

ANTHROPOGENIC CONTAMINANTS AND FISH
HEALTH AT FIVE SITES IN OKLAHOMA

By

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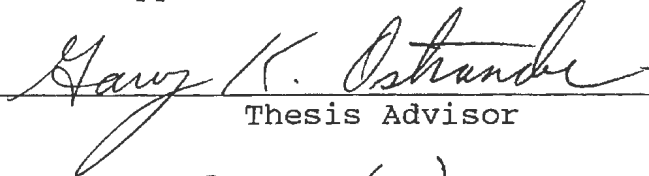
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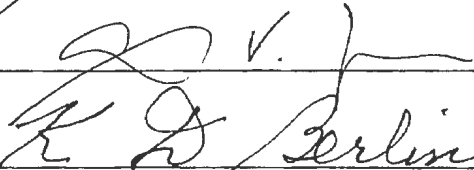
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PREFACE

The primary objective of this study was to provide preliminary information on the carcinogenic potential of some Oklahoma waters. These were meant to be preliminary studies for future research.

I would like to express appreciation to my major advisor, Dr. Gary K. Ostrander, for his guidance and assistance throughout this study. Appreciation is also expressed to my other committee members, Dr.'s Al Zale and K. Darrell Berlin, for their assistance and encouragement.

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CHAPTER I

INTRODUCTION

This thesis is presented in three chapters that are intended to be read and published as separate manuscripts. Therefore, many of the methods are reproduced in each chapter. They are written in thesis format for writer convenience.

CHAPTER II

A NOVEL CASE OF NEUROFIBROMA IN GIZZARD

SHAD, DOROSOMA CEPEDIANUM

Introduction

A neoplasm is an indifferentiated cell mass arising from normal cells, often for no apparent reason. Many studies have been conducted to determine what the causes are for some neoplasms exhibited in aquatic organisms, fish in particular. The primary causes that have been examined to date are: chemical contaminants, genetic abnormalities, and viruses.

Since the first report in 1941 of an epidemic of epidermal neoplasms in the brown bullhead, Ameiurus nebulosus (Lucké and Schlumberger, 1941), and a subsequent report by Dawe (1964) suggesting that neoplasms in the white sucker, Catostomus commersoni, may have been caused by chemical pollutants, many researchers have focused their investigations on determining causes of lesions in aquatic organisms (e.g., Malins et al., 1988; Myers et al., 1987; Murchelano and Wolke, 1985; McCain et al., 1982). Recent studies suggest that some neoplasms (e.g. hepatic) are caused by xenobiotics, specifically the aromatic

hydrocarbons, in the aquatic environment (e.g., Fabacher et al., 1991; Myers et al., 1991; Vogelbein et al., 1990; Baumann et al., 1987).

Many fish populations in areas that are heavily industrialized have shown an increased prevalence of liver neoplasms. Puget Sound, Washington (Malins et al., 1987; Malins et al., 1984), San Pedro Bay, California (Eganhouse and Kaplan, 1982), Boston Harbor, Massachusetts, and the North American Great Lakes (Fabacher et al., 1991; Baumann et al., 1987) have been the focus of such studies. Incidences of 10-80% (Baumann et al., 1990; Vogelbein et al., 1990; Murchelano and Wolke, 1985), and in some extreme cases 100% (House of Representatives, 1983), of neoplasms were found in some fish populations. For example, Black (1987) reported that 100% of the sauger in the Torch Lake, Michigan, had liver cancers. Aromatic hydrocarbons are found in many aquatic systems (Eisler, 1987) and their effects on aquatic organisms have been observed (Neff, 1985). For example, in 1975, tumor surveys were begun in the Puget Sound, Washington, because large numbers of liver tumors were being reported in the English Sole, Parophrys vetulus (Pierce et al., 1978; McCain et al., 1977). Through these studies it was shown that aromatic hydrocarbons, chlorinated butadienes, and polychlorinated biphenyls are the major types of compounds found in association with neoplasm bearing fish (Reviewed in Myers et al., 1990). To

date, this is the most complete data set supporting the theory of chemically-induced neoplasms in feral fish populations. Greater than 2,000 compounds have been identified in the sediment in this area (Malins et al., 1984). The 45 sampling stations in the Puget Sound have provided a sediment pollution profile containing such compounds as benzo[a]pyrene, benz[a]anthracene, heavy metals, polychlorinated biphenyls, and other chlorinated hydrocarbons (Malins et al., 1984). This area continues to be studied intensively.

A genetic based etiology is observed in research conducted on platyfish and swordfish. These fish show no occurrences of neoplasia in wild populations yet when interpopulational and interspecific crosses are performed, spontaneous neoplasia occurs in the progeny in the forms of melanomas, pterinophoromas, neuroblastomas, thyroid carcinomas, kidney carcinomas, and reticulosarcomas (Anders and Anders, 1978). The development of these neoplasia is linked to an oncogene designated as the "tumor gene". It was discovered that negative not positive regulation of the gene was responsible for the mediation of the neoplasms (Anders et al., 1984).

Viruses are suggested as another cause of neoplasms in fish. Lymphoma in the northern pike and neurofibromatosis in the bicolor damselfish are two examples of lesions in which this type of etiology is possible since other causes

such as hydrocarbons have been systematically ruled out. These two types of lesions have been reported in fish populations occurring in waters without obvious contamination sources. These have been reported, in the case of the lymphoma (Sonstegard, 1976), and suggested in the case of the damselfish (Schmale and Hensley, 1988), may be caused by a virus. In feral populations found living in reefs near Florida, the tumor incidence was higher in areas where fish were densely populated. This was indicative of a transmissible agent such as a virus.

The focus of this study was the resident gizzard shad population in Arbuckle Lake, Oklahoma. Raised, black lesions on the head, sides, and fins were reported by Jimmie Pigg of the Oklahoma Department of Health (G. K. Ostrander, personal communication). The primary objectives were to: 1) conduct chemical profiles of the water and sediment and determine if any anthropogenic contaminants were present that might be responsible for the lesions; 2) sample the shad population and determine what proportion of the population was exhibiting the lesions and if there were any other measurable differences (i.e. morphological) in the population; 3) determine by histological examination the type and extent of lesions being exhibited by the fish.

Materials and Methods

Site Description

Arbuckle Lake is located in the Arbuckle Mountains in south central Oklahoma in Murray county (Figure 1). This lake is located in an area where natural sulfur springs and asphalt formations occur in the typical hydrogeology. A preliminary survey (Jimmie Pigg, personal communication) revealed possible malignant neoplasms occurring in the feral gizzard shad population in this lake. As this was an unusual occurrence, it merited further study. The two sampling stations were at the Guy Sandy boat access on the northwestern side of the lake in N $\frac{1}{2}$ SW $\frac{1}{4}$ S7 T1S R3E. Station 1 was along the western shore of a northern arm named the Guy Sandy arm because the inflow is from Guy Sandy Creek. Station 2 was in a small cove immediately to the west of the boat ramp. Alternate sampling stations were surveyed but produced almost no shad per net night therefore these stations were abandoned for the remainder of the study.

