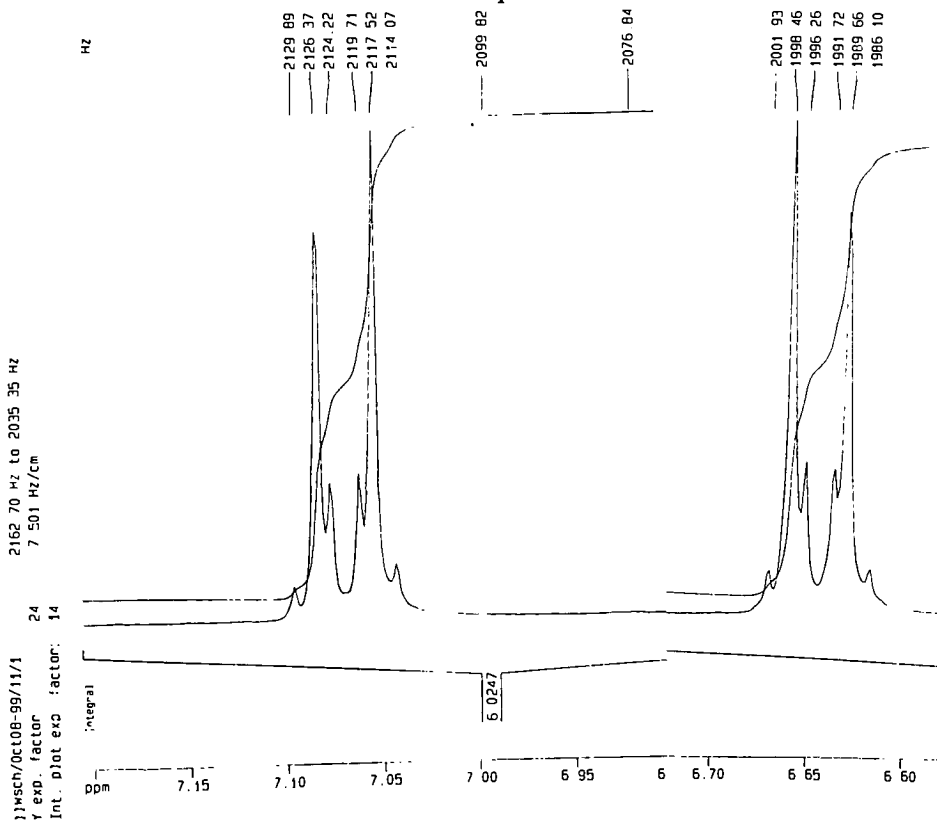
**PHENOL + AQUEOUS HCl + DMSO**
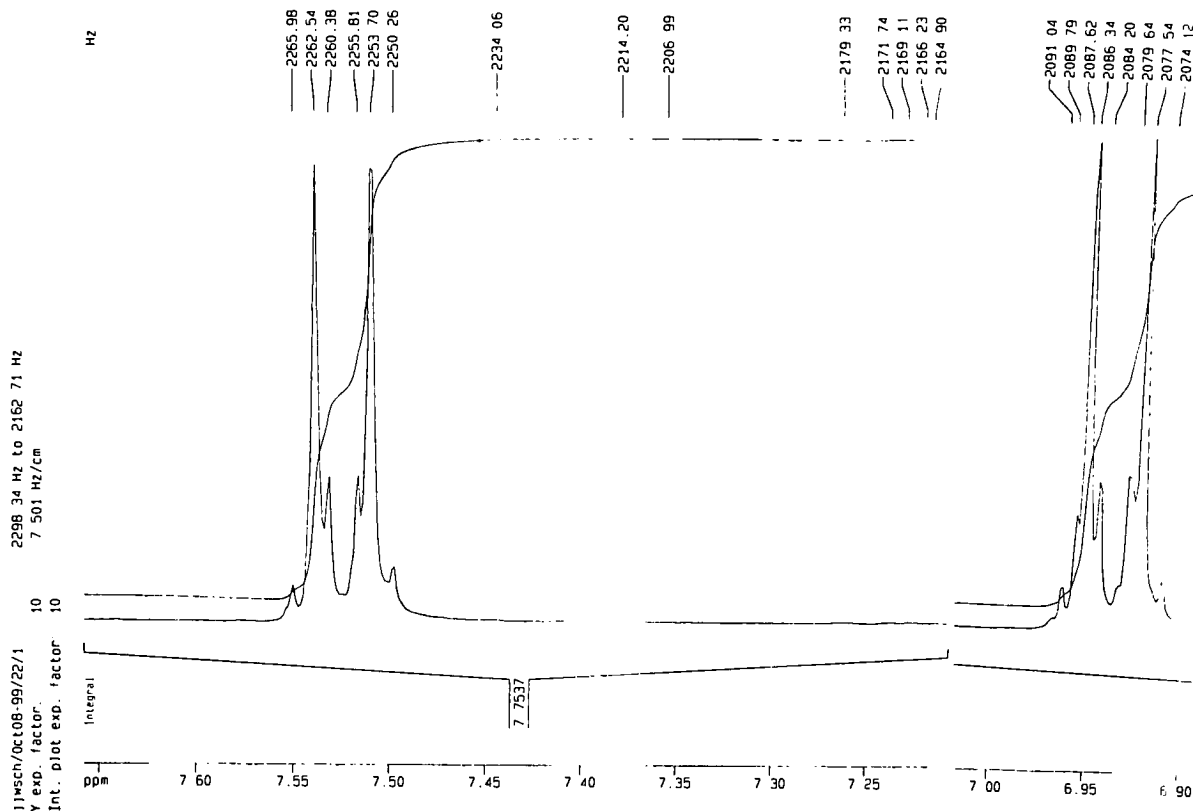
# PHENOL + AQUEOUS HBr + DMSO
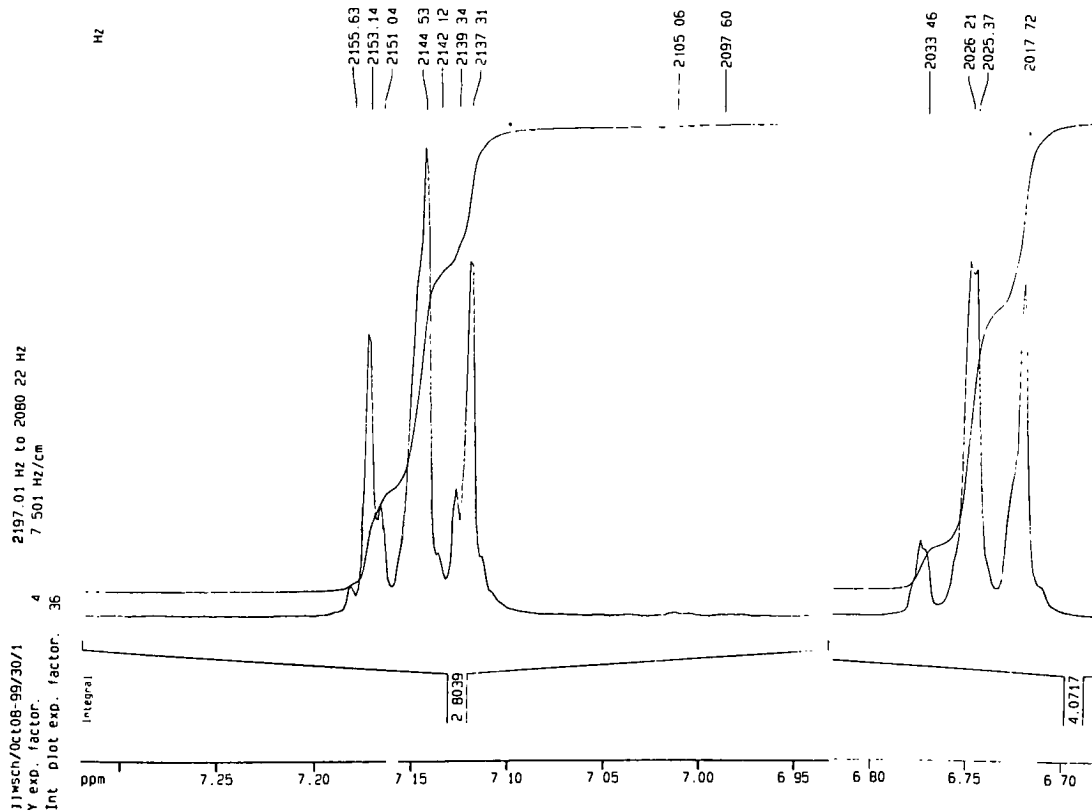
# PHENOL + AQUEOUS HI + DMSO

## Spectrum 15.

Hz

2207.08
2199.89
2192.50
2169.46
2161.72
2153.58
2149.20
2141.50
2131.60
2128.99
2121.25
2117.95
2113.16
2110.95
2097.20
2093.69
2088.86
2085.72
2075.12
2057.45
2048.90
2042.16
2039.65
2037.62
2034.68
2032.64
2019.26
2017.03
2014.05
2012.04
2010.33
2008.25
2005.56
1971.73
1930.23
1926.87

2377.36 Hz to 1924.11 Hz
22.503 Hz/cm changed!

4
1

Integral

53.506

jjwsch/Jul16-99/1/1
Y exp. factor:
Int. plot exp. factor.

ppm  7.8  7.7  7.6  7.5  7.4  7.3  7.2  7.1  7.0  6.9  6.8  6.7  6.6  6.5

# ANISOLE (STANDARD) + DMSO

## Spectrum 16.

Hz

2232.54
2230.29
2222.14

2197.59
2189.53
2180.53
2169.40
2160.33
2152.53
2149.00
2146.83
2142.21
2140.07
2136.51
2120.10
2116.57
2112.53
2109.17
2100.89
2087.03
2078.56
2069.59
2066.09
2062.74
2058.36
2055.74
2049.18
2041.62
2038.01
2035.77
2031.17
2029.05
2025.55
2009.00

2281.99 Hz to 1935.37 Hz
15.002 Hz/cm changed!

3
1

Integral

42.399

jjwsch/Oct08-99/105/1
Y exp. factor:
Int. plot exp. factor:

ppm  7.5  7.4  7.3  7.2  7.1  7.0  6.9  6.8  6.7

# ANISOLE + GASEOUS HCL + DMSO

Spectrum 17.

Current Data Parameters
NAME            Jul24-00
EXPNO               1
PROCNO              1

F2 - Acquisition Parameters
Date_          500000
Time            16 05
INSTRUM          spect
PROBHD    5 mm ONP 1H
PULPROG          zg30
TD              32768
SOLVENT          DMSO
NS                 16
DS                  2
SWH         6172 839 Hz
FIDRES      0 188380 Hz
AQ        2 6542580 sec
RG                256
DW            81 000 usec
DE             6 00 usec
TE             300 0 K
D1        1 00000000 sec
P1             9 00 usec
DE             6 00 usec
SF01      300 1318534 MHz
NUC1               1H
PL1           -5 00 dB

F2 - Processing parameters
SI              16384
SF        300 1300000 MHz
WDW                EM
SSB                 0
LB             0 30 Hz
GB                  0
PC               1 00

10 NMR plot parameters
CX            20 00 cm
F1P           11 000 ppm
F1          3301 43 Hz
F2P          -1 000 ppm
F2          -300 13 Hz
PPMCM       0 60000 ppm/cm
HZCM       180 07800 Hz/cm

Integral: 11 225    11 403    35 808    22 884    5 109    13 572

ppm   10      8      6      4      2      0
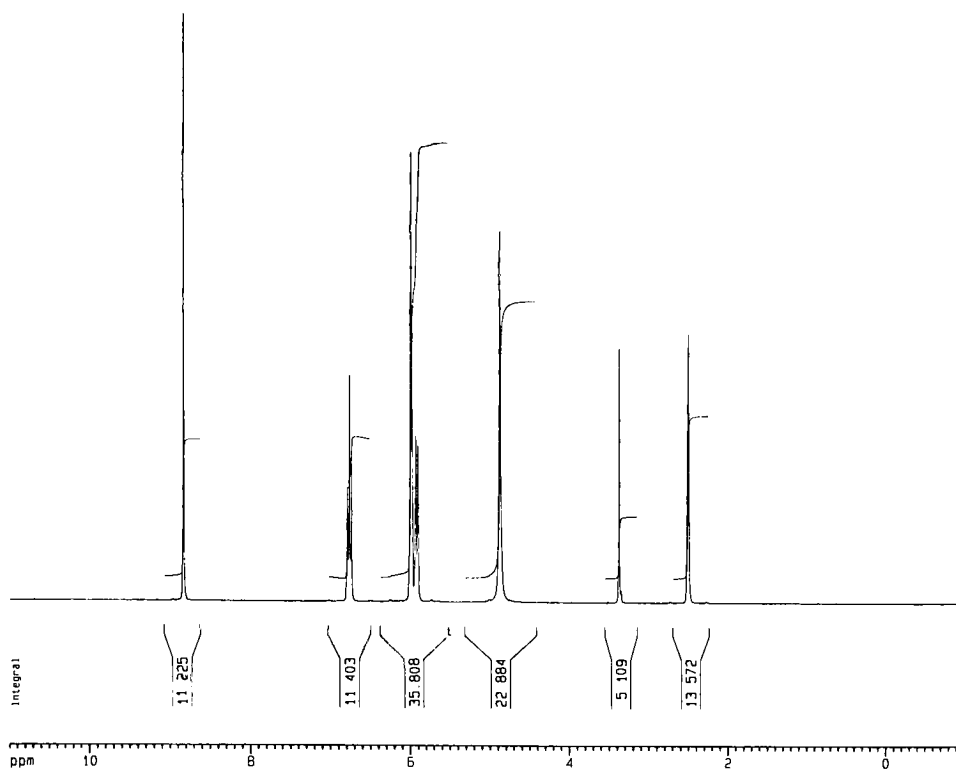
## O-PHENYL PHENOL (STANDARD) + DMSO

Spectrum 18.

Current Data Parameters
NAME            Jul24-00
EXPNO               2
PROCNO              1

F2 - Acquisition Parameters
Date_          500000
Time            16 18
INSTRUM          spect
PROBHD    5 mm ONP 1H
PULPROG          zg30
TD              32768
SOLVENT          DMSO
NS                 16
DS                  2
SWH         6172 839 Hz
FIDRES      0 188380 Hz
AQ        2 6542580 sec
RG               28.5
DW            81 000 usec
DE             6 00 usec
TE             300 0 K
D1        1 00000000 sec
P1             9 00 usec
DE             6 00 usec
SF01      300 1318534 MHz
NUC1               1H
PL1           -5 00 dB

F2 - Processing parameters
SI              16384
SF        300 1300393 MHz
WDW                EM
SSB                 0
LB             0 30 Hz
GB                  0
PC               1 00

10 NMR plot parameters
CX            20 00 cm
F1P           11 000 ppm
F1          3301 43 Hz
F2P          -1 000 ppm
F2          -300 13 Hz
PPMCM       0 60000 ppm/cm
HZCM       180 07802 Hz/cm

Integral: 98 907    1 093

ppm   10      8      6      4      2      0
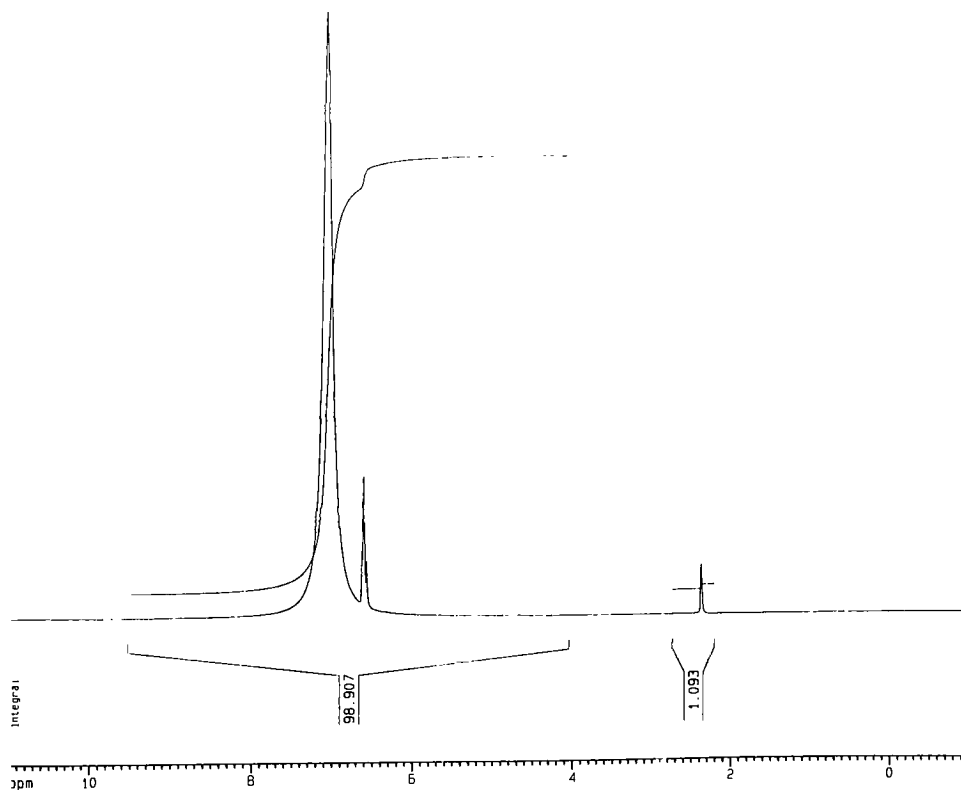
## O-PHENYL PHENOL + AQUEOUS HCl + DMSO

Spectrum 19.
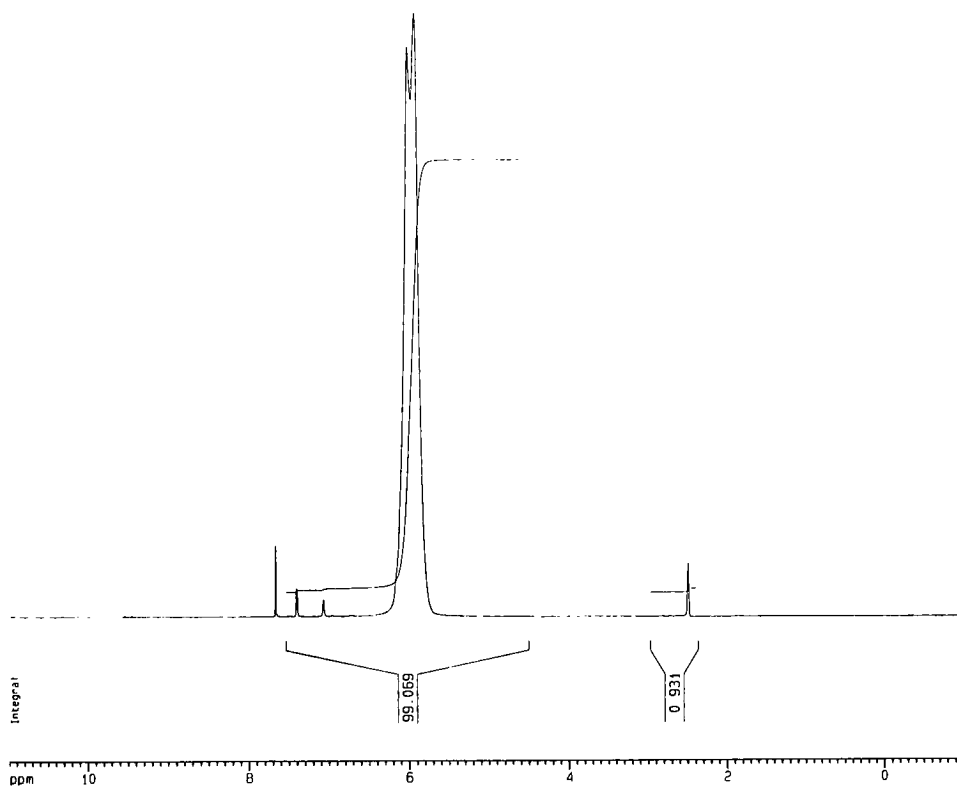
Current Data Parameters
NAME        Jun19-00
EXPNO              7
PROCNO             1

F2 - Acquisition Parameters
Date_         500000
Time           17 18
INSTRUM        spect
PROBHD   5 mm QNP  1H
PULPROG         zg30
TD             32768
SOLVENT         DMSO
NS                16
DS                 2
SWH      6172 839 Hz
FIDRES   0 180380 Hz
AQ     2 6542580 sec
RG              25.4
DW         81 000 usec
DE          6.00 usec
TE           300 0 K
D1    1 00000000 sec
P1          9 00 usec
DE          6 00 usec
SFO1   300 1318534 MHz
NUC1              1H
PL1         -5 00 dB

F2 - Processing parameters
SI             16384
SF     300 1300000 MHz
WDW               EM
SSB                0
LB          0 30 Hz
GB                 0
PC              1.00

10 NMR plot parameters
CX          20.00 cm
F1P        11 000 ppm
F1        3301 43 Hz
F2P        -1 000 ppm
F2        -300 13 Hz
PPMCM     0 60000 ppm/cm
HZCM     180 07800 Hz/cm

Integral

99.069

0.931

ppm    10        8        6        4        2        0

## O-PHENYL PHENOL + AQUEOUS HBr + DMSO

Spectrum 20.