Sampling of fish populations

Arbuckle Lake was sampled with gill nets and seines. Three 100-yard gill nets, six feet deep with 2 to 2.5 inch bar length mesh were set overnight and checked at dawn. Two of the nets were set in tandem along the western shore starting at the boat ramp and continuing north at

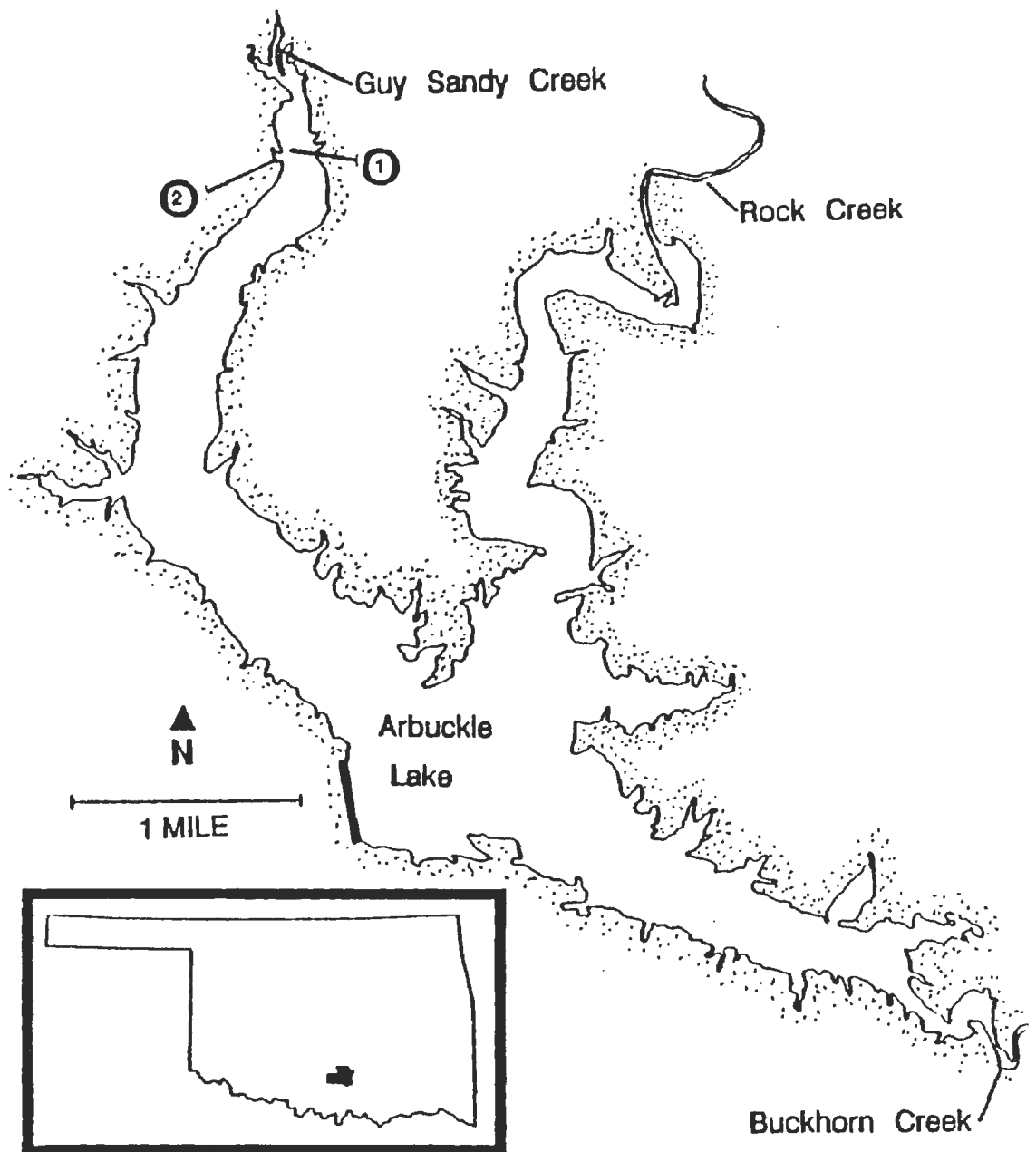


Figure 1: Location of Murray County and sampling stations on Arbuckle Lake.

approximately five feet in depth. The third net was set across the small cove on the western side of the boat ramp. All fish were removed from the nets and immediately examined. All shad plus any other species exhibiting possible lesions were transferred to shore for further examination and histologic processing. The nets were then set for multiple two-hour sets during the day. Those with visible lesions were necropsied and the lesions and livers were fixed for processing. Muscle and liver tissues were taken as a control from a shad with no visible lesions. The tissues were sectioned to five millimeter thicknesses and fixed in 10% neutral buffered formalin (1:10 tissue to fixative) for 24-48 hours. Tissues were then washed with water overnight and transferred to 70% ethanol. Lesions were removed and placed in Bouin's fixative for two hours, transferred to 3% glutaraldehyde for three hours, and finally transferred to cacodylate buffer (pH = 7.2) in preparation for electron microscopy.

Relative weights (W_r) were calculated using standard weight regressions derived from Texas Parks and Wildlife Department age and growth data collected statewide (Childress, 1991). The relative weight is a percentage of the standard weight that is calculated on a logarithm (base-10) as the 75th-percentile of the weight on the log of the midpoint of the length-class. The equation is:

$$\text{Equation 1: } \log_{10}W_s = a + b \log_{10}L$$

where W_s is the standard weight, a is the regression intercept, b is the regression coefficient, and L is the total length. The intercept and regression coefficient calculated by Childress were -4.860 and 2.932 respectively.

Lengths, weights, and relative weight differences were analyzed using a general linear model (GLM) procedure in the Statistical Analysis System (SAS, 1985) software. The GLM procedure is an analysis of variance modified for unbalanced data. The measurements were compared between fish with lesions and fish without lesions and combining all sampling dates.

Water Samples

Three samples of four liters of water were collected in pre-cleaned, four-liter amber bottles. Samples were stored in the dark at 4°C until the time of extraction. Four liters of water were extracted on a Carbon-18 (C18) bonded solid phase extraction column (Bond Elut, Analytichem International, Product #607306). The columns were conditioned by passing two column volumes (12 ml) of methylene chloride through under a slight positive pressure, followed by two column volumes of reagent-grade water. The columns were not allowed to dry from this point on. The columns were then connected to a two-liter separatory funnel, and four liters of water were passed through under a slight vacuum. The columns were air dried under vacuum for

five minutes to remove residual water prior to elution. Compounds were eluted with 40 ml of methylene chloride. This eluate was then passed through a 5 g column of sodium sulfate to remove excess water and rinsed twice with 2 ml of methylene chloride. The eluate was then concentrated by rotary evaporation in a 60°C water bath to 15 ml, transferred to a 15 ml concentrator tube, and finally concentrated to 1 ml in a 60°C water bath with nitrogen purge. The sample was then analyzed by gas chromatography/mass spectrometry. A VG Analytical TS-250 mass spectrometer connected to a Hewlett-Packard 5890A gas chromatograph was used for the analysis. A 30-meter, 0.32-mm inner diameter capillary column with an SE-54 bonded phase was used in the chromatograph. One to three microliters, depending on concentration, was injected and the sample was subjected to a programmed temperature gradient which raised the temperature 10°/minute from 50° to 280° C. The spectra of each peak was compared to reference spectra contained in the National Bureau of Standards (NBS) on-line library (Milne and Heller, 1978; Heller and Milne, 1980).

Sediment Samples

Sediment samples were collected at the same locations described above. Three replicate samples of one liter each were taken in pre-cleaned amber bottles with teflon lid

liners (Scientific Specialties Service, Product #B71132). The samples were stored at -40°C until extraction. In pilot studies, two separate methods of sediment extraction were employed. One was used by Malins and colleagues (1980) in studying Puget Sound sediments and the other by Fabacher and co-workers (1988) in studying Black River sediments. These methods were compared for their ability to resolve compounds and the better of the two, that which provided the best separation of our compounds (Fabacher et al., 1988), by our lab, was used with slight modifications as described below for the remainder of the study.

Dry Sediment Extraction with Fractionation on Neutral Alumina

The sediments were thawed and air dried under a hood for 24 hours prior to extraction. A 100 g aliquot of sediment was then powdered in a blender (Hamilton Beach, model #585-3). The sediment was extracted twice with 100 ml of a benzene:methanol (60:40) and twice with 100 ml of methylene chloride. At each step the slurry was shaken for two hours at 400 rpm on an orbital shaker. The sediment was allowed to settle out for 20 minutes, and the solvent was then decanted off the top into a 500 ml solvent rinsed bottle. The extracts were concentrated with a rotary evaporator in a 60°C water bath to about 10 ml. This solution was transferred to a 15 ml concentrator tube with

two, 2 ml rinses of methylene chloride. Finally, the sample was concentrated at 60°C under a continuous nitrogen stream to one ml. The sample was then fractionated on an alumina column.