Current Data Parameters
NAME          Jun19-00
EXPNO                5
PROCNO               1

F2 - Acquisition Parameters
Date_           500000
Time             16 40
INSTRUM          soect
PROBHD    5 mm ONP  1H
PULPROG           zg30
TO               32768
SOLVENT           DMSO
NS                  16
OS                   2
SWH         6172 839 Hz
F1ORES     0 188380 Hz
AO        2.6542580 sec
RG                 181
OW          81 000 usec
DE           6 00 usec
TE             300 0 K
O1      1 00000000 sec
P1          9.00 usec
DE           6 00 usec
SFO1   300 1318534 MHz
NUC1                1H
PL1           -5 00 dB

F2 - Processing parameters
SI               16384
SF     300 1300638 MHz
WOW                 EM
SSB                  0
LB            0.30 Hz
GB                   0
PC                1 00

10 NMR plot parameters
CX           20 00 cm
F1P         11 000 ppm
F1          3301 43 Hz
F2P         -1 000 ppm
F2          -300 13 Hz
PPMCM    0 60000 ppm/cm
HZCM     180 07803 Hz/cm
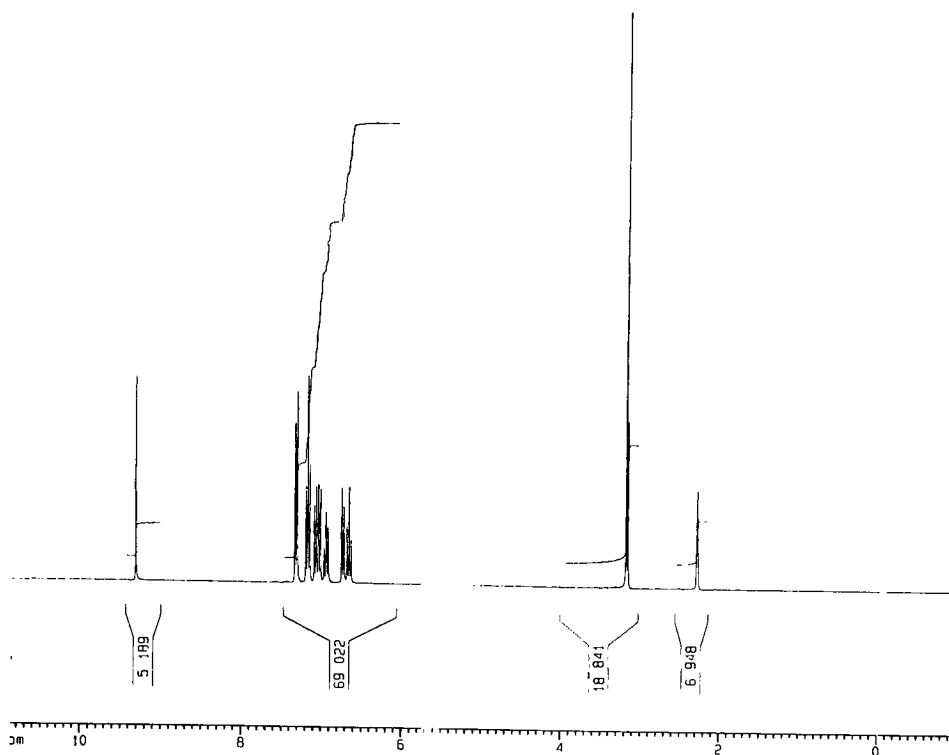
## 3-AMINO PHENOL (STANDARD) + DMSO

Spectrum 21.

Current Data Parameters
NAME          Jun19-00
EXPNO                4
PROCNO               1

F2 - Acquisition Parameters
Date_           500000
Time             16 29
INSTRUM          soect
PROBHD    5 mm ONP  1H
PULPROG           zg30
TO               32768
SOLVENT           DMSO
NS                  16
OS                   2
SWH         6172 839 Hz
F1ORES     0 188380 Hz
AO        2 6542580 sec
RG                35 9
OW          81 000 usec
OE           6 00 usec
TE             300 0 K
O1      1 00000000 sec
P1          9 00 usec
OE           6 00 usec
SFO1   300 1318534 MHz
NUC1                1H
PL1           -5 00 dB

F2 - Processing parameters
SI               16384
SF     300 1300000 MHz
WOW                 EM
SSB                  0
LB            0 30 Hz
GB                   0
PC                1 00

10 NMR plot parameters
CX           20 00 cm
F1P         11 000 ppm
F1          3301 43 Hz
F2P         -1 000 ppm
F2          -300 13 Hz
PPMCM    0 60000 ppm/cm
HZCM     180 07800 Hz/cm
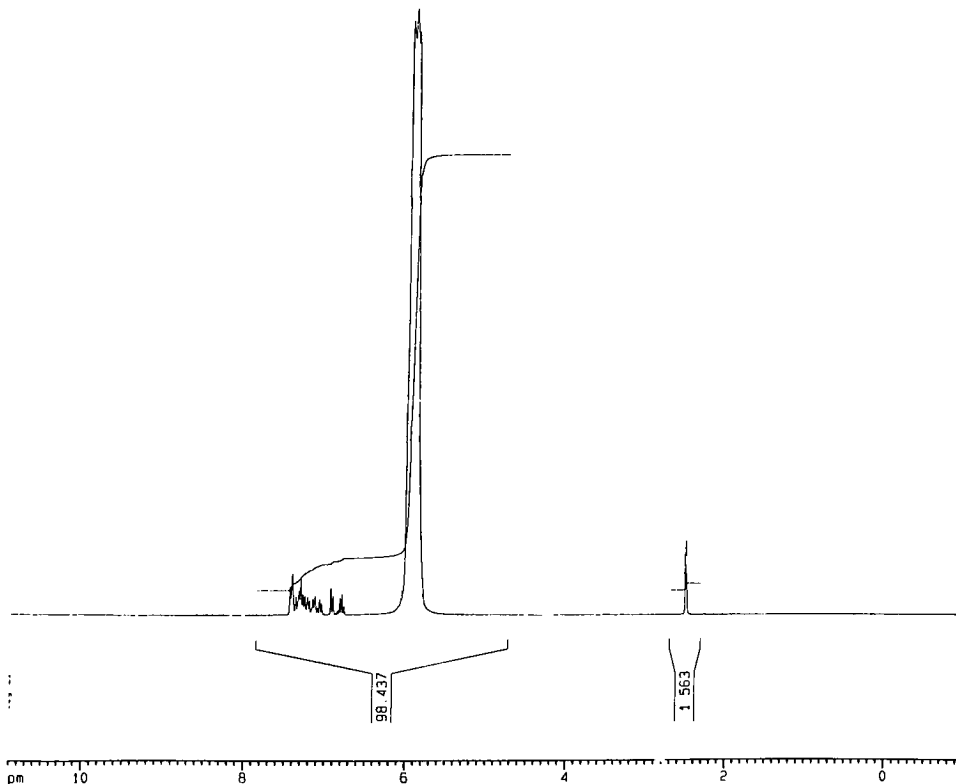
## 3-AMINO PHENOL + AQUEOUS HCl + DMSO

Spectrum 22.

Current Data Parameters
NAME            Jun19-00
EXPNO                  6
PROCNO                 1

F2 - Acquisition Parameters
Date_             500000
Time               16 51
INSTRUM            spect
PROBHD      5 mm ONP  1H
PULPROG             zg30
TD                 32768
SOLVENT             DMSO
NS                    16
DS                     2
SWH          6172 839 Hz
FIDRES      0 188380 Hz
AD          2 6542580 sec
RG                  28 5
DW            81 000 usec
DE             6 00 usec
TE             300 0 K
D1        1 00000000 sec
P1             9 00 usec
DE             6 00 usec
SFO1     300 1318534 MHz
NUC1                  1H
PL1            -5 00 dB

F2 - Processing parameters
SI                 16384
SF       300 1300000 MHz
WDW                   EM
SSB                    0
LB              0 30 Hz
GB                     0
PC                  1 00

1D NMR plot parameters
CX              20 00 cm
F1P            11 000 ppm
F1            3301 43 Hz
F2P            -1 000 ppm
F2            -300 13 Hz
PPMCM      0 60000 ppm/cm
HZCM       180 07800 Hz/cm

Integral

98 847          1 153

ppm    10        8        6        4        2        0

**3-AMINO PHENOL + AQUEOUS HBr + DMSO**

APPENDIX B

# GC Spectrum 1. Area Percent Report

B1

ANIHBR.D  PANKAJ.M  Thu Jul 13 15:26:49 2000
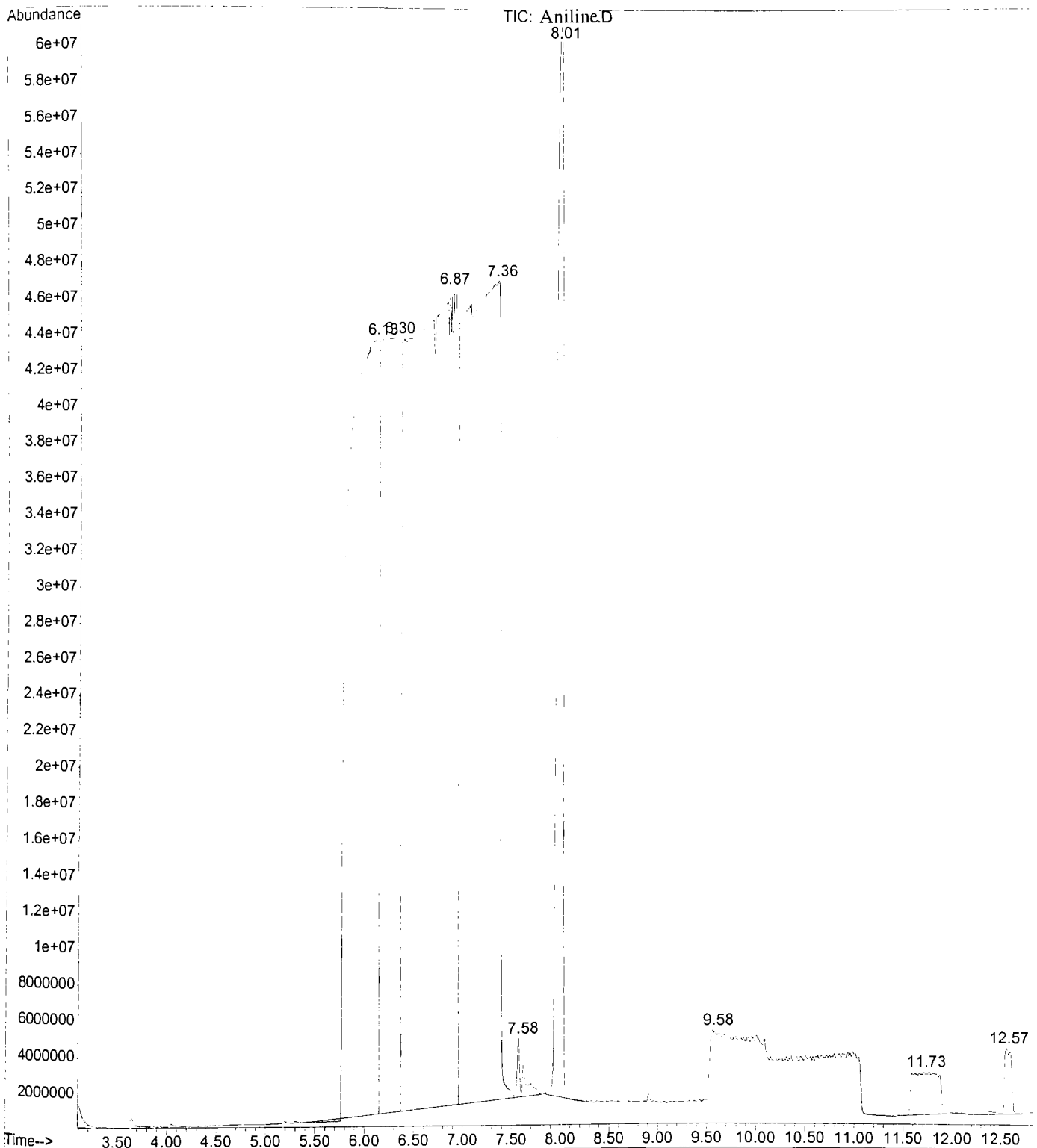
Library Searched : C:\DATABASE\NIST98.L
Quality          : 93
ID               : Aniline          MS Spectrum 1.

Abundance                                    Scan 652 (7.956 min): Aniline.D

                                              93
9000

8000

7000

6000
                              66
5000

4000

3000

2000
                   39
1000            28      52        78
                                      104 117 133 147  163 177 191  207  223 237 251  267 281 295 311 325  341
   0
m/z-->    0    20   40   60   80  100  120  140  160  180  200  220  240  260  280  300  320  340

Abundance                                    #117842: Aniline
                                              93
9000

8000

7000

6000

5000

4000
                              66
3000

2000           39
            28      52
1000      15           78
        2
   0
m/z-->    0    20   40   60   80  100  120  140  160  180  200  220  240  260  280  300  320  340

Data File : C:\HPCHEM\1\DATA\ANHCL.D      Vial: 1      B2
Acq On    : 13 Jul 2000  16:13            Operator:
Sample    :                               Inst    : RIT Chemi
Misc      :                               Multiplr: 1.00
                                          Sample Amount: 0.00

MS Integration Params: autoint1.e
Method    : C:\HPCHEM\1\5973\PANKAJ7.M (Chemstation Integrator)
Title     :



ANHCL.D   PANKAJ7.M   Thu Jul 13 16:26:43 2000

Library Searched : C:\DATABASE\NIST98.L
Quality          : 93
ID               : p-Chloroaniline     MS Spectrum 2.



Scan 156 (8.854 min): ANHCL.D

#121726: p Chloroaniline

Data File : C:\HPCHEM\1\DATA\ANHBR.D                    Vial: 1
Acq On    : 13 Jul 2000  15:35                          Operator:
Sample    :                                             Inst    : RIT Chemi
Misc      :                                             Multiplr: 1.00
                                                        Sample Amount: 0.00

MS Integration Params: autoint1.e
Method    : C:\HPCHEM\1\5973\PANKAJ.M (Chemstation Integrator)
Title     :



ANHBR.D  PANKAJ.M  Thu Jul 13 15:48:48 2000
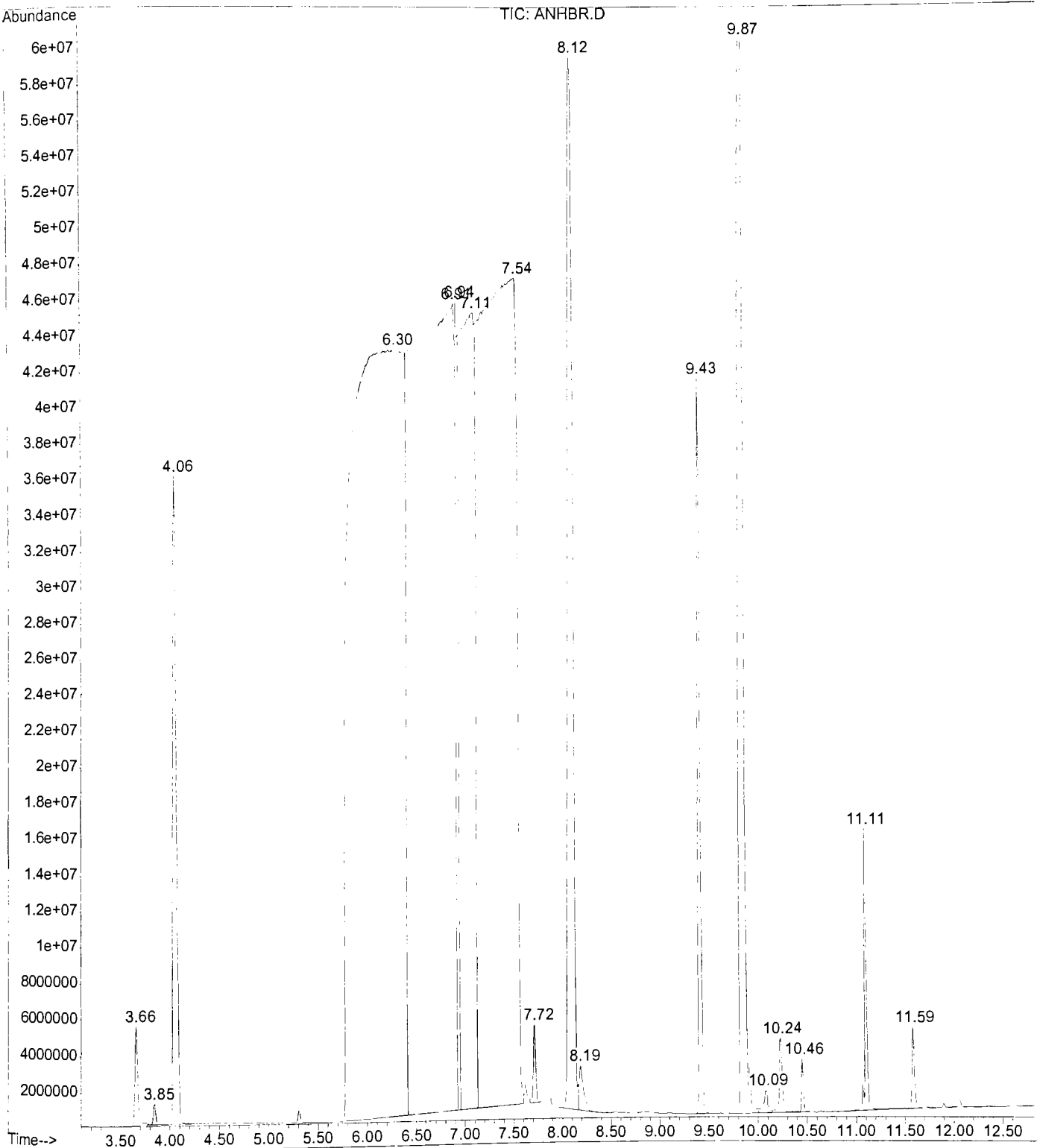
Library Searched : C:\DATABASE\NIST98.L
Quality          : 94
ID               : Aniline, 4 - bromo-        MS Spectrum 3.