An 11 x 250 mm glass chromatography column with a 200 ml solvent reservoir was fitted with a glass wool plug and filled with 9 g of neutral alumina previously activated at 200°C for 12 hours. Enough N-hexane was added to cover the alumina and the solution was shaken to remove any air bubbles. A small layer of sand was placed on top of the alumina to prevent disturbance when adding sample or solvent. Samples were applied and eluted with 400 ml of N-hexane. This first fraction contained mostly aliphatic hydrocarbons. The column was then eluted with 1000 ml of benzene. This fraction contained mostly aromatic hydrocarbons. Finally, the column was eluted with 1550 ml of chloroform and this final fraction contained mostly the nitrogen containing aromatic hydrocarbons. All three fractions were reduced by rotary evaporation to about 15 ml and resultant solutions were concentrated on a 60°C water bath under a nitrogen stream to one ml. The samples were then analyzed by gas chromatography/mass spectrometry. A VG Analytical TS-250 mass spectrometer was used with the same conditions as described earlier for the water analysis. One to three microliters, depending on concentration, were injected and the sample was subjected to a programmed

temperature gradient (10° /minute from 50° to 280° C). The spectra of each peak was compared to reference spectra, contained in the NBS on-line library (Milne and Heller, 1978; Heller and Milne, 1980) for compound identification.

Results

Water and Sediment Sampling

No anthropogenic contaminants detectable by our methods were identified in any water samples. One positively identified compound, 2-methyl-1(1,1-dimethyl)propanoic acid which is a decomposition product of wood, was detected in the sediment samples. No compounds were detected above detection limits ($<0.005\mu\text{g/l}$) in water samples taken for benzene, toluene, ethylbenzene, and xylene (BTEX) analysis.

Fish Sampling

A total of 374 adult and approximately 200 juvenile gizzard shad were captured. Forty-seven other individuals were captured comprising six taxa (Table I). Lesions that were taken from gizzard shad were sent to Dr. William Hawkins of the Gulf Coast Research Laboratory and Dr. John Harshbarger, Director of the Registry of Tumors in Lower Vertebrates, Smithsonian Institute, for classification. They both concluded that the lesion was a neurofibroma. Based on our sampling of the lake, there was a 17.63% occurrence of neurofibroma tumors in the adult gizzard shad

population (Note: subsequent studies and sampling suggest close to 21-22% incidences). This percentage was a mean percentage of the eight sampling trips which ranged from 4-20%. This was probably a very conservative estimate because it was based on lesions that could be seen with the unaided eye. The lesions were observed in adult fish with a mean length of 33.37 cm (Table II). Thus, we estimated the fish to be 2 to 3 years in age. Among the 200 juveniles seined along shore and examined, no lesions were observed. Gizzard shad comprised 91% of fish captured as was expected based on our sampling methodology. Statistically significant differences were observed in the length, weight, and relative weights (Table III) using a general linear models procedure in SAS, which is a modified analysis of variance for unbalanced data. The fish without lesions were shorter (342 mm vs. 351 mm) and weighed less (414 g vs. 434 g) but their relative weight was higher (111% vs. 107%) than that of fish with lesions.

TABLE I
FISH SPECIES AND NUMBERS

Species	Numbers	%
*Gizzard Shad		
(<u>Dorosoma cepedianum</u>) (Adult)	374	91
Largemouth Bass		
(<u>Micropterus salmoides</u>)	8	2
White Crappie		
(<u>Pomoxis annularis</u>)	10	2
Carp		
(<u>Cyprinus carpio</u>)	12	2
Shortnose Gar		
(<u>Lepisosteus oculatus</u>)	4	1
White Bass		
(<u>Morone chrysops</u>)	12	2
Spotted Sucker		
(<u>Minytrema melanops</u>)	1	<1

* In, addition, approximately 200 juvenile fish
were seined along shore.

TABLE II

GIZZARD SHAD TUMOR FREQUENCIES IN
ARBUCKLE LAKE, DAVIS, OKLAHOMA

Date	# of Shad Caught	# of Shad with Tumors	Length Range (cm)	% Tumors
08-06-91	49	2	28.9 - 33.4	4.08
08-07-91	56	13	26.2 - 34.4	23.21
12-05-91	11	1	27.5 - 30.5	9.09
04-24-92	80	5	30.0 - 40.5	6.25
08-21-92	69	7	27.0 - 30.0	10.14
09-12-92	11	3	33.5 - 36.5	27.27
01-06-93	107	31	31.0 - 39.5	28.97
03-27-93	270	67	29.6 - 39.7	24.81
Totals	653	129	26.2 - 40.5	*16.73

*This is the mean percentage of the eight sampling trips.

Discussion

This is the first epizootic of neurofibroma ever reported in a freshwater species. Sampling of Arbuckle Lake revealed no obvious contamination sources. Since we did not analyze for viruses, heavy metals, or radiation, and no definitive reports are available on this data, we cannot rule them out as possible causes of the lesions. These are currently being investigated.

Similar to reports of lymphoma in northern pike and neurofibromatosis in the damselfish (Sonstegard, 1976; Schmale et al, 1983), the gizzard shad are in apparently pristine water. A similar type of lesion has been previously described in the bicolor damselfish. Lesion prevalence in the damselfish has been reported to be 0.1-23% (Schmale et al. 1983). The population of gizzard shad that we studied exhibited a 17% occurrence. Based on our sampling, there are significant numbers of fish exhibiting neurofibromas and the percentages are comparable to the occurrence of neurofibromatosis in the damselfish. While gizzard shad are consistently caught at this location exhibiting these lesions, this lesion has not been reported in any other Oklahoma species (Jimmie Pigg, personal communication). Results of sampling in four locations at several different times showed that the gizzard shad population was highly concentrated in the area of the lake that we were sampling in. It is unknown why this was so,

but correlates to data reported by Schmale and colleagues (1983) that the lesions they observed were more prevalent in concentrated populations.

Fish with lesions were longer and weighed more but their relative weight was less. This can be explained by making the assumption that fish exhibiting lesions were older than fish without thus they were longer and heavier. However, the fact that they were affected with the lesion possibly reduced their overall fitness and thus reduced their relative weight. The relative weight would be reduced in fish not utilizing their biomass intake optimally (Wege and Anderson, 1978). The etiology and transmissibility of the neurofibroma in this population of gizzard shad is currently being studied.

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APPENDICES

APPENDIX A

MORPHOMETRIC MEASUREMENTS

Gizzard Shad weights, lengths, standard
weights(W_s)*, and relative weights(W_R)*
without lesions

ACCESSION WEIGHT		LENGTH		
#	(g)	(mm)	W_s	W_R
3	380	355	414.2567	91.7305
7	370	346	384.2122	96.3009
10	206	328	328.5051	62.7083
11	440	359	428.0918	102.7817
12	360	337	355.6402	101.2259
13	440	356	417.6874	105.3419
14	322	319	302.7710	06.3510
15	400	332	340.3901	117.5122
16	322	327	325.5772	98.9012
17	540	376	490.2874	110.1395
18	338	323	314.0377	107.6304
19	348	324	316.8969	109.8149
20	440	358	424.6049	103.6257
21	302	316	294.4981	102.5473
22	320	313	286.3756	111.7414
23	360	325	319.7732	112.5798
24	408	342	371.3339	109.8742
25	370	338	358.7433	103.1378
26	460	353	407.4510	112.8970
27	400	348	390.7602	102.3646
28	440	351	400.7195	109.8025
29	380	330	334.4128	113.6320
30	510	369	464.0036	109.9130
31	598	395	566.5297	105.5549
32	400	346	384.2122	104.1091
33	390	343	374.5263	104.1315
34	390	337	355.6402	109.6614
35	400	336	352.5549	113.4575
36	440	346	384.2122	114.5201
37	364	336	352.5549	103.2463
38	400	349	394.0616	101.5070
39	420	346	384.2122	109.3146
40	400	353	407.451	98.1713
41	370	332	340.3901	108.6988
42	330	332	340.3901	96.9476
43	380	345	380.9654	99.7465
44	360	332	340.3901	105.7610

ACCESSION WEIGHT		LENGTH		
#	(g)	(mm)	W_s	W_R
45	410	346	384.2122	106.7119
46	360	325	319.7732	112.5798
47	450	345	380.9654	118.1209
48	380	340	365.0028	104.1088
49	460	347	387.4771	118.7167
50	360	325	319.7732	112.5798
51	360	328	328.5051	109.5873
52	370	340	365.0028	101.3691
53	442	352	404.0760	109.3854
54	400	355	414.2567	96.5584
55	450	352	404.0760	111.3652
56	420	348	390.7602	107.4828
57	430	348	390.7602	110.0419
58	400	341	368.1594	108.6486
59	400	343	374.5263	106.8016
60	380	337	355.6402	106.8496
61	360	338	358.7433	100.3503
62	400	335	349.4873	114.4534
63	370	327	325.5772	113.6443
64	340	339	361.8641	93.9579
65	420	346	384.2122	109.3146
66	380	344	377.7368	100.5991
67	318	316	294.4981	107.9803
68	395	332	340.3901	116.0433
69	540	369	464.0036	116.3784
89	430	347	387.4771	110.9743
90	390	346	384.2122	101.5064
91	470	352	404.0760	116.3147
92	380	339	361.8641	105.0118
94	400	340	365.0028	109.5882
95	410	353	407.4510	100.6256
96	460	361	435.1221	105.7175
97	500	367	456.6683	109.4887
98	450	360	431.5975	104.2638
99	460	355	414.2567	111.0423
100	500	360	431.5975	115.8487
101	400	338	358.7433	111.5003
102	440	351	400.7195	109.8025
103	400	337	355.6402	112.4732
104	350	330	334.4128	104.6611
106	480	354	410.8446	116.8325
112	400	330	334.4128	119.6127
113	360	332	340.3901	105.7610

ACCESSION WEIGHT		LENGTH		
#	(g)	(mm)	W_s	W_R
114	400	354	410.8446	97.3604
115	300	296	243.1237	123.3940
116	400	338	358.7433	111.5003
117	460	347	387.4771	118.7167
118	340	319	302.7710	112.2961
119	380	330	334.4128	113.6320
120	330	315	291.7740	113.1012
121	380	335	349.4873	108.7307
122	400	338	358.7433	111.5003
123	380	337	355.6402	106.8496
124	360	315	291.7740	123.3832
125	360	326	322.6666	111.5703
126	370	320	305.5623	121.0882
127	330	322	311.1956	106.0426
128	410	331	337.3927	121.5201
129	380	334	346.4373	109.6880
130	440	320	305.5623	143.9968
131	550	362	438.6655	125.3803
132	430	348	390.7602	110.0419
133	350	316	294.4981	118.8463
134	300	301	255.3624	117.4801
135	320	317	297.2390	107.6575
136	350	326	322.6666	108.4711
137	330	331	337.3927	97.8088
139	590	361	435.1221	135.5941
140	440	351	400.7195	109.8025
148	480	365	449.4099	106.8067
149	320	308	273.1686	117.1438
150	360	315	291.7740	123.3832
151	320	318	299.9966	106.6679
152	480	343	374.5263	128.1619
153	420	335	349.4873	120.1760
154	380	321	308.3705	123.2284
155	350	320	305.5623	114.5429
156	450	336	352.5549	127.6397
157	470	350	397.3814	118.2743
158	360	312	283.7013	126.8940
159	600	370	467.7001	128.2873
160	360	316	294.4981	122.2419
161	460	337	355.6402	129.3442
162	380	332	340.3901	111.6366
163	430	307	270.5763	158.9201
164	410	323	314.0377	130.5576

ACCESSION WEIGHT		LENGTH		
#	(g)	(mm)	W_s	W_R
165	620	376	490.2874	126.4564
166	480	358	424.6049	113.0463
167	350	319	302.7710	115.5989
168	440	350	397.3814	110.7249
169	440	344	377.7368	116.4832
170	310	310	278.4021	111.3497
171	380	327	325.5772	116.7158
172	360	325	319.7732	112.5798
173	400	329	331.4503	120.6818
174	360	330	334.4128	107.6514
175	620	383	517.5339	119.7989
176	440	354	410.8446	107.0965
177	440	340	365.0028	120.5470
178	440	350	397.3814	110.7249
179	320	310	278.4021	114.9417
180	380	341	368.1594	103.2162
181	360	321	308.3705	116.7427
182	540	366	453.0296	119.1975
183	560	374	482.6803	116.0188
184	560	372	475.1513	117.8572
187	500	350	397.3814	125.8237
188	540	375	486.4740	111.0028
189	360	325	319.7732	112.5798
190	420	348	390.7602	107.4828
192	540	357	421.1368	128.2244
193	380	348	390.7602	97.2463
194	360	326	322.6666	111.5703
195	500	359	428.0918	116.7974
196	480	355	414.2567	115.8702
195	400	336	352.5549	113.4575
197	410	346	384.2122	106.7119
200	440	346	384.2122	114.5201
201	460	359	428.0918	107.4536
203	400	334	346.4373	115.4610
256	320	325	319.7732	100.0709
257	560	368	460.3263	121.6528
258	350	338	358.7433	97.5628
259	640	390	545.7597	117.2677
260	410	340	365.0028	112.3279
261	560	371	471.4160	118.7911
262	390	346	384.2122	101.5064
263	390	339	361.8641	107.7753
264	370	331	337.3927	109.6645

ACCESSION WEIGHT		LENGTH		
#	(g)	(mm)	W_s	W_r
265	380	334	346.4373	109.6880
266	530	370	467.7001	113.3205
267	500	368	460.3263	108.6186
268	400	340	365.0028	109.5882
269	370	334	346.4373	106.8014
270	390	337	355.6402	109.6614
271	350	329	331.4503	105.5965
272	490	360	431.5975	113.5317
273	450	353	407.4510	110.4427
274	390	355	414.2567	94.1445
275	430	351	400.7195	107.3070
276	450	364	445.8094	100.9400
277	420	338	358.7433	117.0754
278	360	328	328.5051	109.5873
279	430	347	387.4771	110.9743
280	510	361	435.1221	117.2085
281	450	355	414.2567	108.6283
282	500	370	467.7001	106.9061
283	350	328	328.5051	106.5432
284	550	376	490.2874	112.1791
285	400	338	358.7433	111.5003
286	500	360	431.5975	115.8487
287	510	355	414.2567	123.1121
288	340	327	325.5772	104.4299
289	380	325	319.7732	118.8342
290	400	342	371.3339	107.7198
291	450	361	435.1221	103.4193
292	400	342	371.3339	107.7198
293	410	345	380.9654	107.6213
294	460	354	410.8446	111.9645
295	400	342	371.3339	107.7198
296	390	323	314.0377	124.1889
297	440	343	374.5263	117.4817
298	480	357	421.1368	113.9772
299	400	351	400.7195	99.8204
300	440	353	407.4510	107.9884
301	370	333	343.4049	107.7445
302	350	351	400.7195	87.3428
303	500	361	435.1221	114.9103
304	410	358	424.6049	96.5603
305	390	312	283.7013	137.4685
306	350	321	308.3705	113.4999
307	460	356	417.6874	110.1302