B3

Abundance

Scan 909 (9.878 min): ANHBR.D

171

9000

8000

7000

6000

5000

4000

3000

2000  39

1000  52

28    65   92

75   106   117   130   143   156   184 194 207 221 231   249   267   281 292 302 313 325   341

m/z-->    20    40    60    80    100    120    140    160    180    200    220    240    260    280    300    320    340

Abundance

#124909:  Aniline,4-bromo-

171

9000

8000  65

7000

6000  92

5000

4000

3000

2000  39

1000  52

15   28   81   106 117  130  143  156

m/z-->    20    40    60    80    100    120    140    160    180    200    220    240    260    280    300    320    340

Data File : C:\HPCHEM\1\DATA\NMETHHCl.D                Vial: 1
Acq On    : 13 Jul 2000  17:54                         Operator:
Sample    :                                            Inst    : RIT Chemi
Misc      :                                            Multiplr: 1.00
                                                       Sample Amount: 0.00

MS Integration Params: autoint1.e
Method    : C:\HPCHEM\1\5973\PANKAJ7.M (Chemstation Integrator)
Title     :



NMETHHBR.D   PANKAJ7.M   Thu Jul 13 18:07:28 2000

Abundance

Scan 219 (9.324 min): NMETHHCL.D
140

9000

8000

7000

6000

5000        77

4000              127

3000

2000

1000    51 63        99
    28 39         88   111    154163 177 191  207 221  239249  267 281 295  313 327 341
  0
m/z-->  20    40    60    80   100   120   140   160   180   200   220   240   260   280   300   320   340

Abundance

#63690: Aniline, 4-chloro-N-methyl-
140

9000

8000

7000

6000

5000

4000

3000

2000        77

1000    50   63     99 111
     40            126
  0
m/z-->  20    40    60    80   100   120   140   160   180   200   220   240   260   280   300   320   340

NMETHHCL.D  PANKAJ7.M  Thu Jul 13 18:52:55 2000

Library Searched : C:\DATABASE\NIST98.L
Quality : 47
ID : Aniline, 4- bromo- N - methyl-     MS Spectrum 5.

Abundance                                Scan 285 (9.816 min): NMETHHBR.D
                                                    184

9000

8000

7000

6000

5000                          77
                                        105
4000

3000

2000              51
                      63          91
1000         39                              140
        28                                            157
     0                              117 129              170    194 207 221    239249    267  281291302313  327  341
m/z-->      20    40    60    80   100   120   140   160   180   200   220   240   260   280   300   320   340

Abundance                          #79589: Aniline, 4-bromo-N-methyl-
                                                    187

9000

8000                          106

7000

6000

5000

4000                     77

3000
                  52
2000
          28  39
1000              63
        18              93
     0                         117        157
m/z-->      20    40    60    80   100   120   140   160   180   200   220   240   260   280   300   320   340

# GC Spectrum 6. Area Percent Report

```
Data File : C:\HPCHEM\1\DATA\P2.D                    Vial: 1
Acq On    :   4 Jan 2000  14:47                      Operator: Pankaj
Sample    : Aniline, 4- bromo- N,N - dimethyl-       Inst    : RIT Chemi
Misc      :                                          Multiplr: 1.00
                                                     Sample Amount: 0.00

MS Integration Params: autoint1.e
Method    : C:\HPCHEM\1\5973\PANKAJ.M  (Chemstation Integrator)
Title     :
```

le           : C:\HPCHEM\1\DATA\P2.D
erator       : Pankaj
quired       : 4 Jan 2000  14:47      using AcqMethod PANKAJ
strument     :   RIT Chemi
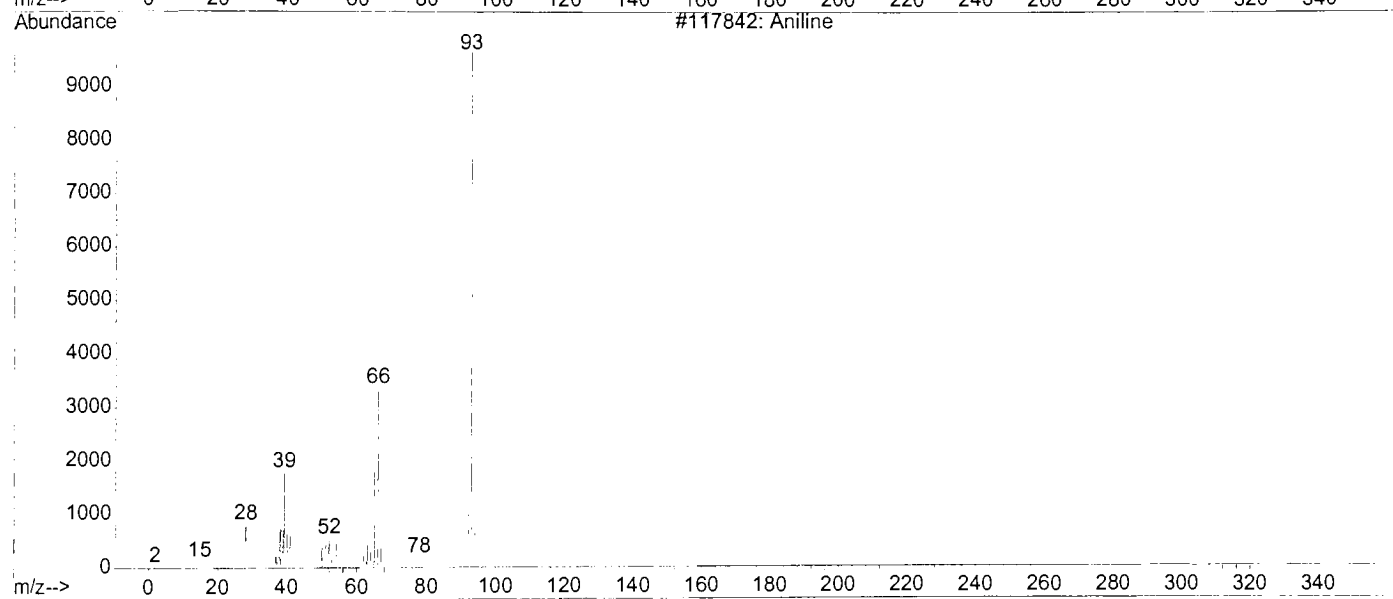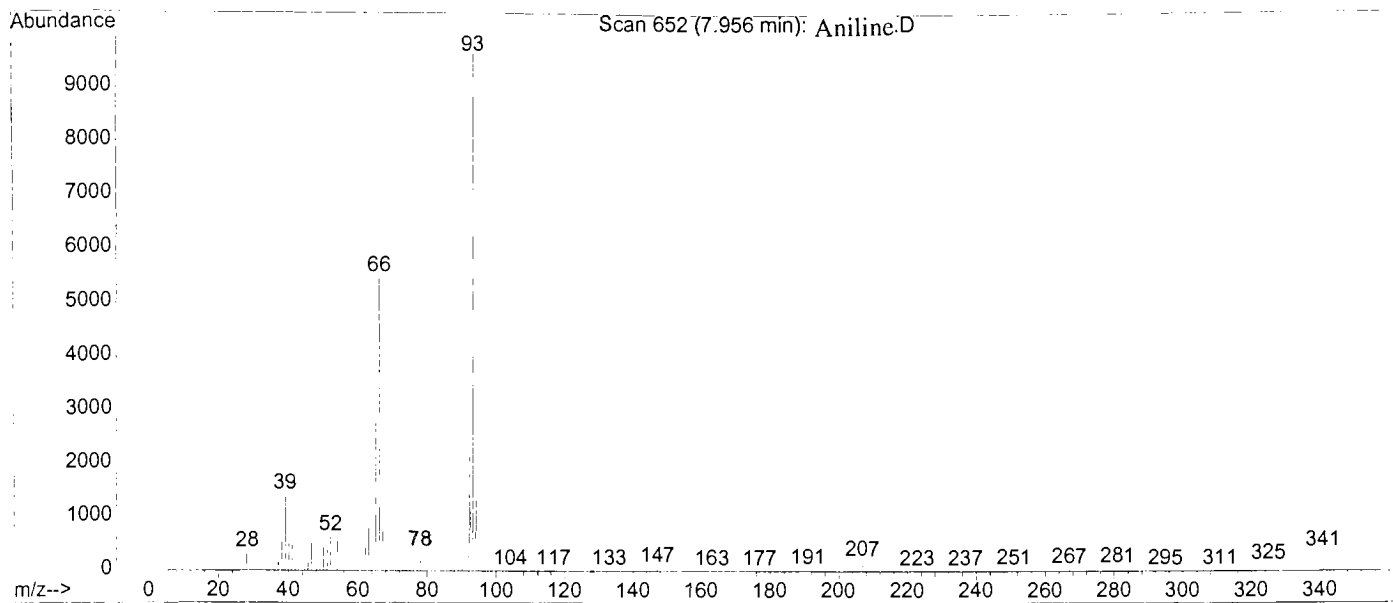mple Name: Aniline, 4- bromo- N,N - dimethyl-      MS Spectrum 6.
sc Info      :
al Number: 1

Abundance                                          TIC: P2.D
1e+08                                          7,77

9e+07

8e+07

7e+07

6e+07

5e+07

4e+07

3e+07

2e+07

1e+07
                7.68                                    7.83        7.88        7.92
Time-->  7.64    7.66    7.68    7.70    7.72    7.74    7.76    7.78    7.80    7.82    7.84    7.86    7.88    7.90    7.92

Abundance                              Scan 800 (7.765 min): P2.D Aniline, 4-bromo-N,N-dimethyl-
                                                             198
6000000
                                  118
5500000

5000000

4500000

4000000
                                              183
3500000
                      77    104
3000000
                                      157
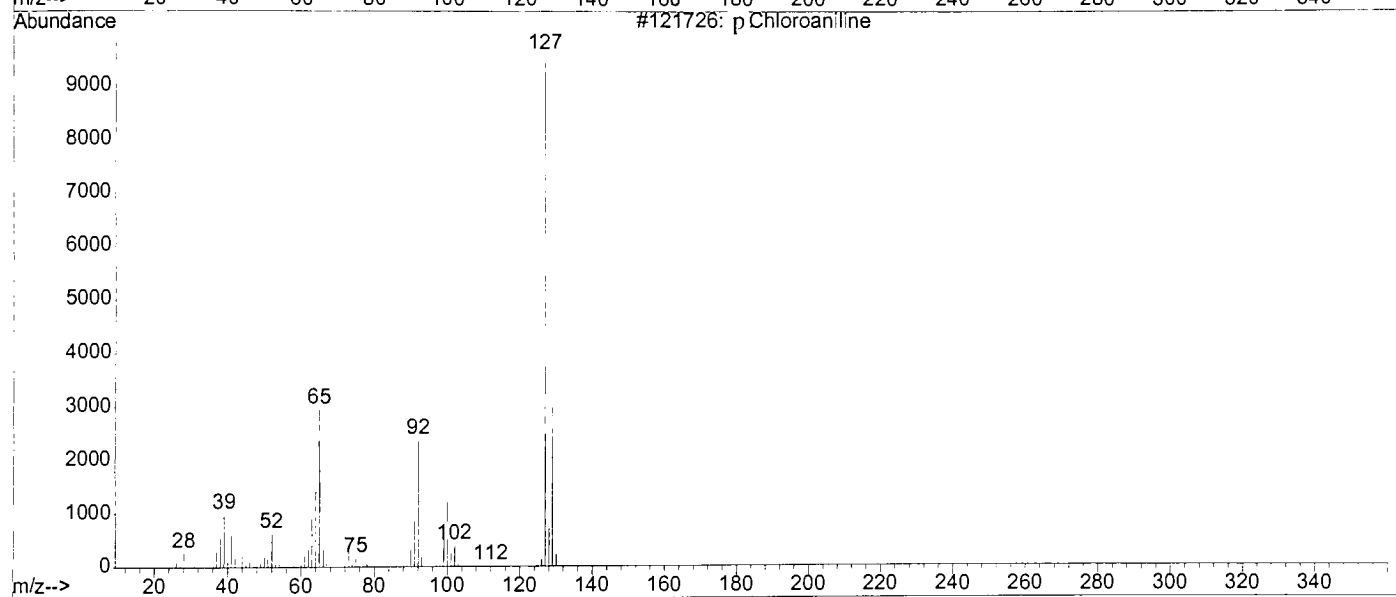2500000

2000000

              50   63        91
1500000

1000000
          39
500000                          129  143   171              208 221  236   252  267  281    298  311  325   341
      28
   0
m/z-->  10  20  30  40  50  60  70  80  90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 350

.le        : C:\HPCHEM\1\DATA\P2.D
)erator    : Pankaj
:quired    :  4 Jan 2000  14:47      using AcqMethod PANKAJ
1strument  :     RIT Chemi
1mple Name : Aniline, 4- bromo- N,N - dimethyl-      MS Spectrum 6.
.sc Info   :
.al Number : 1



TIC: P2.D

Abundance
1e+08 — 7.77

9e+07

8e+07

7e+07

6e+07

5e+07

4e+07

3e+07

2e+07

1e+07 —  7.68                                     7.83           7.88        7.92

Time--> 7.64  7.66  7.68  7.70  7.72  7.74  7.76  7.78  7.80  7.82  7.84  7.86  7.88  7.90  7.92

Abundance                    Scan 800 (7.765 min): P2.D Aniline, 4-bromo-N,N-dimethyl-

6000000              198  199  200  201

5500000

5000000

4500000

4000000

3500000

3000000

2500000

2000000                                   202

1500000

1000000

500000       196                        203                                                               215
0    194          196  197  198  199  200  201  202  203  204  205  207 208  209  210  211 212  213 214

m/z-->  194  195  196  197  198  199  200  201  202  203  204  205  206  207  208  209  210  211  212  213  214  215

Data File : C:\HPCHEM\1\DATA\P20.D                    Vial: 1
Acq On    :  9 Mar 2000  10:42                        Operator:
Sample    : phenol+hcl                                Inst    : RIT Chemi
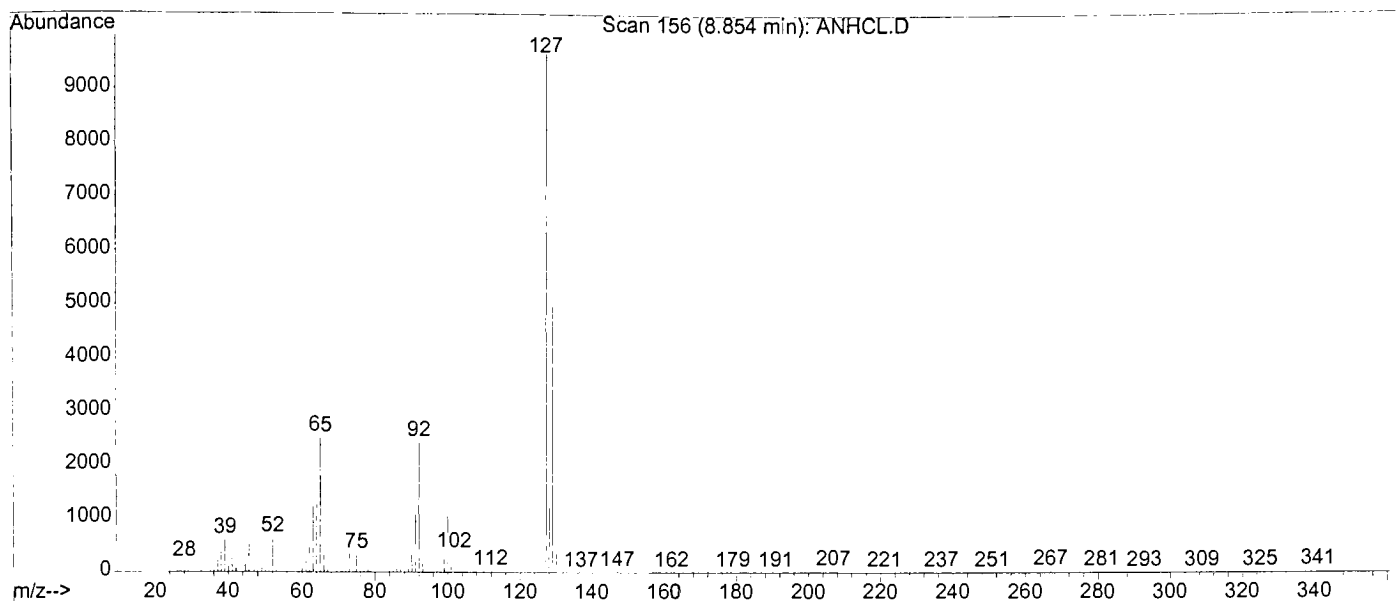Misc      :                                           Multiplr: 1.00
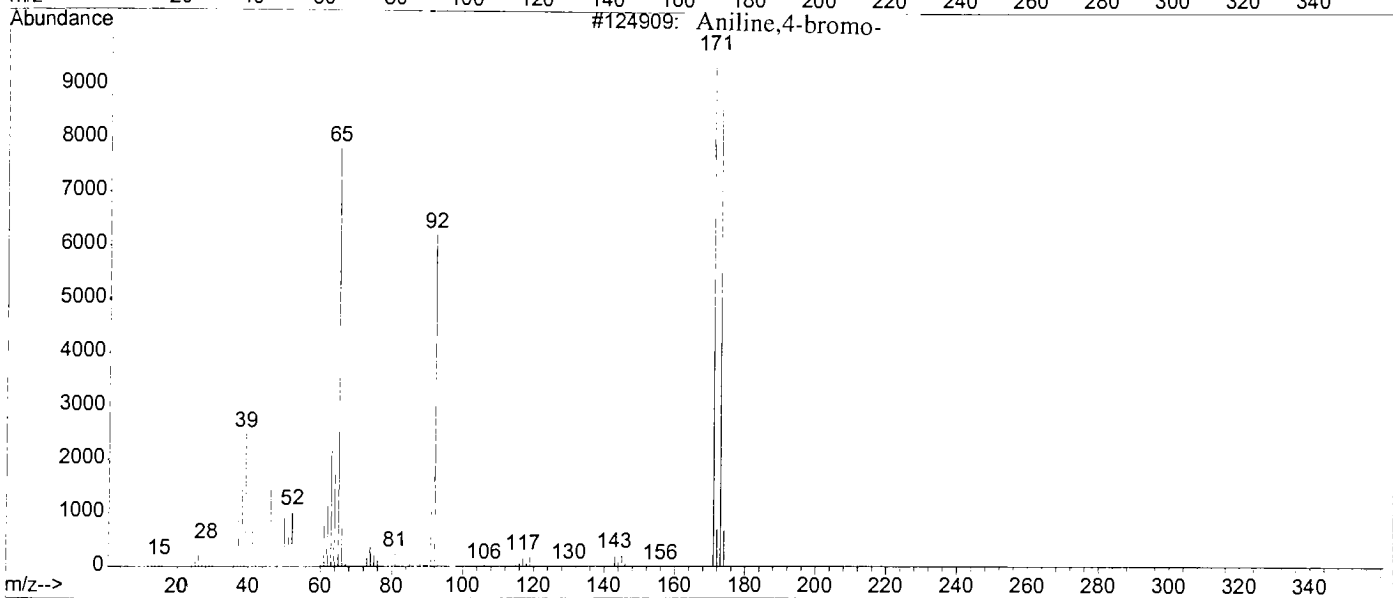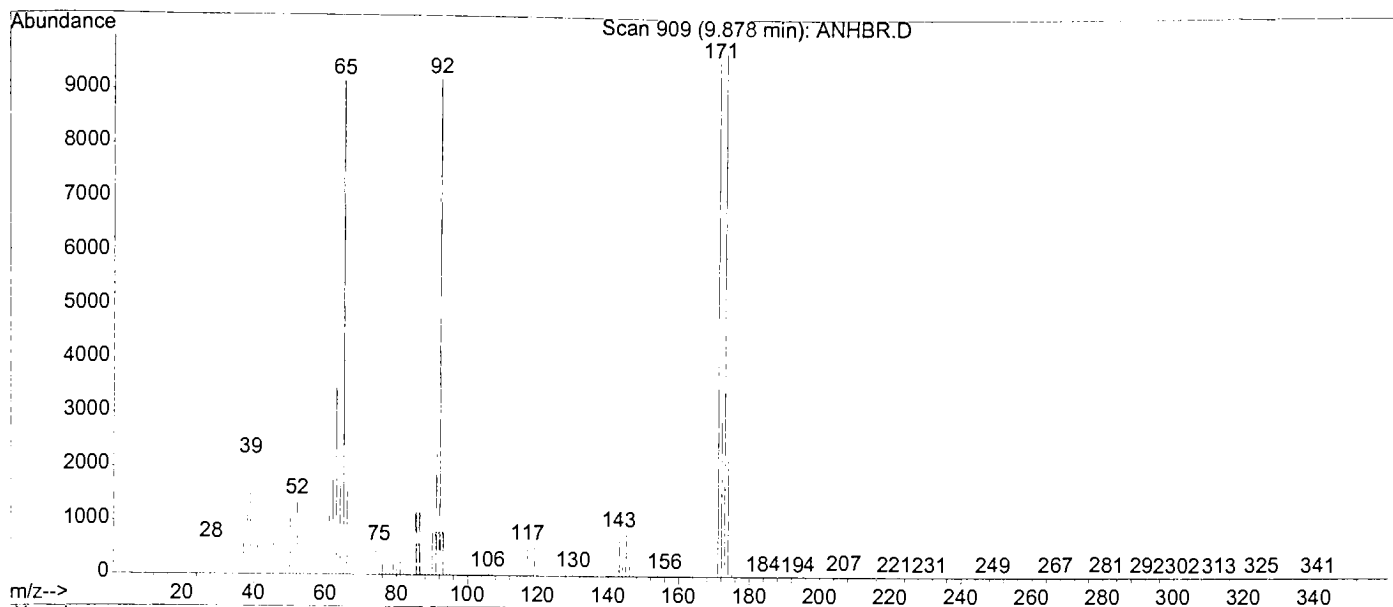                                                      Sample Amount: 0.00