ACCESSION WEIGHT		LENGTH		
#	(g)	(mm)	W_s	W_R
308	510	361	435.1221	117.2085
309	380	339	361.8641	105.0118
310	400	331	337.3927	118.5562
311	450	345	380.9654	118.1209
312	330	335	349.4873	94.4240
313	510	362	438.6655	116.2617
314	340	335	349.4873	97.2853
315	510	350	397.3814	128.3402
316	480	365	449.4099	106.8067
317	400	331	337.3927	118.5562
318	380	332	340.3901	111.6366
319	360	331	337.3927	106.7006
320	450	358	424.6049	105.9809
321	500	361	435.1221	114.9103
322	480	369	464.0036	103.4475
323	340	326	322.6666	105.3719
324	470	356	417.6874	112.5243
325	330	319	302.7710	108.9933
326	340	320	305.5623	111.2703
327	330	322	311.1956	106.0426
328	330	323	314.0377	105.0829
329	430	359	428.0918	100.4457
330	420	341	368.1594	114.0810
331	460	357	421.1368	109.2282
332	460	355	414.2567	111.0423
333	490	354	410.8446	119.2665
334	350	326	322.6666	108.4711
335	480	351	400.7195	119.7845
336	360	328	328.5051	109.5873
337	390	332	340.3901	114.5744
338	360	327	325.5772	110.5728
339	410	349	394.0616	104.0446
340	480	353	407.4510	117.8056
341	320	320	305.5623	104.7250
342	400	341	368.1594	108.6486
343	390	331	337.3927	115.5923
344	320	338	358.7433	89.2003
345	490	356	417.6874	117.3126
346	420	345	380.9654	110.2462
347	390	337	355.6402	109.6614
348	390	330	334.4128	116.6223
349	560	373	478.9060	116.9332
350	560	373	478.9060	116.9332

ACCESSION WEIGHT		LENGTH		
#	(g)	(mm)	W_s	W_R
351	380	331	337.3927	112.6284
352	350	332	340.3901	102.8232
353	400	334	346.4373	115.4610
354	410	342	371.3339	110.4128
355	340	320	305.5623	111.2703
356	370	331	337.3927	109.6645
357	360	332	340.3901	105.7610
358	470	355	414.2567	113.4562
359	350	332	340.3901	102.8232
360	490	365	449.4099	109.0319
361	400	353	407.4510	98.1713
362	480	352	404.0760	118.7895
363	380	334	346.4373	109.6880
364	400	340	365.0028	109.5882
365	290	312	283.7013	102.2202
366	410	345	380.9654	107.6213
367	480	359	428.0918	112.1255
368	510	365	449.4099	113.4821
369	370	338	358.7433	103.1378
370	450	349	394.0616	114.1953
371	340	330	334.4128	101.6708
372	410	348	390.7602	104.9237
373	470	345	380.9654	123.3708
374	490	362	438.6655	111.7024
MEANS	415	342	373.6249	110.9447

*(Based on standard weight curves of Childress, 1991)

Gizzard Shad with lesions

ACCESSION WEIGHT		LENGTH		
#	(g)	(mm)	W_s	W_R
1	320	310	278.4021	114.9417
2	320	325	319.7732	100.0709
4	320	355	414.2567	77.2467
5	364	341	368.1594	98.8702
6	360	341	368.1594	97.7837
8	360	328	328.5051	109.5873
9	440	350	397.3814	110.7249
70	400	340	365.0028	109.5882
71	460	360	431.5975	106.5808
72	390	344	377.7368	103.2465
73	450	355	414.2567	108.6283
74	350	322	311.1956	112.4695

ACCESSION WEIGHT		LENGTH		
#	(g)	(mm)	W_s	W_R
75	440	350	397.3814	110.7249
76	410	358	424.6049	96.5603
77	460	365	449.4099	102.3564
78	440	355	414.2567	106.2143
79	425	352	404.0760	105.1782
80	390	344	377.7368	103.2465
81	400	347	387.4771	103.2319
82	460	359	428.0918	107.4536
83	372	348	390.7602	95.1990
84	410	345	380.9654	107.6213
85	370	339	361.8641	102.2483
86	370	330	334.4128	110.6417
87	420	343	374.5263	112.1416
88	540	370	467.7001	115.4586
93	490	369	464.0036	105.6026
105	460	360	431.5975	106.5808
107	360	332	340.3901	105.7610
108	500	359	428.0918	116.7974
109	320	320	305.5623	104.7250
110	480	356	417.6874	114.9185
111	280	308	273.1686	102.5008
138	590	361	435.1221	135.5941
141	480	365	449.4099	106.8067
142	320	308	273.1686	117.1438
143	390	333	343.4049	113.5686
144	340	328	328.5051	103.4992
145	390	323	314.0377	124.1889
146	340	320	305.5623	111.2703
147	480	367	456.6683	105.1091
183	560	374	482.6803	116.0188
184	560	372	475.1513	117.8572
189	360	325	319.7732	112.5798
197	400	336	352.5549	113.4575
200	440	346	384.2122	114.5201
202	400	352	404.0760	98.9912
204	620	384	521.5058	118.8865
205	400	347	387.4771	103.2319
206	360	361	435.1221	82.7354
207	400	348	390.7602	102.3646
208	400	341	368.1594	108.6486
209	520	381	509.6500	102.0308
210	420	344	377.7368	111.1885
211	600	380	505.7379	118.6385

ACCESSION WEIGHT		LENGTH		
#	(g)	(mm)	W_s	W_p
212	300	311	281.0434	106.7451
213	590	382	513.5820	114.8794
214	440	352	404.0760	108.8904
215	500	363	442.2280	113.0639
216	360	397	574.9814	62.6107
217	460	355	414.2567	111.0423
218	600	381	509.6500	117.7279
219	460	352	404.0760	113.8400
220	380	351	400.7195	94.8294
221	380	366	453.0296	83.8797
222	400	363	442.2280	90.4510
223	370	340	365.0028	101.3691
224	460	353	407.4510	112.8970
225	400	347	387.4771	103.2319
226	570	381	509.6500	111.8415
227	410	345	380.9654	107.6213
228	490	376	490.2874	99.9413
229	390	346	384.2122	101.5064
230	580	371	471.4160	123.0336
231	410	359	428.0918	95.7738
232	390	350	397.3814	98.1425
233	440	350	397.3814	110.7249
234	500	373	478.9060	104.4046
235	460	356	417.6874	110.1302
236	590	364	445.8094	132.3435
237	560	380	505.7379	110.7293
238	350	347	387.4771	90.3279
239	420	351	400.7195	104.8115
240	420	343	374.5263	112.1416
241	420	353	407.4510	103.0799
242	440	345	380.9654	115.4960
243	410	350	397.3814	103.1754
244	560	367	456.6683	122.6273
245	480	352	404.0760	118.7895
246	410	352	404.0760	101.4661
247	550	361	435.1220	126.4013
248	350	348	390.7602	89.5689
249	400	347	387.4771	103.2319
250	550	376	490.2874	112.1791
251	310	319	302.7710	102.3876
252	500	370	467.7001	106.9061
253	580	364	445.8094	130.1004
MEANS	434	351	405.0440	107.2946

*(Based on standard weight curves of Childress, 1991)

APPENDIX B

CHROMATOGRAMS AND MASS SPECTRA

The following are chromatograms from one water sample and three sediment fractions. There is also one mass spectra for a compound positively identified in the sediment sample. The accession labels and page numbers are as follows:

Accession Label	Page
ARBW-1 = Water sample	36
ARBS-1 = Sediment fraction #1	37-38
ARBS-2 = Sediment fraction #2	39
ARBS-3 = Sediment fraction #3	40

The mass spectra are presented as two spectra. The top spectra is that of the sample with the peak number in the upper right corner. The bottom spectra is that of the library match corresponding to the sample. The name and molecular formula of the compound are given at the top. Also, the CAS registry number is listed, if available, at the top right following the label "RFN". In the interest of being concise, spectra were only included once per occurrence.