MS Integration Params: autoint1.e
Method    : C:\HPCHEM\1\5973\PANKAJ.M (Chemstation Integrator)
Title     :

Library Searched : C:\DATABASE\NIST98.L
Quality          : 94
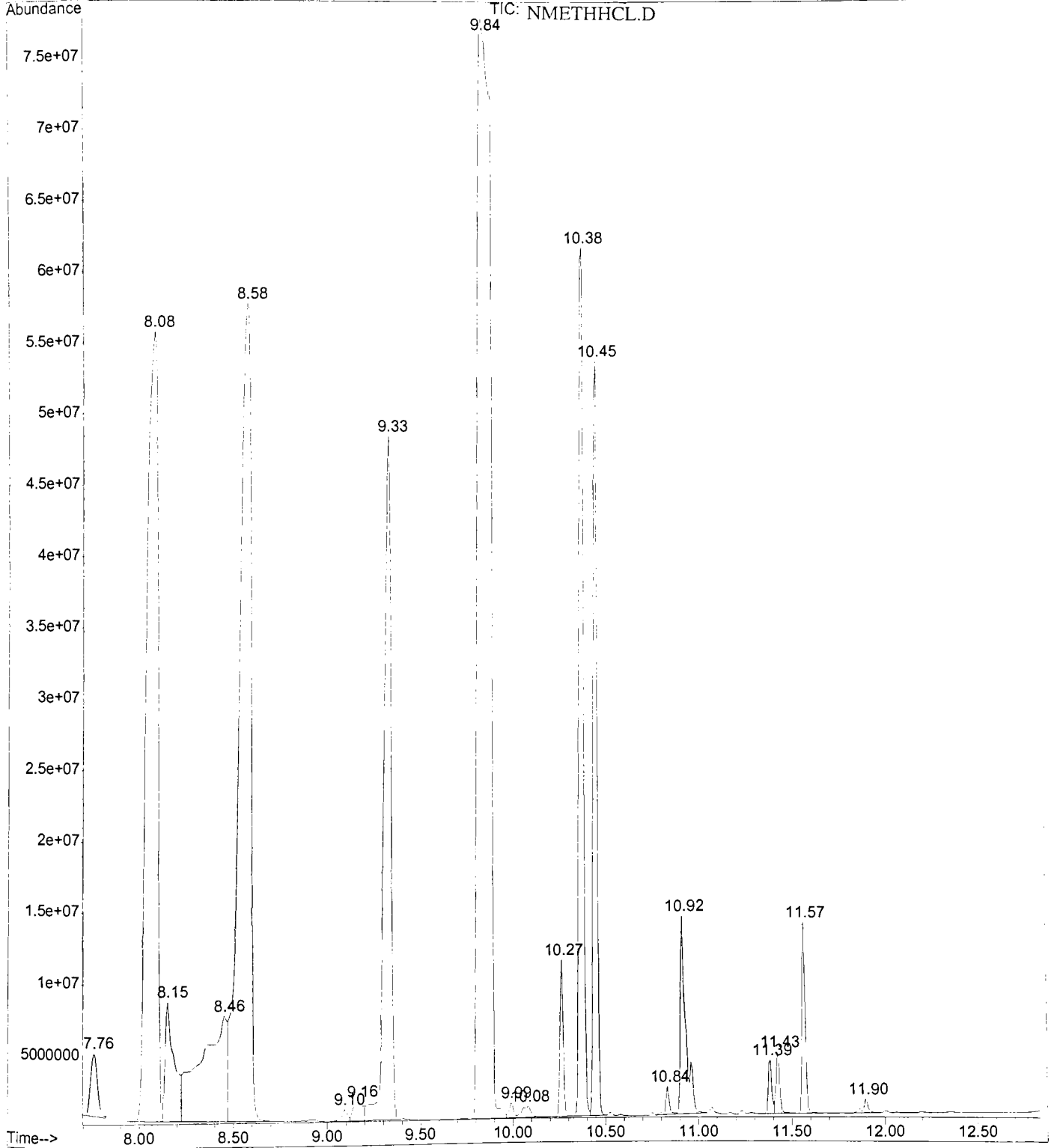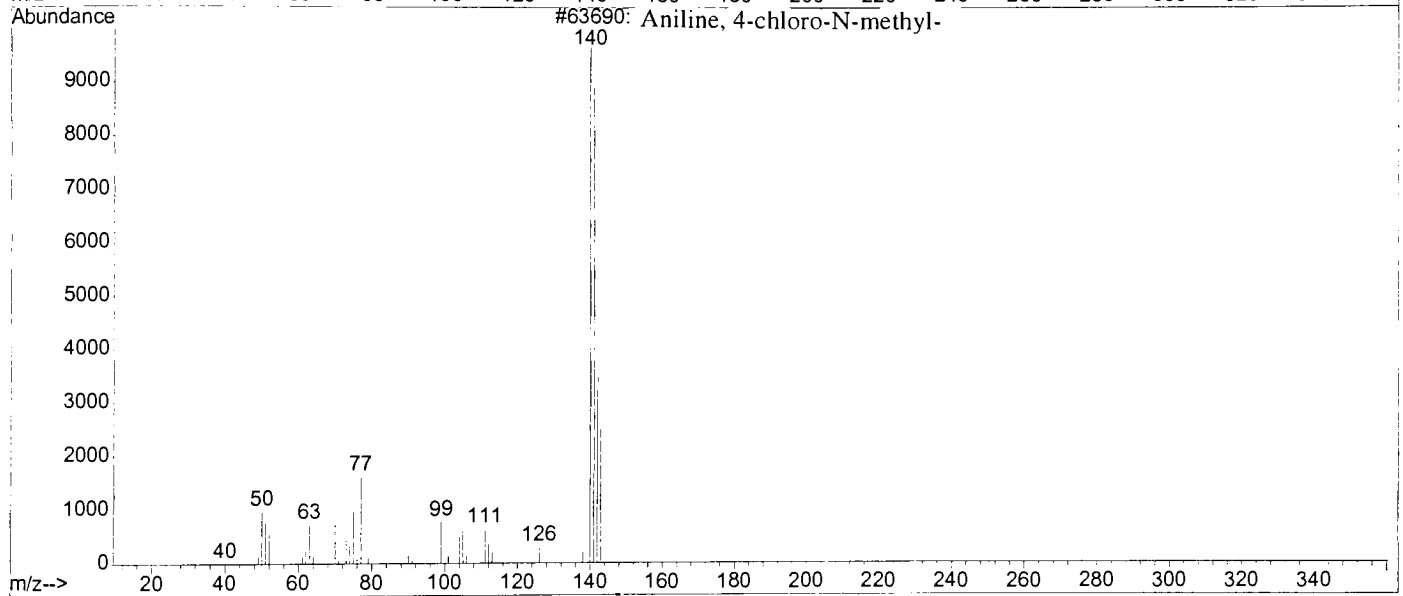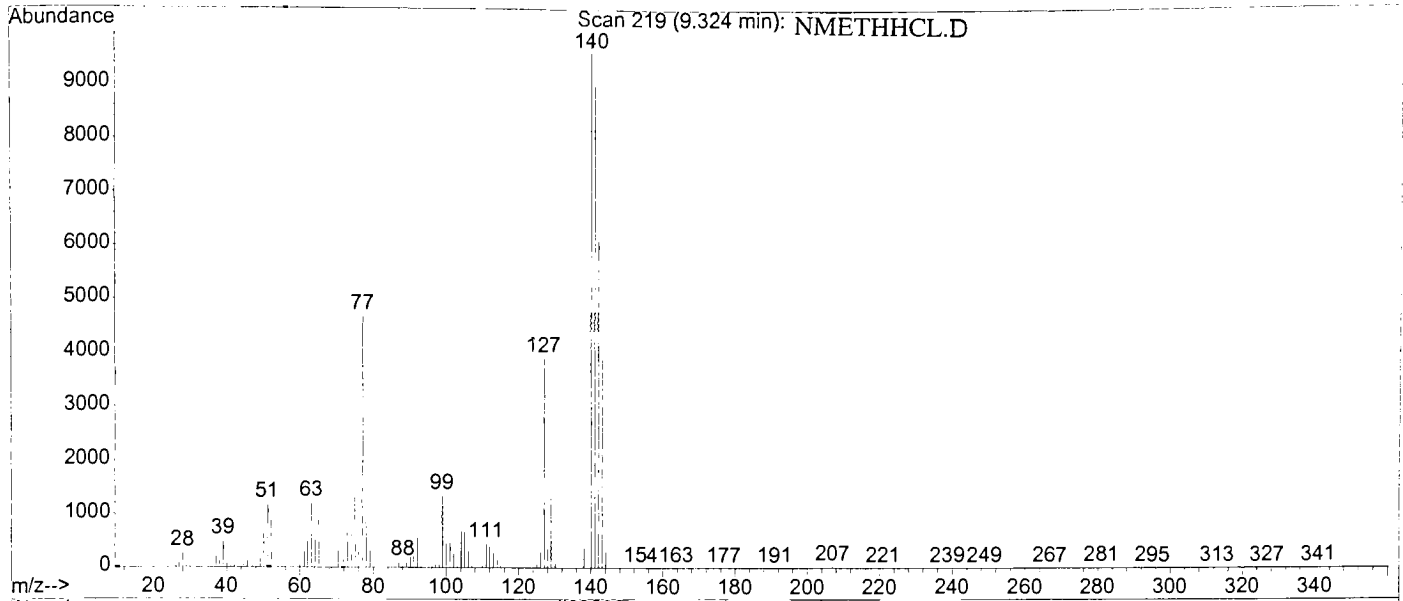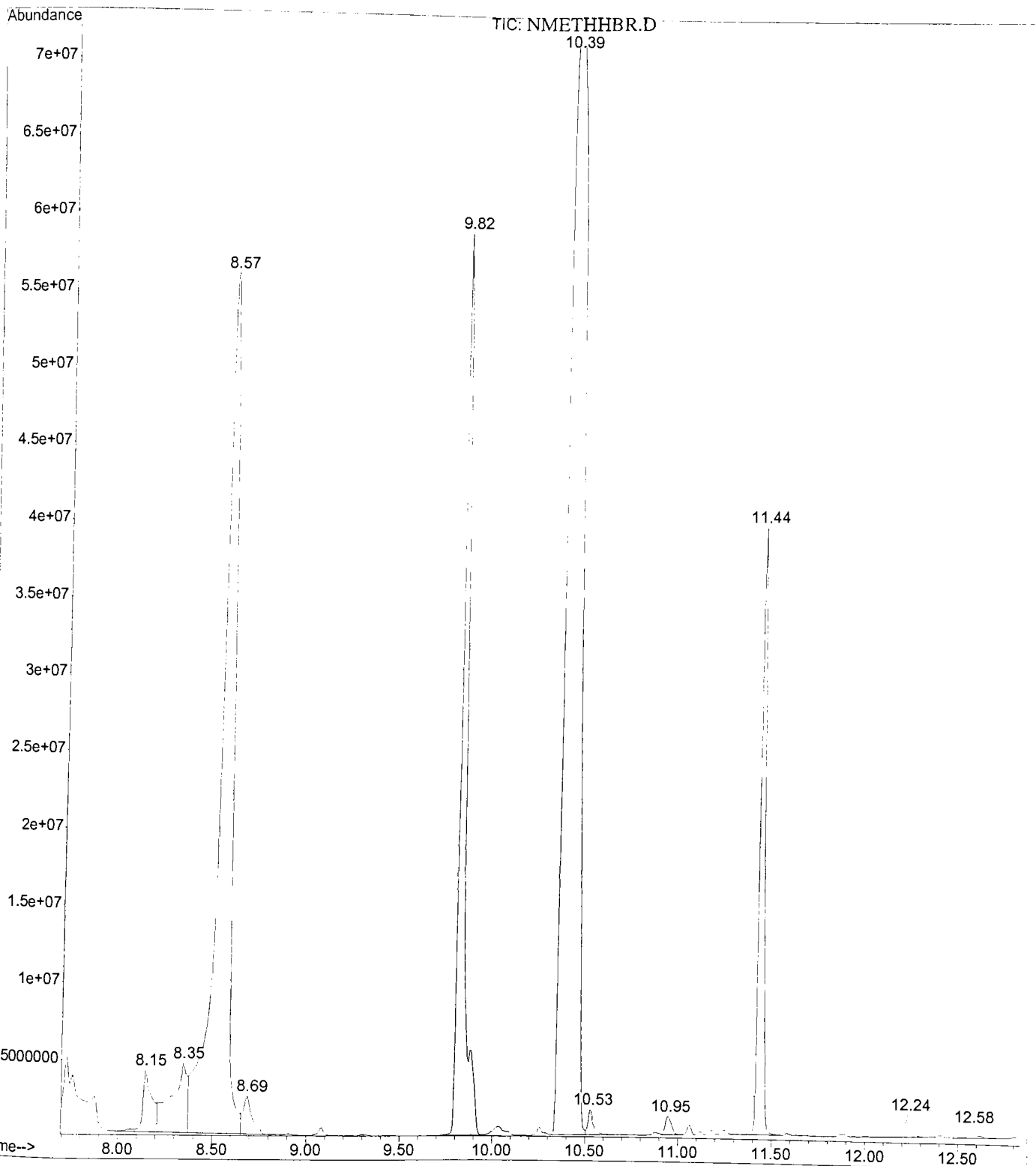ID               : Parachlorophenol   MS Spectrum 7.

B7

Abundance

Scan 589 (6.189 min): P20.D

128

9000

8000

7000

6000

5000

4000

3000

65

2000

1000

100

39

29          50          75

89          110      138  151 162172      191      207217227237 249      267  281 292302312322 333343

0

m/z-->     20     40     60     80     100     120     140     160     180     200     220     240     260     280     300     320     340

Abundance

#57956: Parachlorophenol

128

9000

8000

7000

6000

5000

65

4000

3000

39

2000

1000

100

50          75

29                                89    110

0

m/z-->     20     40     60     80     100     120     140     160     180     200     220     240     260     280     300     320     340

# GC Spectrum 8. Area Percent Report

```
Data File : C:\HPCHEM\1\DATA\P16D.D              Vial: 1
Acq On    : 9 Mar 2000  11:12                    Operator:
Sample    : phenol++++hbr                        Inst    : RIT Chemi
Misc      :                                      Multiplr: 1.00
                                                 Sample Amount: 0.00

MS Integration Params: autoint1.e
Method    : C:\HPCHEM\1\5973\PANKAJ.M (Chemstation Integrator)
Title     :
```
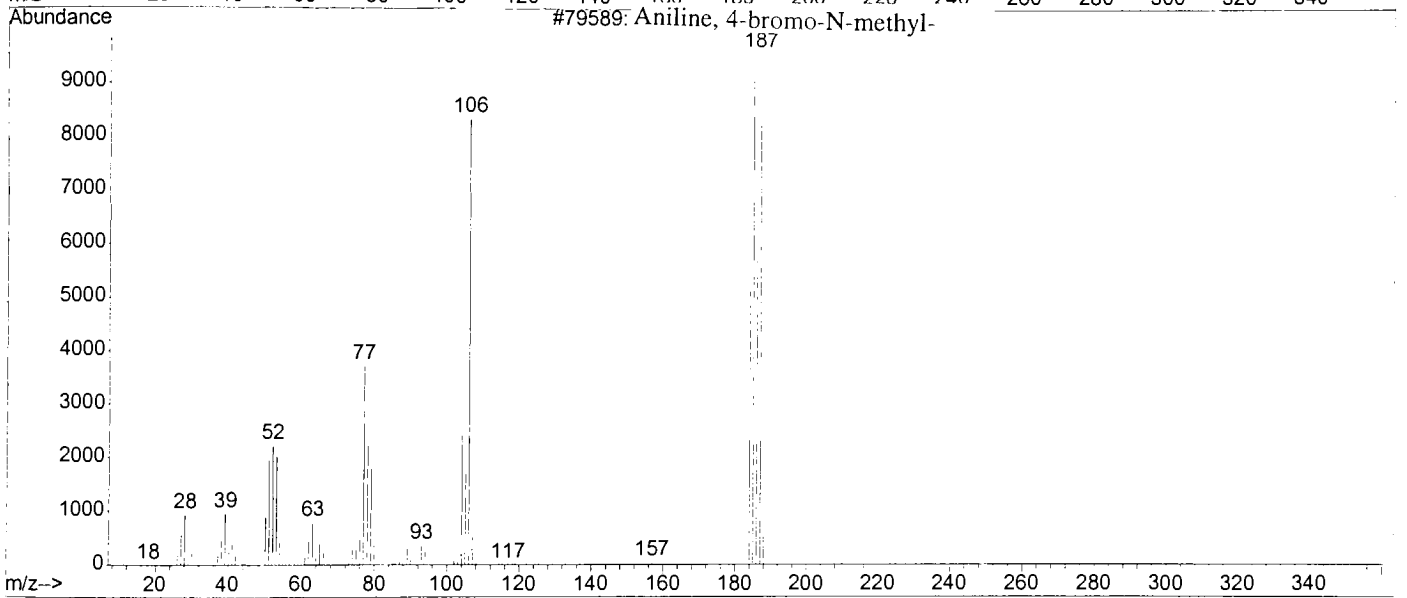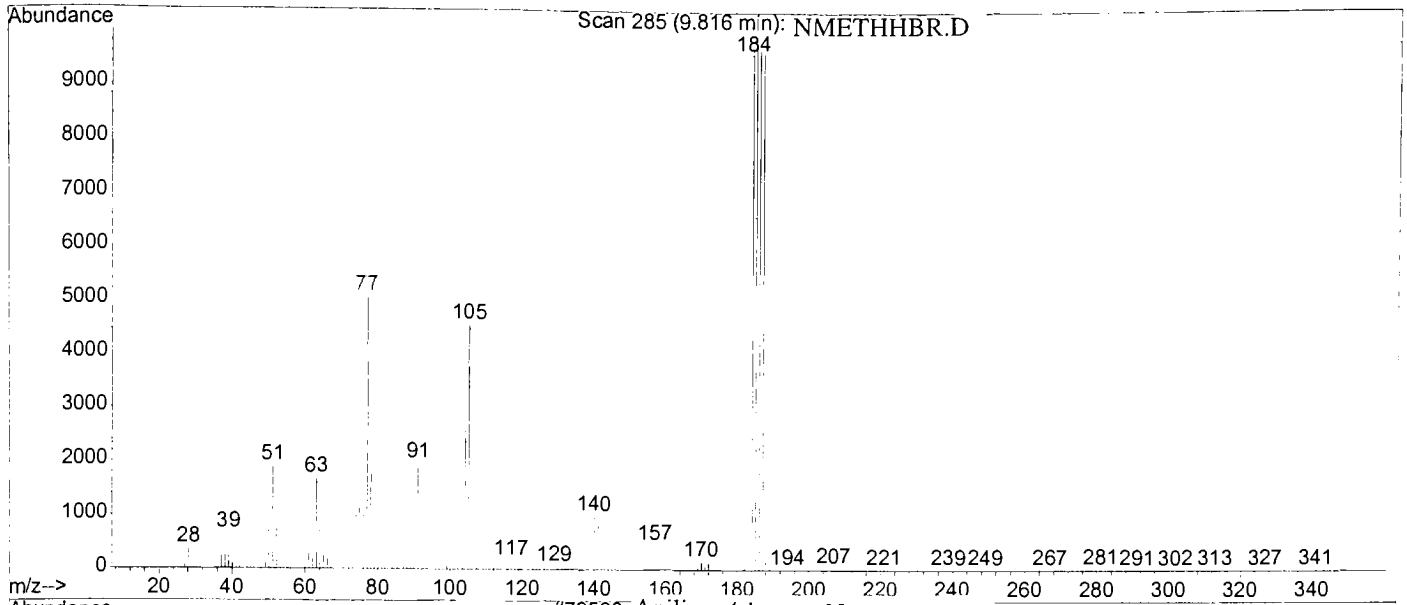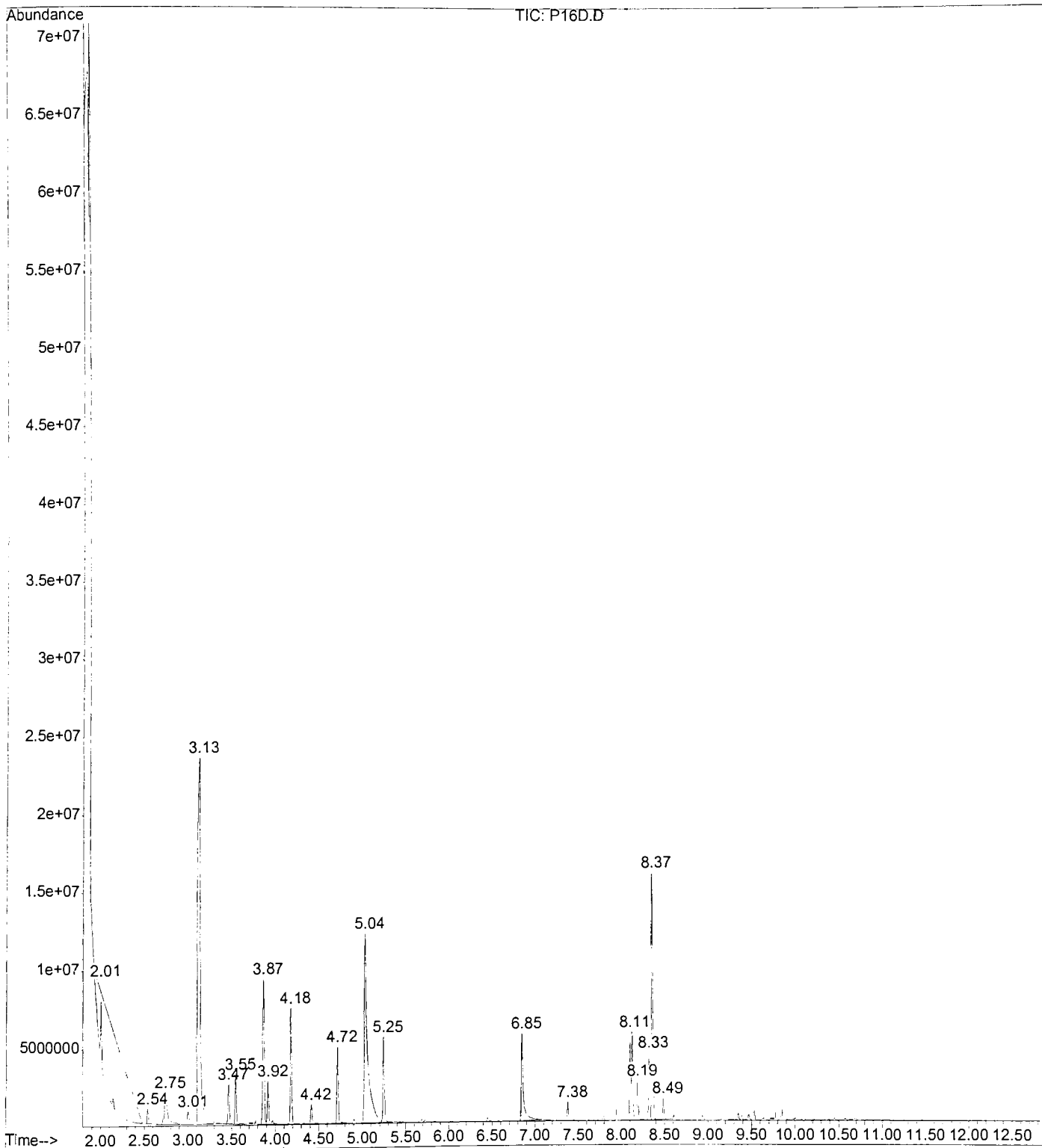
Library Searched : C:\DATABASE\NIST98.L
Quality          : 94
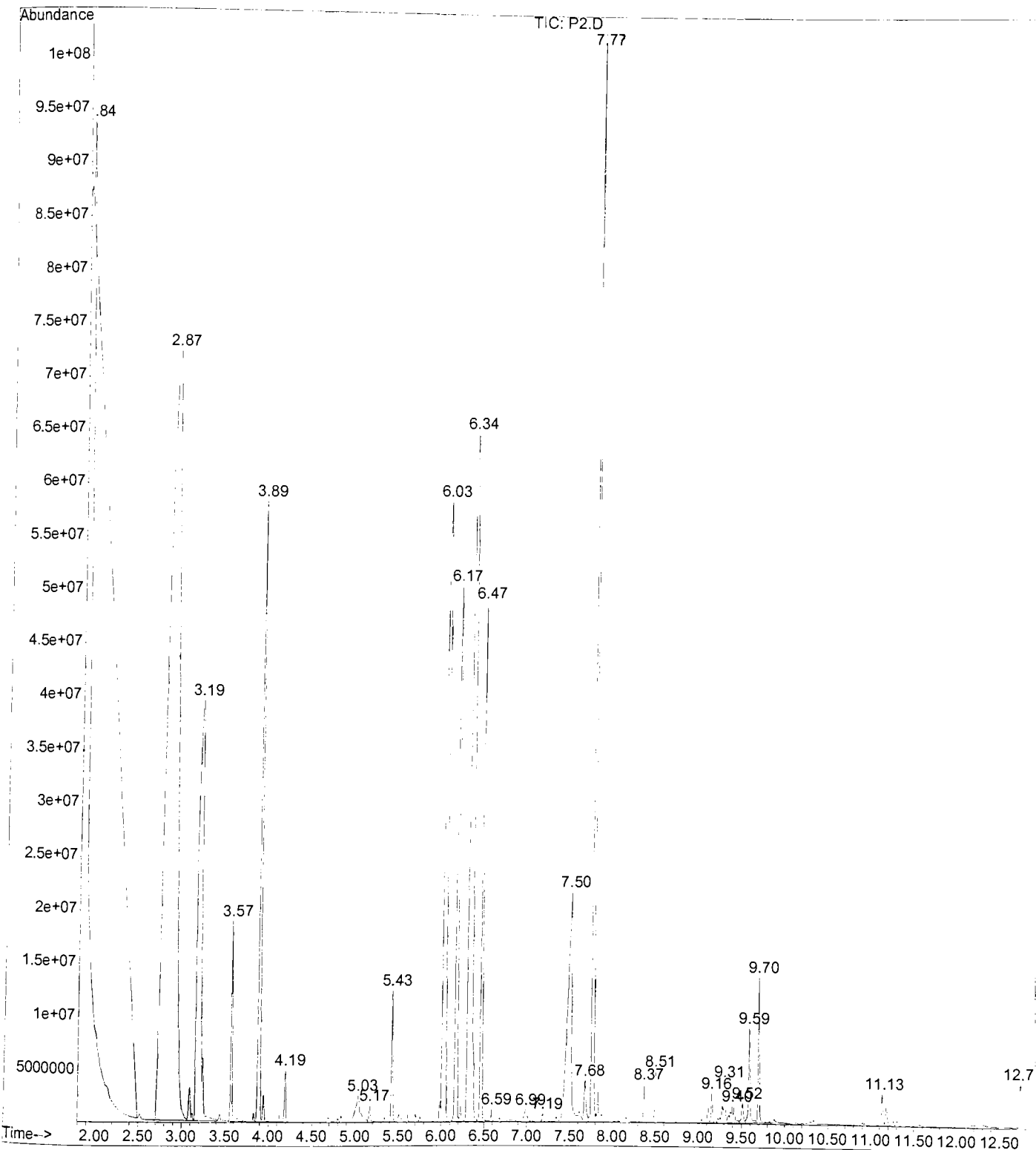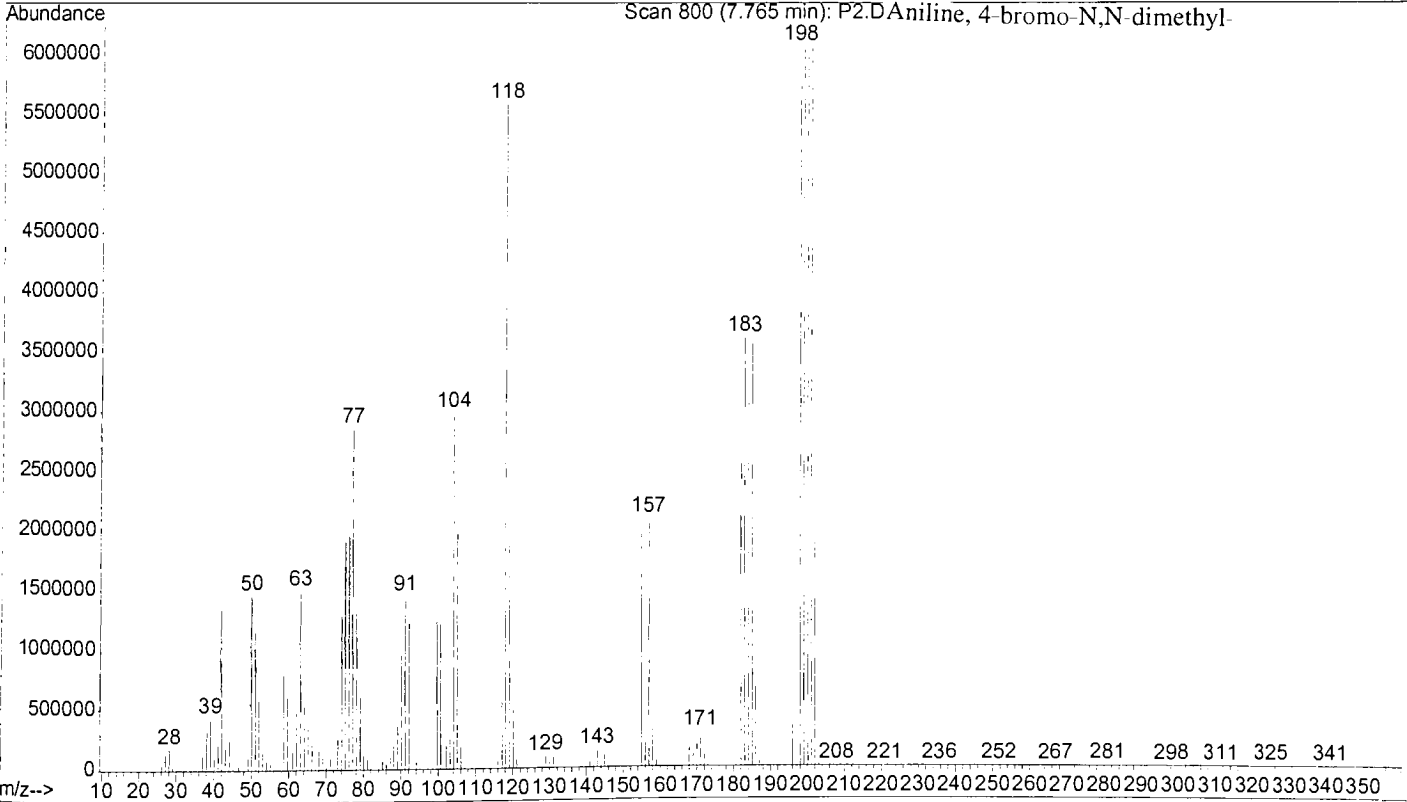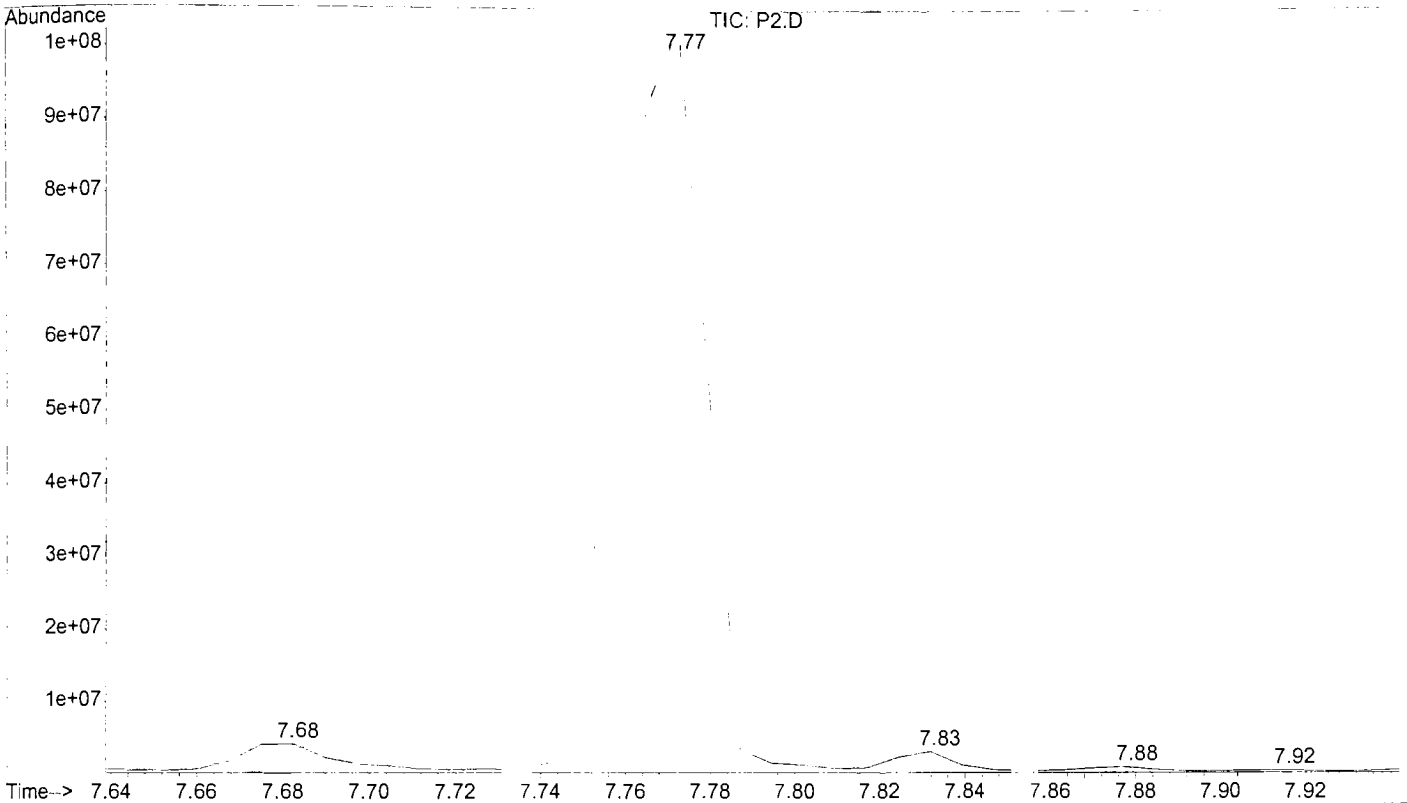ID               : Phenol, 4-bromo-    MS Spectrum 8.

Abundance                                    Scan 678 (6.852 min): P16D.D
                                                      172

9000

8000

7000

6000

5000

4000                    65

3000

2000               93

1000      39

    0    28    50      75      105 117 128   145      157      191  207  221 232   250   267  281 292 302 312 324   341
m/z-->   20    40    60      80    100   120   140   160   180   200   220   240   260   280   300   320   340

Abundance                                    #74993: Phenol, 4-bromo-
                                                      172

9000

8000

7000

6000

5000

4000

3000              65

2000          93

1000     39

         18 28     50     75     104 117  133 145 155
    0
m/z-->   20    40    60      80    100   120   140   160   180   200   220   240   260   280   300   320   340

Library Searched : C:\DATABASE\NIST98.L
Quality : 94
ID : Phenol, 3-bromo-     MS Spectrum 8.