INST 1 METH 1 FILE 6
RUN 1 13 . 27.3 12/17/91
SENSITIVITIES 200 4 0.17
0.42

B

5µl ARBW-1

11.75

B

17.93

19.64

21.12

23.47

25.34

28.49

32.04

36.61

38.37

END

IHP
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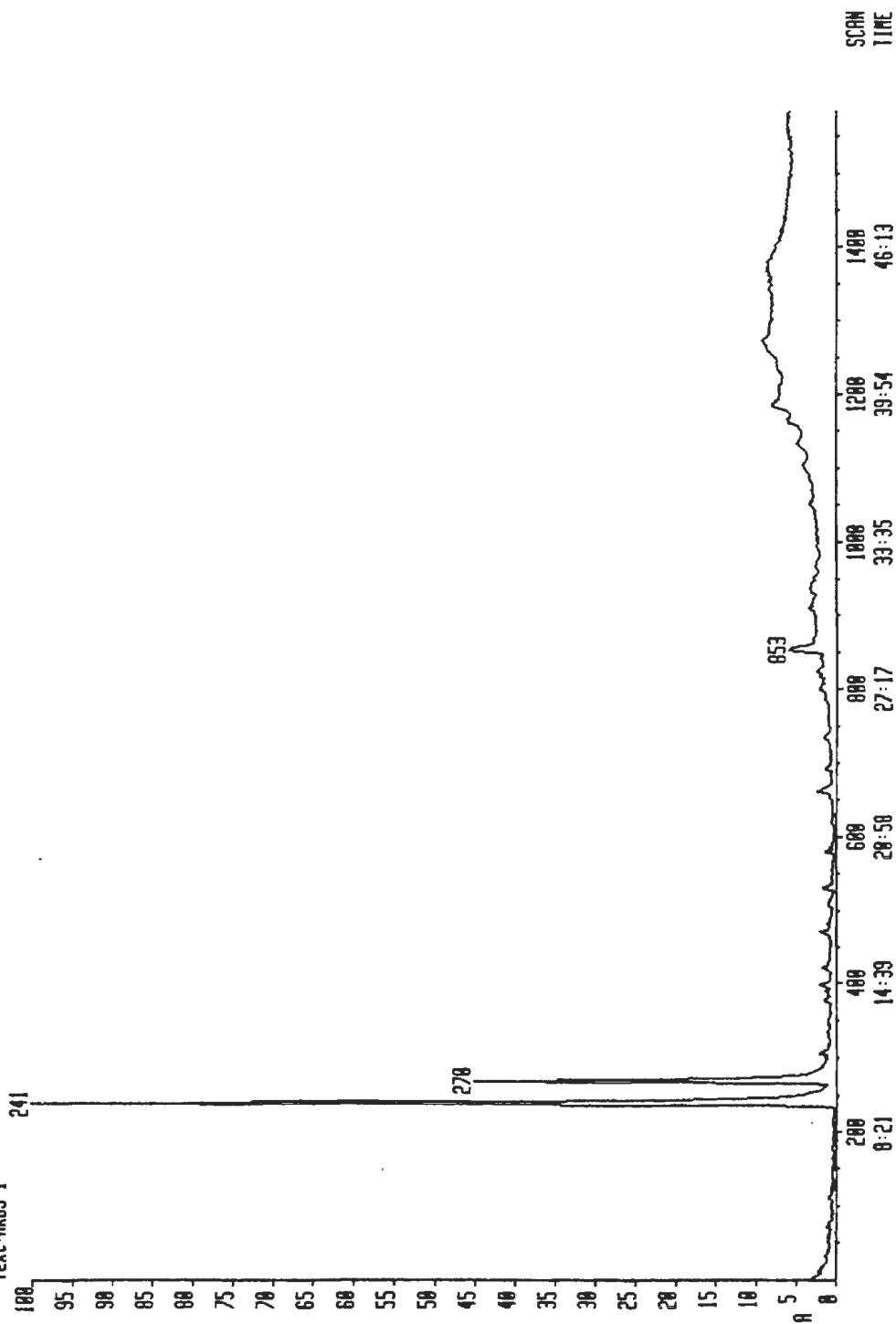
Sys: DEFAULTS

(E1+)

12187207 #1-1584 21-JUL-92 20:56 TS258

Chromatogram Identifiers : A : ATIC

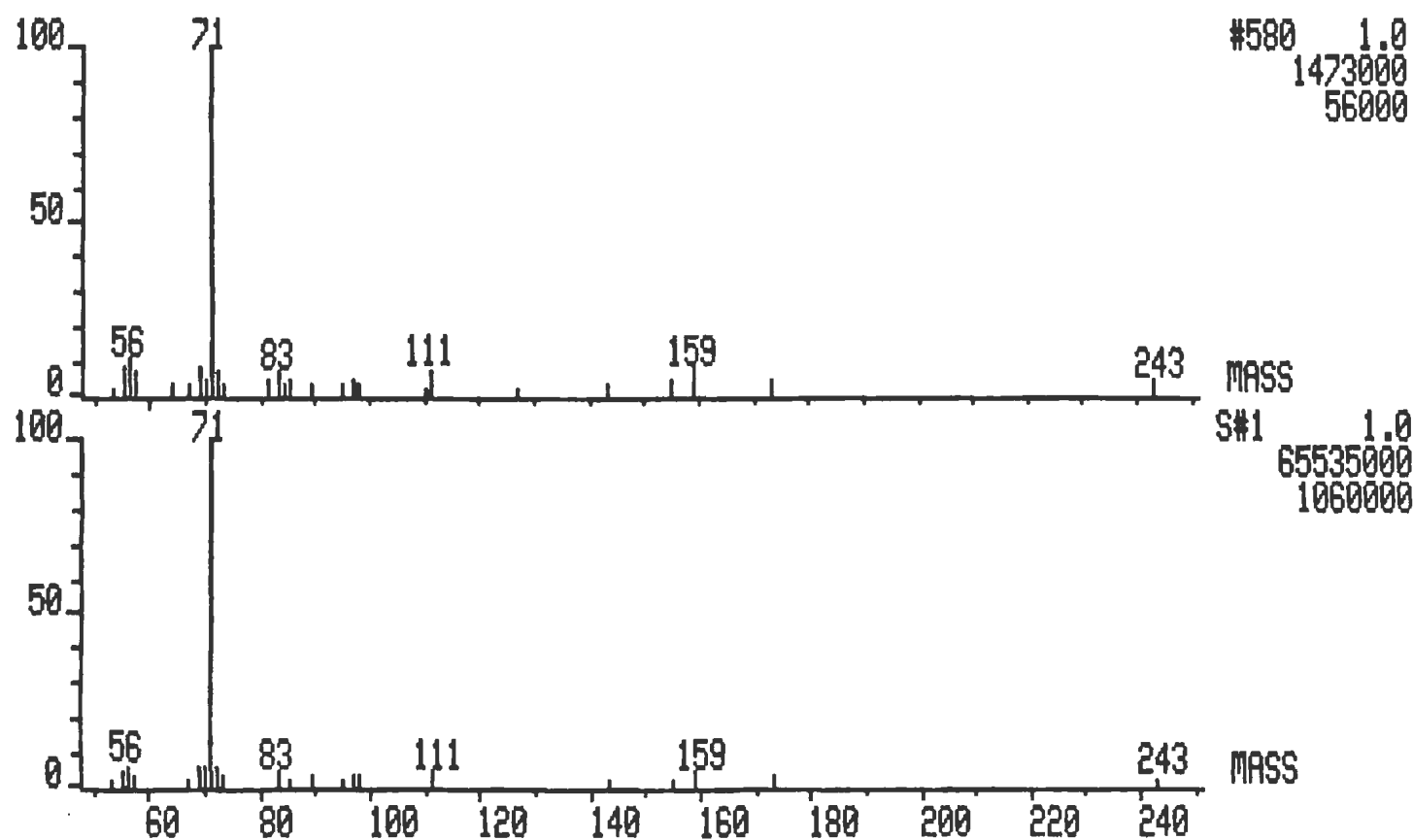
Text: ARBS-1



LIBFITS1#1* x1 Bgd=580 T2107207 +0:00:00

PROPANOIC ACID, 2-METHYL-, 1-(1,1-DIMETHYLETHYL)- p838 M884 r906 RFN:0-00-0

C16.H30.04



T2207204 01-1489 22-JUL-92 13:53 TS250

(EI+)