Abundance                          Scan 678 (6.854 min): P15.D
                                         172

9000

8000

7000

6000

5000

4000
                        65
3000

2000                          93

1000      39
      28        50        75              117   143
0 ─────────────────────────────106────133───155─────191──207217──235──251──267──281──295──311──325──341─
m/z-->   20    40    60    80    100   120   140   160   180   200   220   240   260   280   300   320   340

Abundance                          #74991: Phenol, 3-bromo-
                                         172

9000

8000

7000

6000
                        65
5000

4000                          93

3000

2000      39

1000         53
      26              75        104  117  133 143  157
0 ─────────────────────────────────────────────────────────────────────────────────────────────────────
m/z-->   20    40    60    80    100   120   140   160   180   200   220   240   260   280   300   320   340

# GC Spectrum 9. Area Percent Report

Library Searched : C:\DATABASE\NIST98.L
Quality          : 95
ID               : Benzene, 1-chloro-4-methoxy-    MS Spectrum 9.

Abundance                                    Scan 465 (5.560 min): P32D.D
                                             142

9000

8000

7000

6000                              127

5000

4000               99

3000

2000

1000          63  75
        28 38 50        85      111
     0
m/z-->   20   40   60   80  100  120  140  152 163  179 191  207217227237  252  269 281 291301312322  341
        20   40   60   80  100  120  140  160  180  200  220  240  260  280  300  320  340
Abundance                                    #122976: Benzene, 1-chloro-4-methoxy-
                                             142

9000

8000

7000

6000               99

5000               127

4000

3000
               63
2000      38       73
          50
1000
        27           85   111
     0
m/z-->   20   40   60   80  100  120  140  160  180  200  220  240  260  280  300  320  340

# GC Spectrum 10. Area Percent Report

Data File : C:\HPCHEM\1\DATA\AMINPHCL.D  Vial: 1  B10
Acq On : 13 Jul 2000  20:10
Sample :                                  Operator:
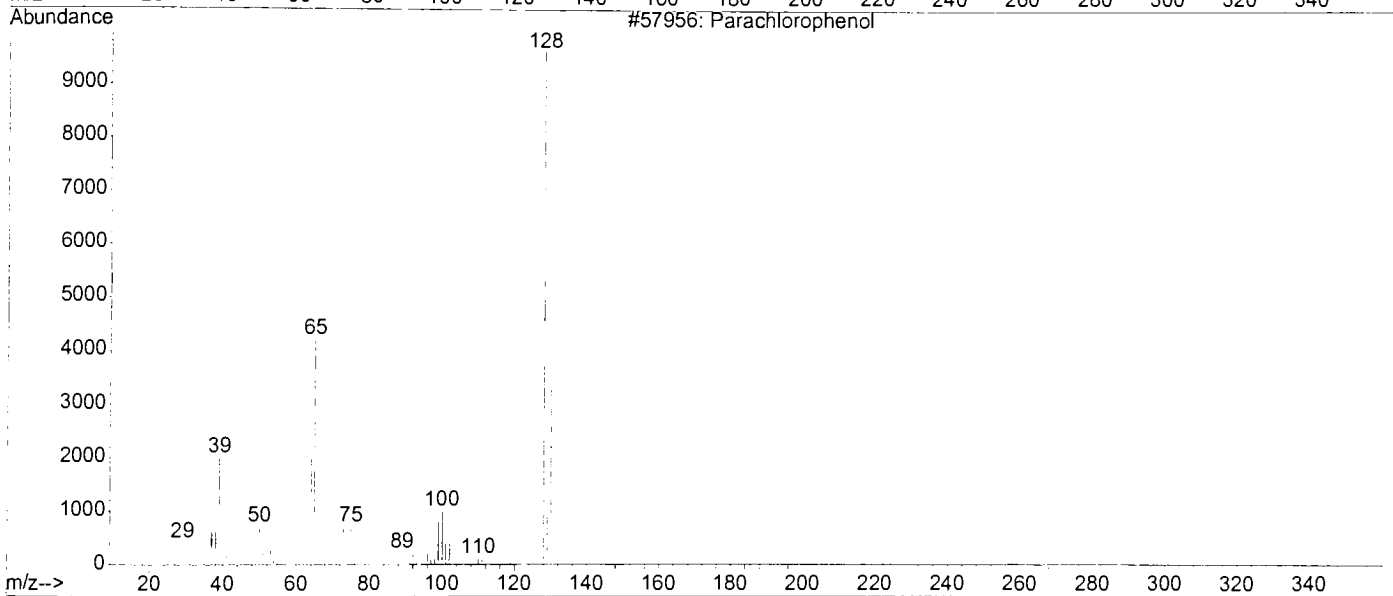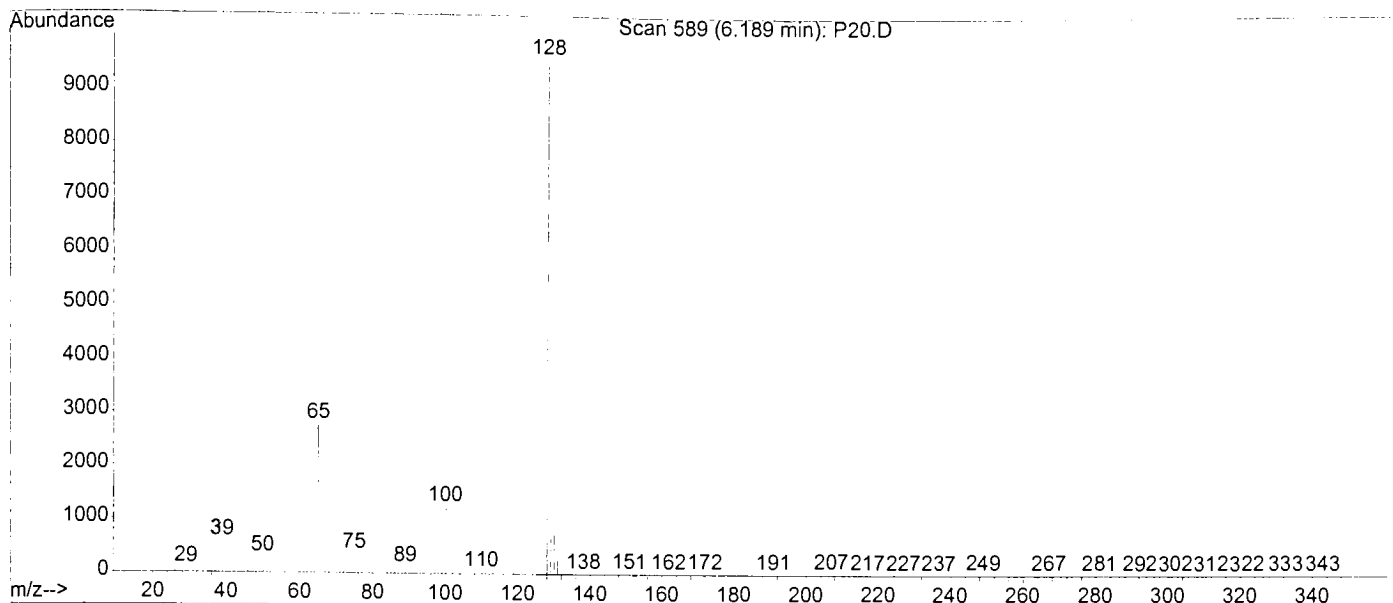Misc :                                    Inst : RIT Chemi
                                          Multiplr: 1.00
                                          Sample Amount: 0.00

MS Integration Params: autoint1.e
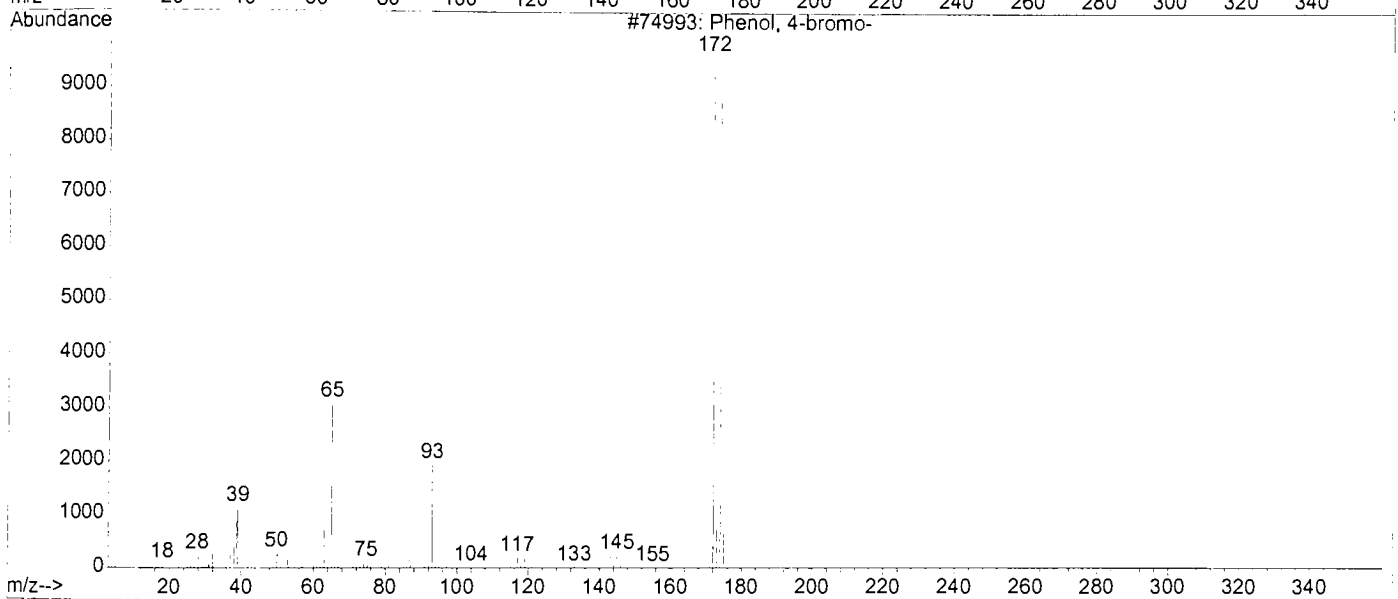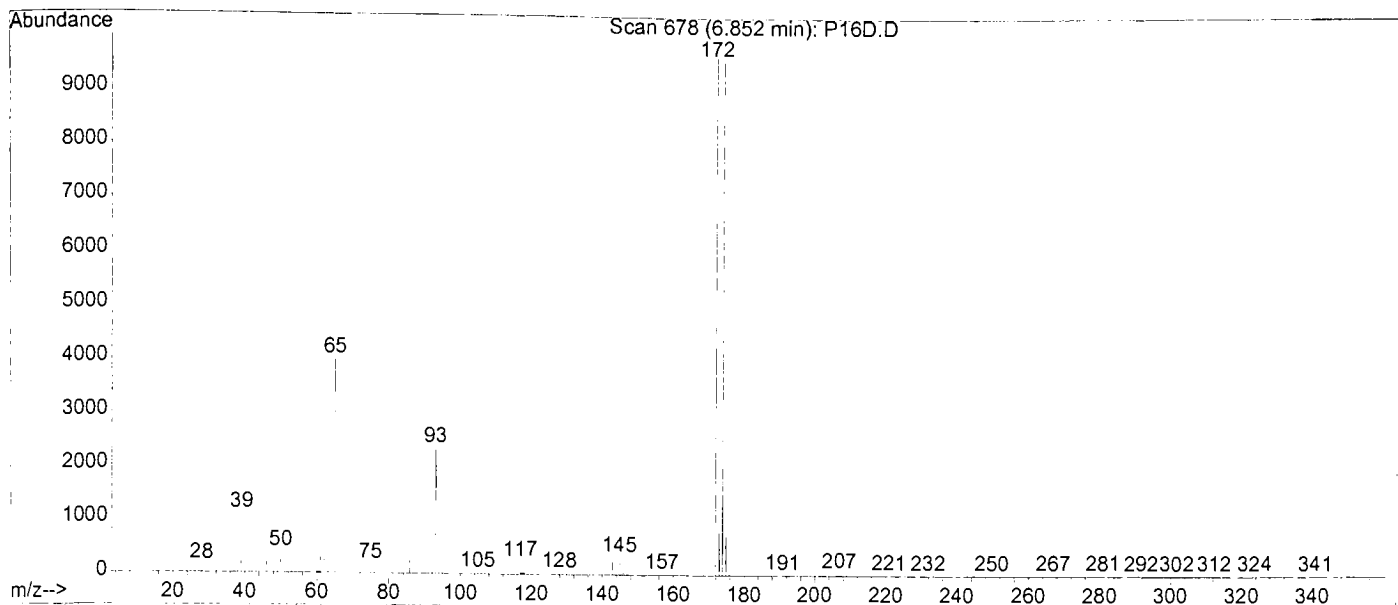Method : C:\HPCHEM\1\5973\PANKAJ.M (Chemstation Integrator)
Title :



AMINPHCL.D  PANKAJ.M  Thu Jul 13 20:23:04 2000
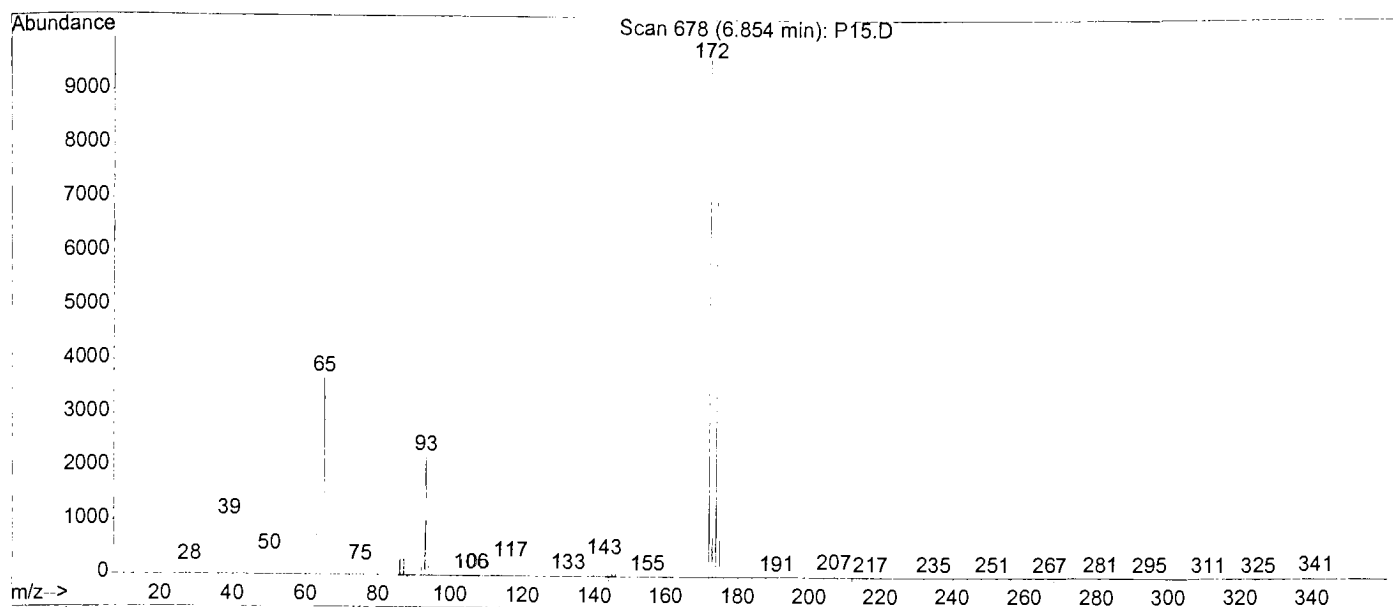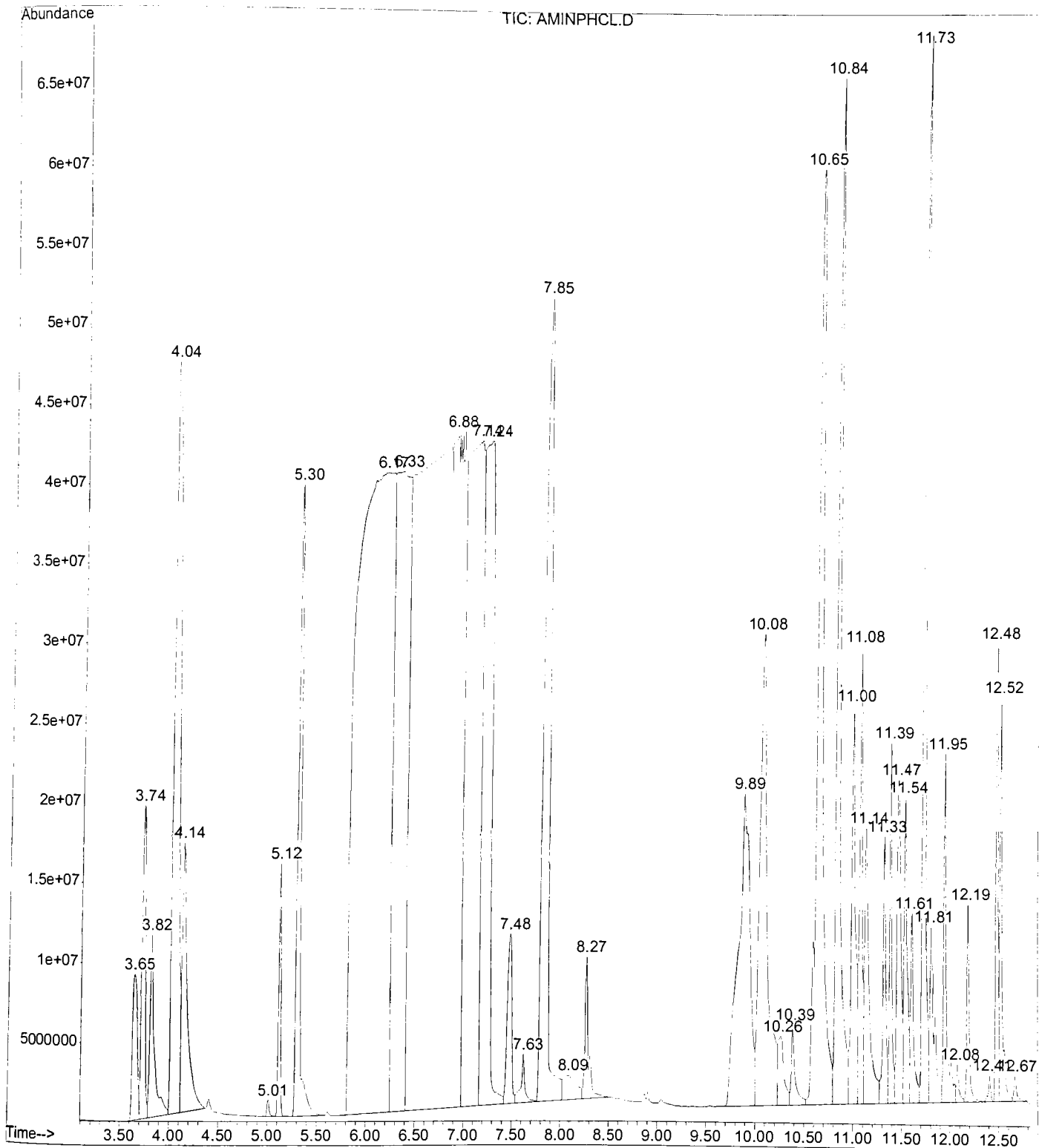
Library Searched : C:\DATABASE\NIST98.L
Quality          : 42
ID               : Phenol, 3-amino-2,4-dichloro-      MS Spectrum 10.