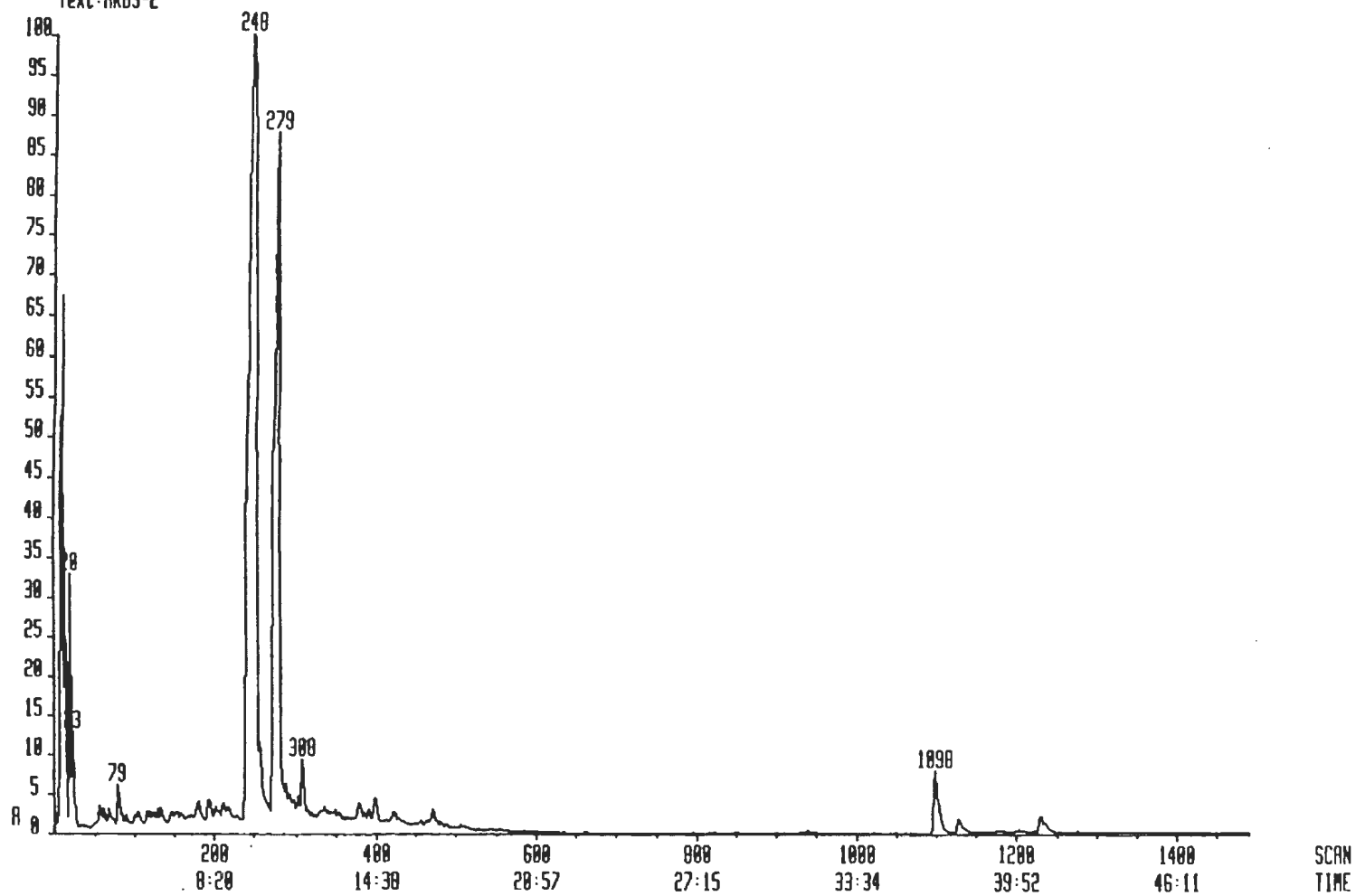
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IHP

Chromatogram Identifiers : A:ATIC

A: 34378

Text:ARBS-2



IMP
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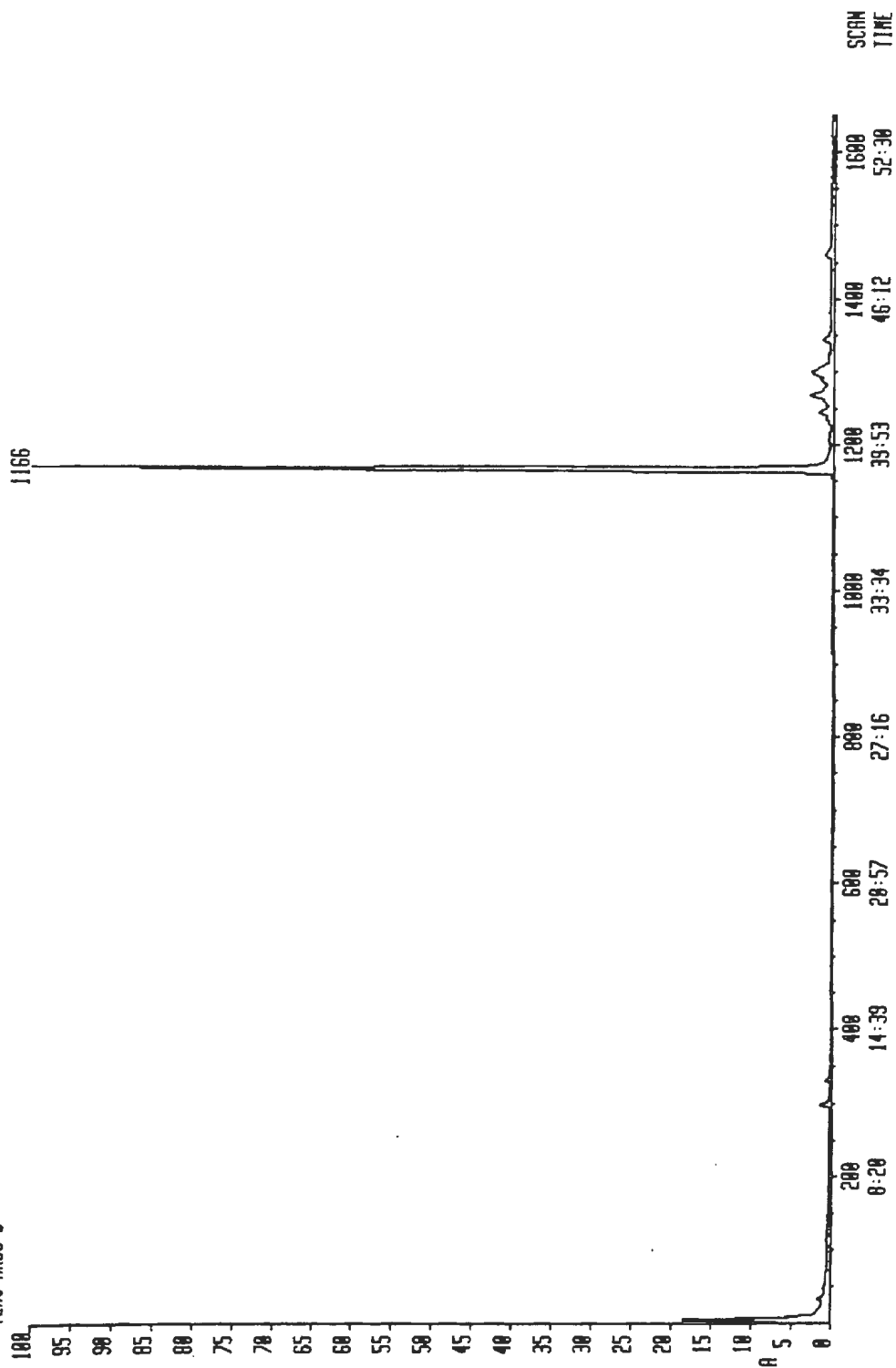
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(E1+)