B10

Scan 1039 (10.848 min): AMINPHCL.D

#76330: Phenol, 3-amino-2,4-dichloro-

Data File : C:\HPCHEM\1\DATA\AMINPHEN.D              Vial: 1        B11
Acq On    : 13 Jul 2000   19:43                      Operator:
Sample    :                                          Inst    : RIT Chemi
Misc      :                                          Multiplr: 1.00
                                                     Sample Amount: 0.00

MS Integration Params: autoint1.e
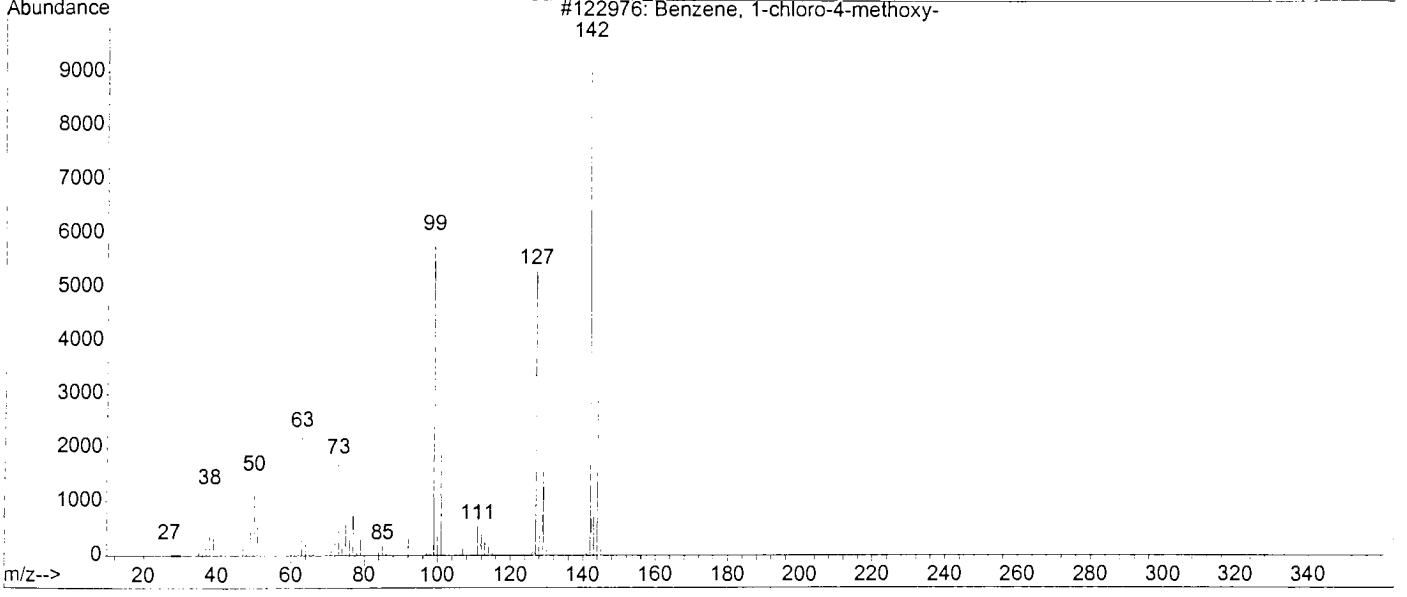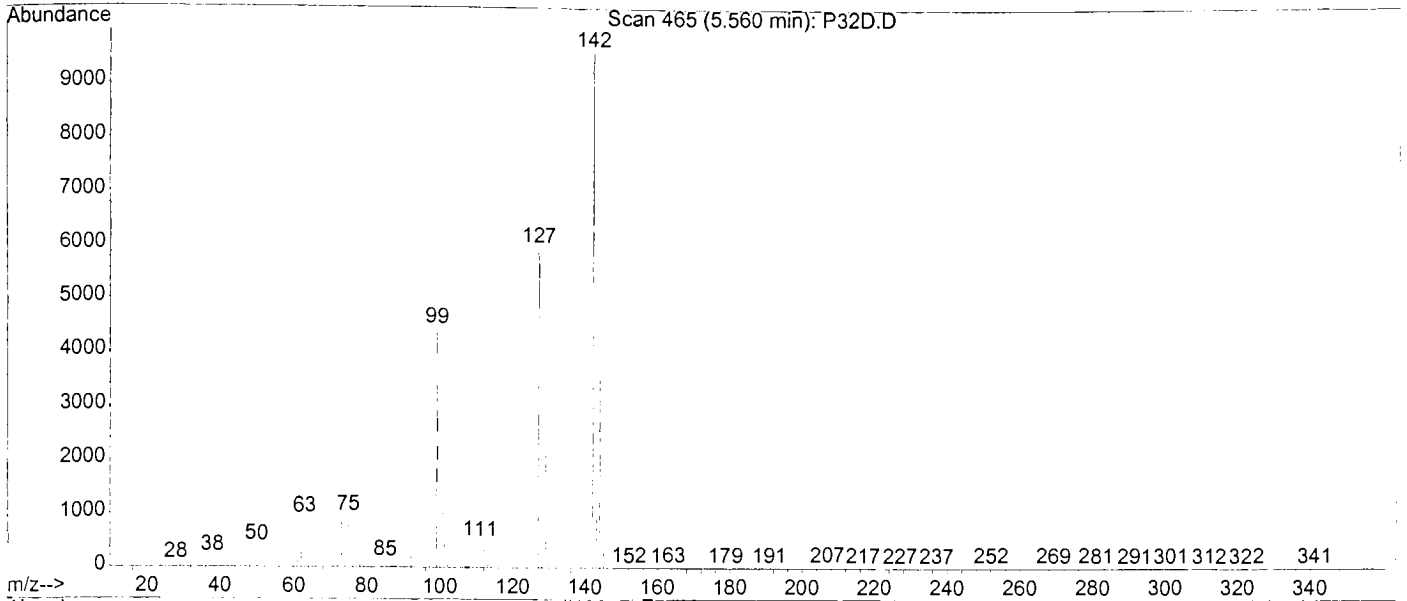Method    : C:\HPCHEM\1\5973\PANKAJ.M (Chemstation Integrator)
Title     :



TIC: AMINPHEN.D

AMINPHEN.D   PANKAJ.M   Thu Jul 13 19:55:56 2000

Library Searched : C:\DATABASE\NIST98.L
Quality          : 25
ID               : Phenol, 3-amino-2,4-dibromo-    MS Spectrum 11.

Scan 1174 (11.858 min): AMINPHEN.D

Abundance

9000

8000

7000

6000

5000

4000

3000

2000

1000

0

m/z-->  20   40   60   80   100  120  140  160  180  200  220  240  260  280  300  320  340

Peaks: 28 38 52 62 78 89 107 117 133 143 158 170 186 197 211 223 238 249 267 281 291 301 313 327 341

#95508: Phenol, 3-amino-2,4-dibromo-

Abundance

9000

8000

7000

6000

5000

4000

3000

2000

1000

0

m/z-->  20   40   60   80   100  120  140  160  180  200  220  240  260  280  300  320  340

Peaks: 39 52 65 78 91 109 146 159 170 185 218 251 267

Data File : C:\HPCHEM\1\DATA\OPHENPHE.D                    Vial: 1      B12
Acq On    : 21 Jun 2000  16:16                             Operator:
Sample    :                                                Inst    : RIT Chemi
Misc      :                                                Multiplr: 1.00
                                                           Sample Amount: 0.00

MS Integration Params: autoint1.e
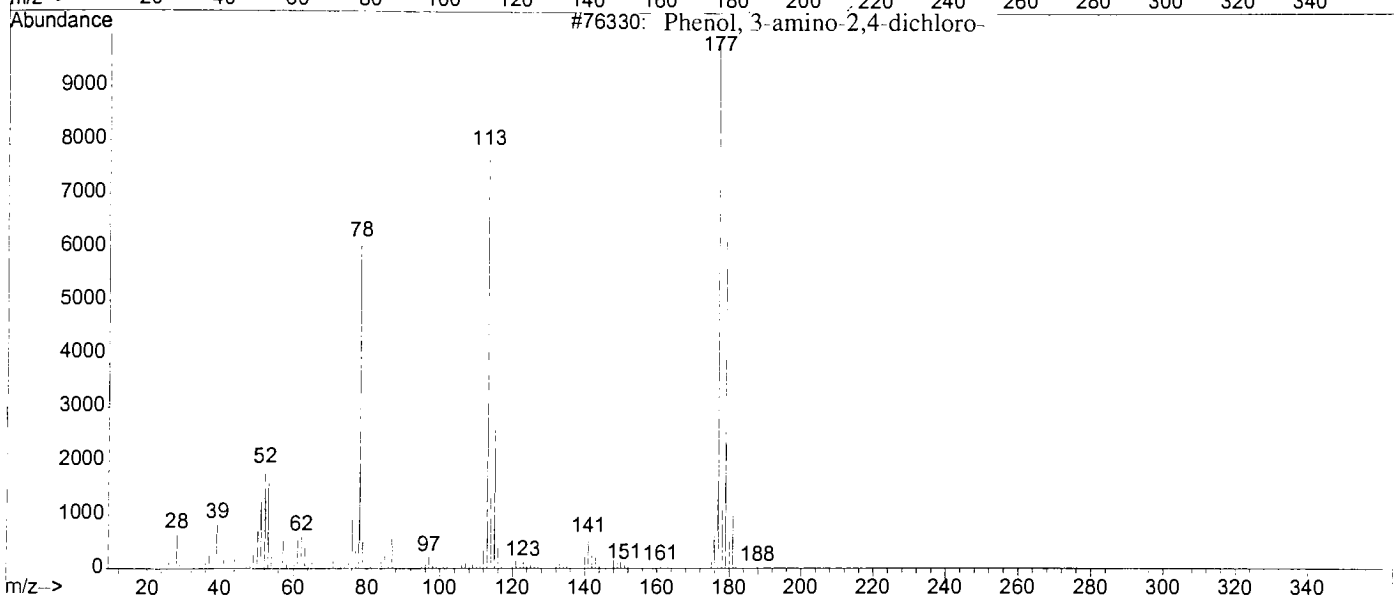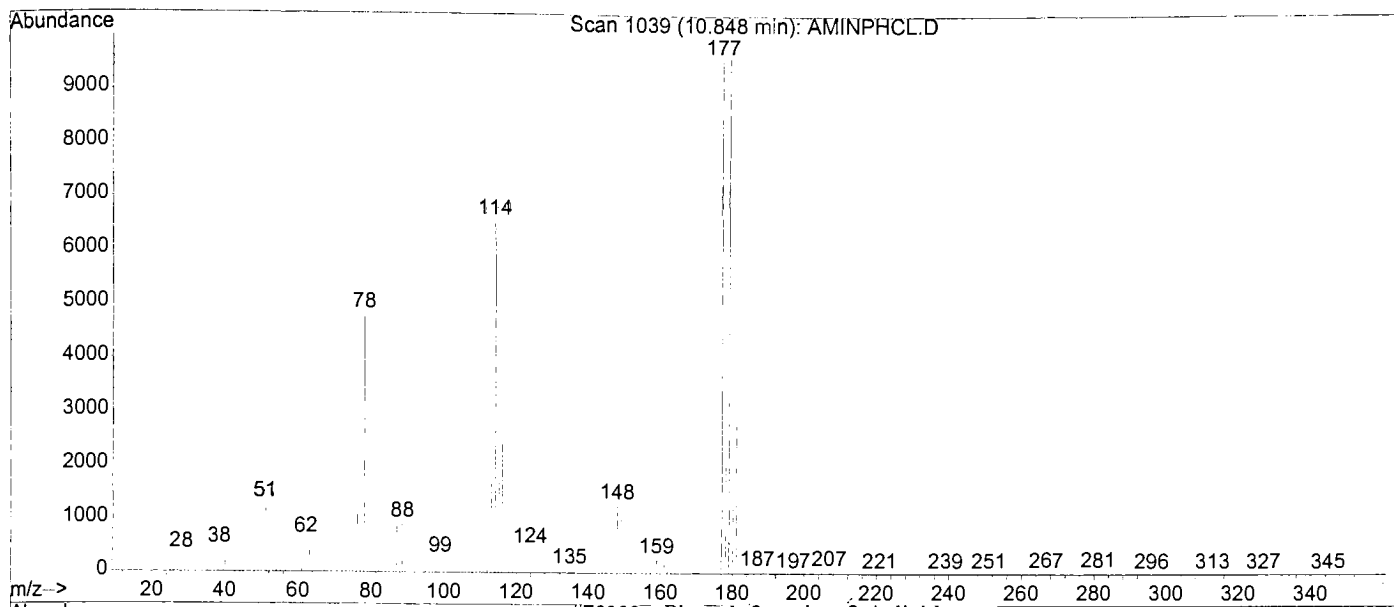Method    : C:\HPCHEM\1\5973\PANKAJ.M (Chemstation Integrator)
Title     :

Library Searched : C:\DATABASE\NIST98.L
Quality          : 90
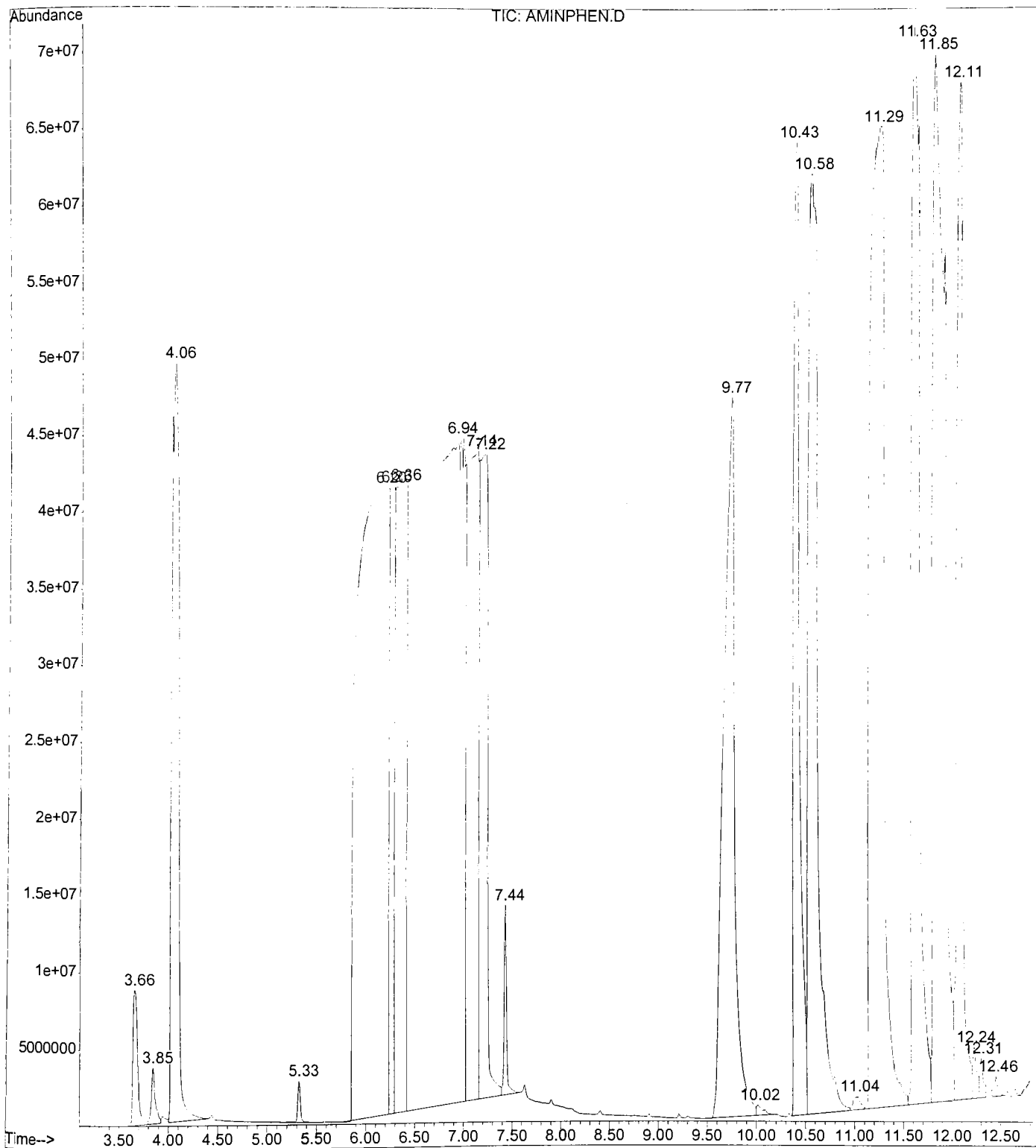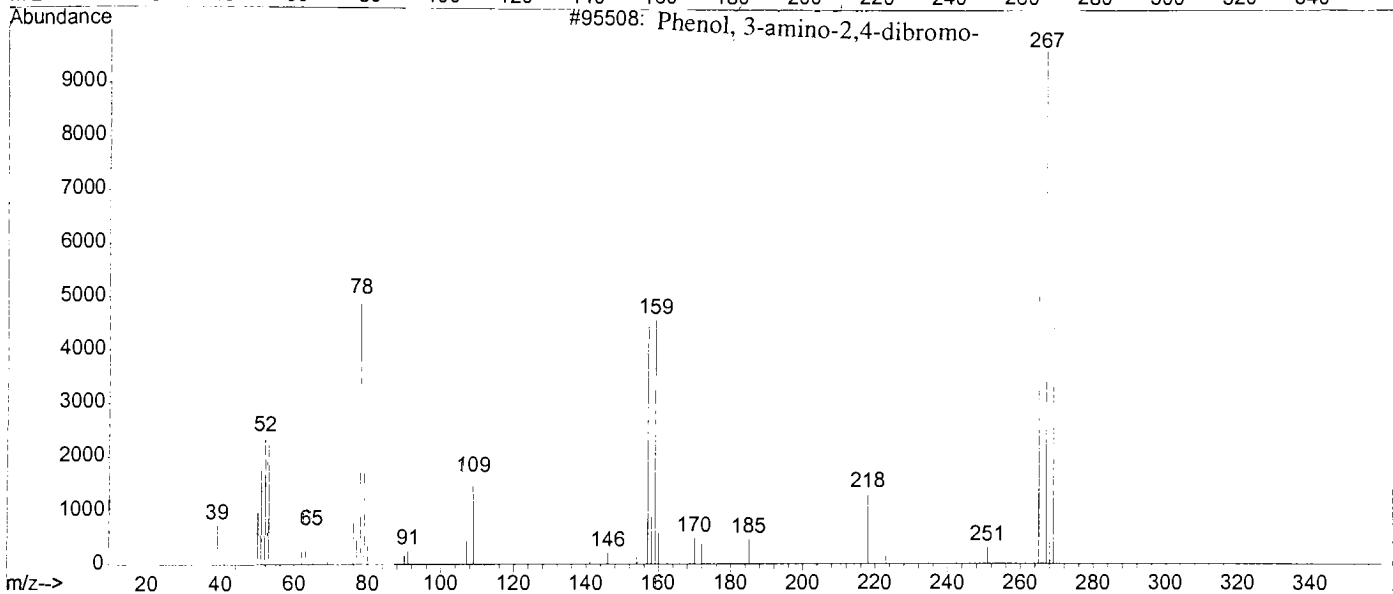ID               : [1,1'-Biphenyl]-2-ol, 3-chloro-     MS Spectrum 12.



Scan 1334 (12.052 min): OPHENPHE.D

#84030: [1,1'-Biphenyl]-2-ol, 3-chloro-
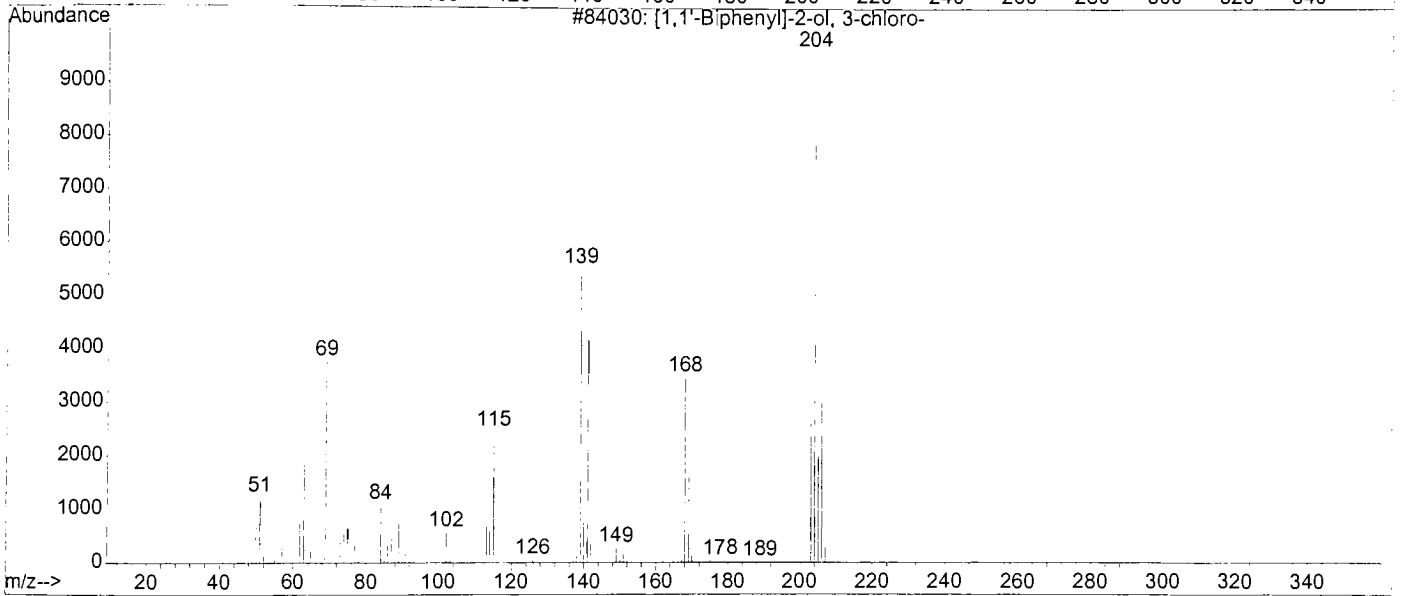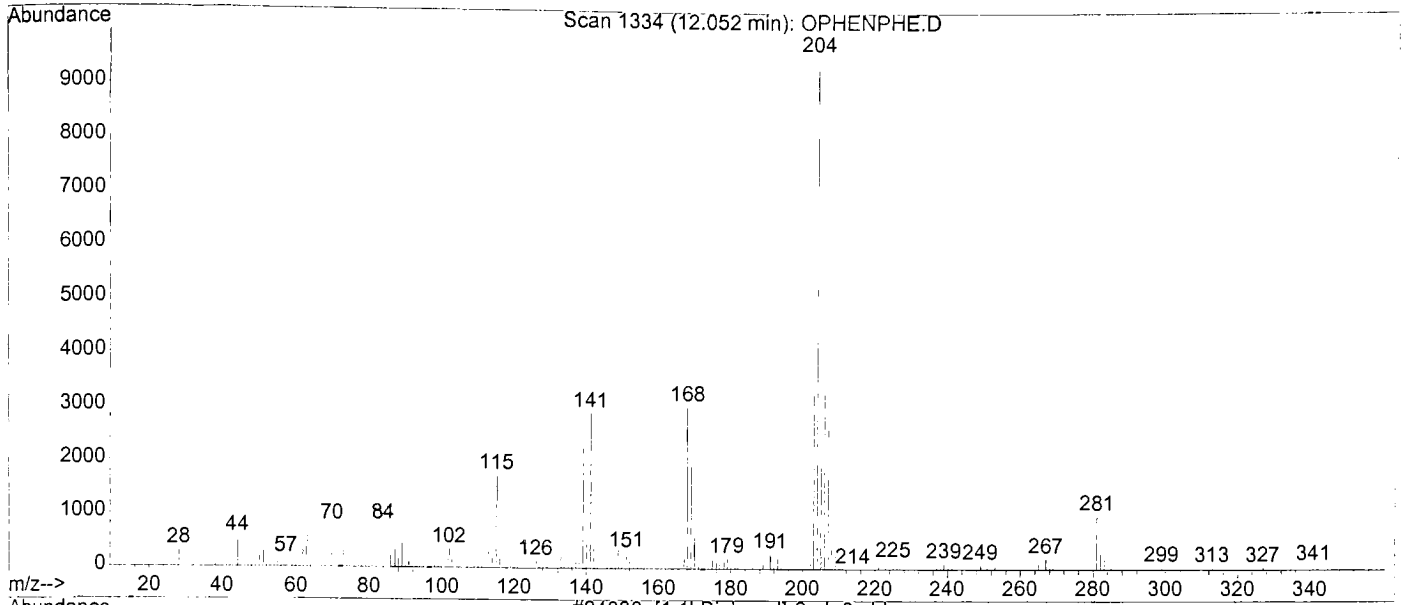
# GC Spectrum 13. Area Percent Report

Data File : C:\HPCHEM\1\DATA\OPHENHBR.D          Vial: 1
Acq On    : 21 Jun 2000  16:57                   Operator:
Sample    :                                      Inst    : RIT Chemi
Misc      :                                      Multiplr: 1.00
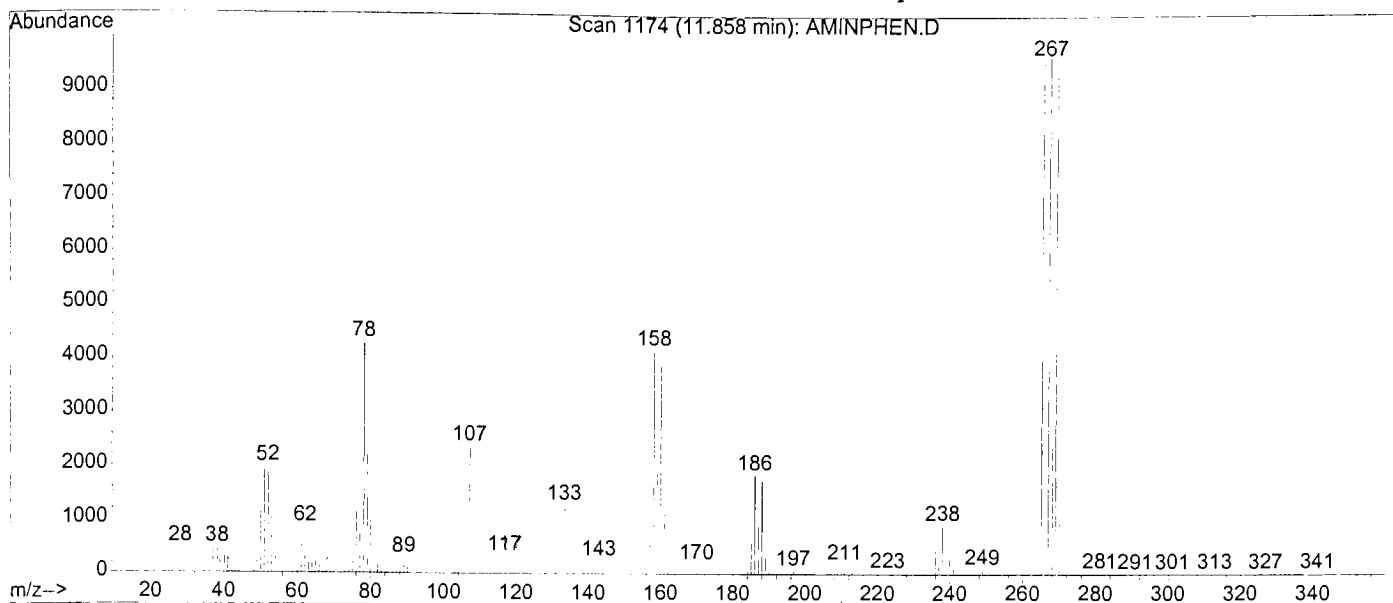                                                 Sample Amount: 0.00

MS Integration Params: autoint1.e
Method    : C:\HPCHEM\1\5973\TRACY.M (Chemstation Integrator)
Title     :



ISOLVENT.D   TRACY.M   Wed Jun 21 17:10:24 2000

Library Searched : C:\DATABASE\NIST98.L
Quality          : 95
ID               : [1,1'-Biphenyl]-2-ol, 3,5-dibromo-    MS Spectrum 13.



Scan 950 (9.182 min): OPHENHBR.D

#63409: [1,1'-Biphenyl]-2-ol, 3,5-dibromo-
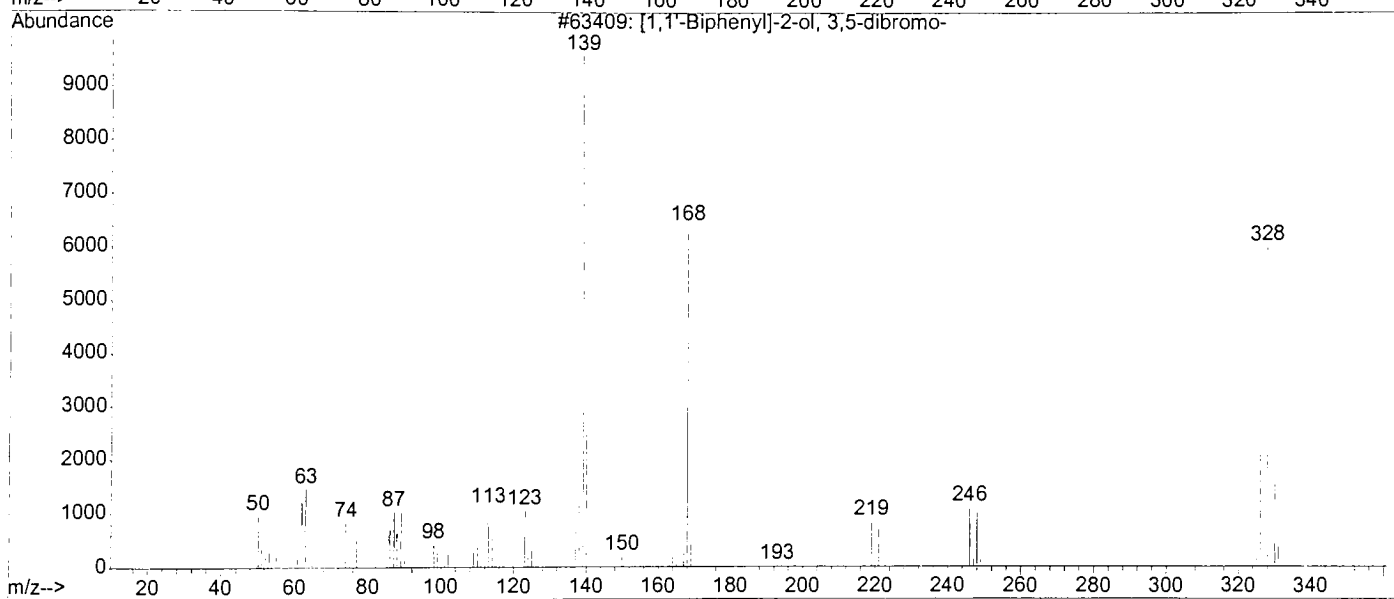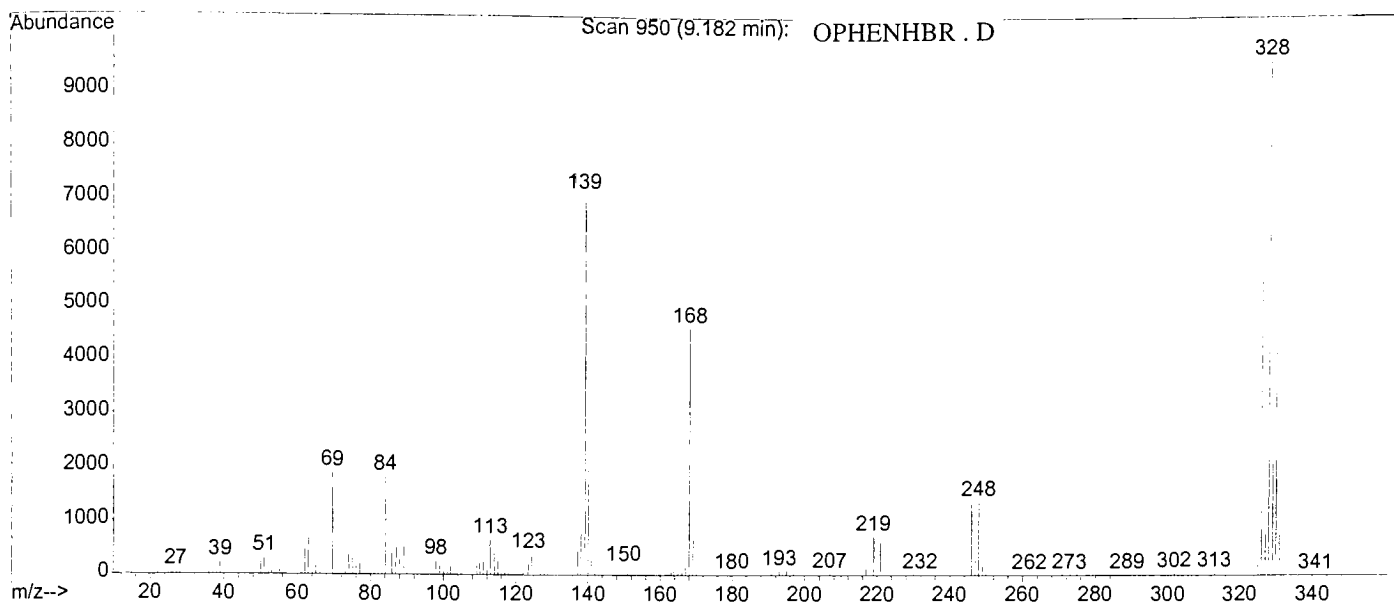
APPENDIX C

Table of NMR Parameters

```
Current Data Parameters
NAME            Jun19-00
EXPNO                  6
PROCNO                 1

F2 - Acquisition Parameters
Date_             500000
Time               16.51
INSTRUM            spect
PROBHD      5 mm QNP  1H
PULPROG             zg30
TD                 32768
SOLVENT             DMSO
NS                    16
DS                     2
SWH          6172.839 Hz
FIDRES       0.188380 Hz
AQ           2.6542580 sec
RG                  28.5
DW            81.000 usec
DE             6.00 usec
TE               300.0 K
D1           1.00000000 sec
P1             9.00 usec
DE             6.00 usec
SFO1       300.1318534 MHz
NUC1                  1H
PL1            -5.00 dB

F2 - Processing parameters
SI                 16384
SF          300 1300000 MHz
WDW                   EM
SSB                    0
LB              0.30 Hz
GB                     0
PC              1.00

1D NMR plot parameters
CX             20.00 cm
F1P            11.000 ppm
F1           3301.43 Hz
F2P            -1.000 ppm
F2           -300.13 Hz
PPMCM        0.60000 ppm/cm
HZCM       180.07800 Hz/cm
```

**APPENDIX D**

Data Analysis Parameters

Method Name: C:\HPCHEM\1\5973\CHRIS.M


Percent Report Settings
----------------------

Sort By: Retention Time

Output Destination
    Screen:  Yes
    Printer: Yes
    File:    No

Integration Events: AutoIntegrate

Generate Report During Run Method:  Yes

Signal Correlation Window: 0.020


Qualitative Report Settings
---------------------------

Peak Location of Unknown: Apex

Library to Search       Minimum Quality
C:\DATABASE\NIST98.L       0

Integration Events: AutoIntegrate

Report Type: Summary

Output Destination
    Screen:  No
    Printer: Yes
    File:    No

Generate Report During Run Method:  Yes


Quantitative Report Settings
----------------------------

Report Type: Summary

Output Destination
    Screen:  Yes
    Printer: No

Method: CHRIS.M            Thu Jul 13 15:56:22 2000              Page: 1

Calibration Last Updated: Mon Nov 20 15:48:49 1995


Reference Window: 10.00 Percent
Non-Reference Window: 5.00 Percent
Correlation Window: 0.02 minutes
Default Multiplier: 1.00
Default Sample Concentration: 0.00

Compound Information
--------------------

------------------------------------------------------------------------------
 1)   mass 284                                     ( )

Ret. Time   7.28 min., Extract & Integrate from   6.78 to   7.78 min.

Signal        Rel Resp.    Pct. Unc.(rel)     Integration
Tgt  283.90                                   *** AUTO ***

Lvl ID    Conc ( )    Response
3              1.000       371748
4             10.000      3849624
5            100.000     18762380
2              0.100        31150

Qualifier Peak Analysis ON
Curve Fit: Avg. RF
------------------------------------------------------------------------------
 2)   mass 283                                     ( )

Ret. Time   7.28 min., Extract & Integrate from   6.78 to   7.78 min.

Signal        Rel Resp.    Pct. Unc.(rel)     Integration
Tgt  283.90                                   *** AUTO ***

Lvl ID    Conc ( )    Response
3              1.000       371748
4             10.000      3849624
5            100.000     18762380
2              0.100        31150

Qualifier Peak Analysis ON
Curve Fit: Avg. RF
------------------------------------------------------------------------------
 3)   mass 247                                     ( )

Ret. Time   7.28 min., Extract & Integrate from   6.78 to   7.78 min.

Signal        Rel Resp.    Pct. Unc.(rel)     Integration
Tgt  246.90                                   *** AUTO ***

```
        1.000          44562
       10.000         479738
      100.000        2662997
        0.100             -1
```

alifier Peak Analysis ON
rve Fit: Avg. RF

-----------------------------------------------------------------------

) mass 212                              ( )

t. Time    7.28 min., Extract & Integrate from    6.78 to    7.78 min.

gnal        Rel Resp.    Pct. Unc.(rel)    Integration
t  212.00                                  *** AUTO ***

l ID    Conc ( )    Response
          1.000          25707
         10.000         264918
        100.000        1532237
          0.100             -1

alifier Peak Analysis ON
rve Fit: Avg. RF

-----------------------------------------------------------------------


                END OF DATA ANALYSIS PARAMETERS
                -------------------------------

                   Thu Jul 13 15:56:22 2000

```