T2287285 01-1649 22-JUL-92 15:56 TS250

Chromatogram Identifiers : A :ATTC

Text:RRBS-3



CHAPTER III

ANTHROPOGENIC CONTAMINANTS AND FISH
HEALTH IN AN INDUSTRIAL RIVER

Introduction

Since the dawn of the industrial age, there has been an increasing number of anthropogenic contaminants produced. The Environmental Protection Agency reported that larger industries together produce 1,500 tons of chemical wastes per year per capita in the United States (House of Representatives, 1983). This does not account for amount that the smaller industries are producing. They also report that only 5% of that is disposed of commercially. The remaining 95% is stored, treated, or disposed of by the companies themselves hopefully in a legal manner. Industries such as oil refineries, gasoline refineries, coking facilities, chemical plants, pulp mills and paper mills release chemicals in their treated effluent waters. Some of these chemicals are in the classes of compounds that are suspect in the formation of neoplasms in both lower and higher vertebrates. Recent studies suggest that these neoplasms are caused by xenobiotics, specifically the polycyclic aromatic hydrocarbons (PAHs), in the aquatic

environment (e.g., Fabacher et al., 1991; Myers et al., 1991; Vogelbein et al., 1990; Baumann et al., 1987). A report in 1941 of an epidemic of epidermal neoplasms in the brown bullhead, Ameiurus nebulosus (Lucké and Schlumberger, 1941), and a subsequent report by Dawe (1964) suggesting that neoplasms in the white sucker, Catostomus commersoni, may be caused by chemical pollutants, were two of the earliest studies conducted on this subject. Since then, considerable research has focused on the determination of a cause for the observations (e.g., Malins et al., 1988; Myers et al., 1987; Murchelano and Wolke, 1985; McCain et al., 1982).

Many heavily industrialized areas have had an increase in the prevalence of liver neoplasms. Puget Sound, Washington, San Pedro Bay, California, Boston Harbor, Massachusetts, and the North American Great Lakes have been the focus of some of the studies (e.g. Malins et al., 1987; Malins et al., 1984; Eganhouse and Kaplan, 1982; Fabacher et al., 1991; Baumann et al., 1987).

The PAHs and other hydrocarbons found at these sites are mostly non water soluble. They tend to collect in the sediments and can be found there in concentrations up to 1000-fold greater than in the water (Malins and Ostrander, 1991). For this reason, much of the research has focused on benthic species. In 1974, tumor surveys were begun in the Puget Sound, Washington, because large numbers of liver

tumors were being reported in the English Sole, Parophrys vetulus (McCain et al., 1977; Pierce et al., 1978). Through these studies it was shown that aromatic hydrocarbons, chlorinated butadienes, and polychlorinated biphenyls are the major compounds found in association in waters with fish bearing these neoplasms (Myers et al., 1990).

The white croaker, Genyonemus lineatus, was the focus of studies conducted in San Pedro Bay near Los Angeles because of its abundance, benthic association, and wide distribution. Moreover, an increased prevalence of malignant liver lesions was reported in this species compared to fish collected from cleaner waters (Malins et al., 1987).

Boston Harbor is another industrialized coastal waterway in which feral fish populations exhibit cancer, neoplasms, and pre-neoplastic conditions. The winter flounder, Pseudopleuronectes americanus, was shown to have a 10% incidence of pre-neoplastic and neoplastic hepatic lesions (Murchelano and Wolke, 1985).

The Great Lakes have also been the focus of research conducted on benthic species. For example, the brown bullhead population in the Black River, a tributary of Lake Erie, was shown to have from 20 to 50% occurrence of liver neoplasms in fish three and four year old fish (Baumann et al., 1987). Also 100% of the sauger (Stizostedion canadense) population in Torch Lake, Michigan, had

hepatocellular carcinoma, a type of liver cancer (Black et al., 1982).

Abnormalities other than neoplasms and cancer are also observed with elevated levels of chemical contamination. Fin erosion was consistently observed in winter flounder taken from the Boston Harbor area (Murchelano and Wolke, 1985). Fin erosion is seen in both cultured populations and wild populations residing in poor quality water. Grady and colleagues (1992) reported that brown bullheads fed a synthetic PAH, β -naphthoflavone, exhibited greatly reduced body weight and length. The fish fed the β -naphthoflavone averaged about 8 g and 9 cm and those without averaged 25 g and 13 cm. All of the fish were about 0.458 g at the start of the experiment. The fish also had lesions such as fin erosion, clubbed barbels, and damage to the gill membranes. Morphological indexes have been used to evaluate the effects of pollution on the atlantic croaker, Micropogonias undulatus (Burke et al., 1993).

The previously mentioned sites share a number of common factors. First, all are contaminated by anthropogenic chemicals, and resident fish populations appear to be showing a response. These chemicals may be absorbed through the skin or across gill membranes, or ingested with food consumed in and near the sediment; thus, these chemicals can have many potentially adverse effects on fish populations. Observed effects include narcotization, fin erosion,

necrosis, and several forms of neoplasms (Fabacher et al., 1991; Baumann et al., 1987; Malins et al., 1987; Murchelano and Wolke, 1985; Malins et al., 1984; Eganhouse and Kaplan, 1982).

Second, some of these types of compounds have been shown in laboratory studies to induce cancer and morphological differences in fish and mammalian species (e.g. Black et al., 1985; Farber and Cameron, 1980). Black (1985) induced neoplasia in mice and brown bullheads by repeatedly applying extracts of contaminated sediments from the Buffalo River, New York. Although some of the compounds themselves may not be carcinogenic, many metabolites are. In spite of the fact that little is known of the metabolic biotransformation abilities of fish (e.g., P-450), they appear to possess sophisticated systems capable of producing carcinogenic metabolites (Malins and Ostrander, 1991).

Third, the older individuals of these fish populations are most affected by cancer. For example, Baumann (1987) and colleagues showed that less than 2% of two-year old brown bullheads had liver tumors but >11% of three year old fish had tumors. This increase in tumor rate can disrupt the age structure of the population because the older individuals are dying younger than they would naturally either directly because of the cancer or indirectly by predation. Also, these individuals may not be living long enough to reproduce or may be unable to reproduce at all.

These factors can make it difficult for fisheries biologists to manage the populations.

Fourth, all of these sites are heavily industrialized coastal waterways. Most of the previously cited research has been focused on coastal waters. A few isolated exceptions, such as Torch Lake, Michigan, have revealed similar problems exist in inland waters. Contaminant sources such as coking plants, pulp mills, and oil and gas refineries are inherent to inland waterways.

The petroleum industry produces many compounds of the classes previously cited and found in these areas of multiple anthropogenic sources. Such chemicals may be inadvertently introduced into the streams and lakes. In addition, many non-point sources can contribute contaminants to the water. For example, run-off from farm lands treated with pesticides and herbicides can be a source of contaminants (Neary et al., 1993). Also, run-off from municipal stormwater and wastewater drains carry hydrocarbons produced from automobile and refinery exhausts (e.g. Parker et al., 1993; Hansen et al., 1993). Today, many species of fish world-wide are known to exhibit neoplasms. Of the species with liver neoplasms, all of them are bottom feeders (Harshbarger and Clark, 1990). The potentially carcinogenic hydrocarbons tend to collect in the sediments because of their chemical nature, very non-polar, non water-soluble, hydrophobic, and non-ionic. Polycyclic

aromatic hydrocarbons generally are found at concentrations 1000-fold greater in sediments than in water (Malins and Ostrander, 1991). Oklahoma has a large number of oil and gas refineries. Bottom-dwelling families in the midwest include the Cyprinidae, Catastomidae, and Ictaluridae. These fishes are in constant contact with the sediments and are possibly exposed to high concentrations of pollutants.

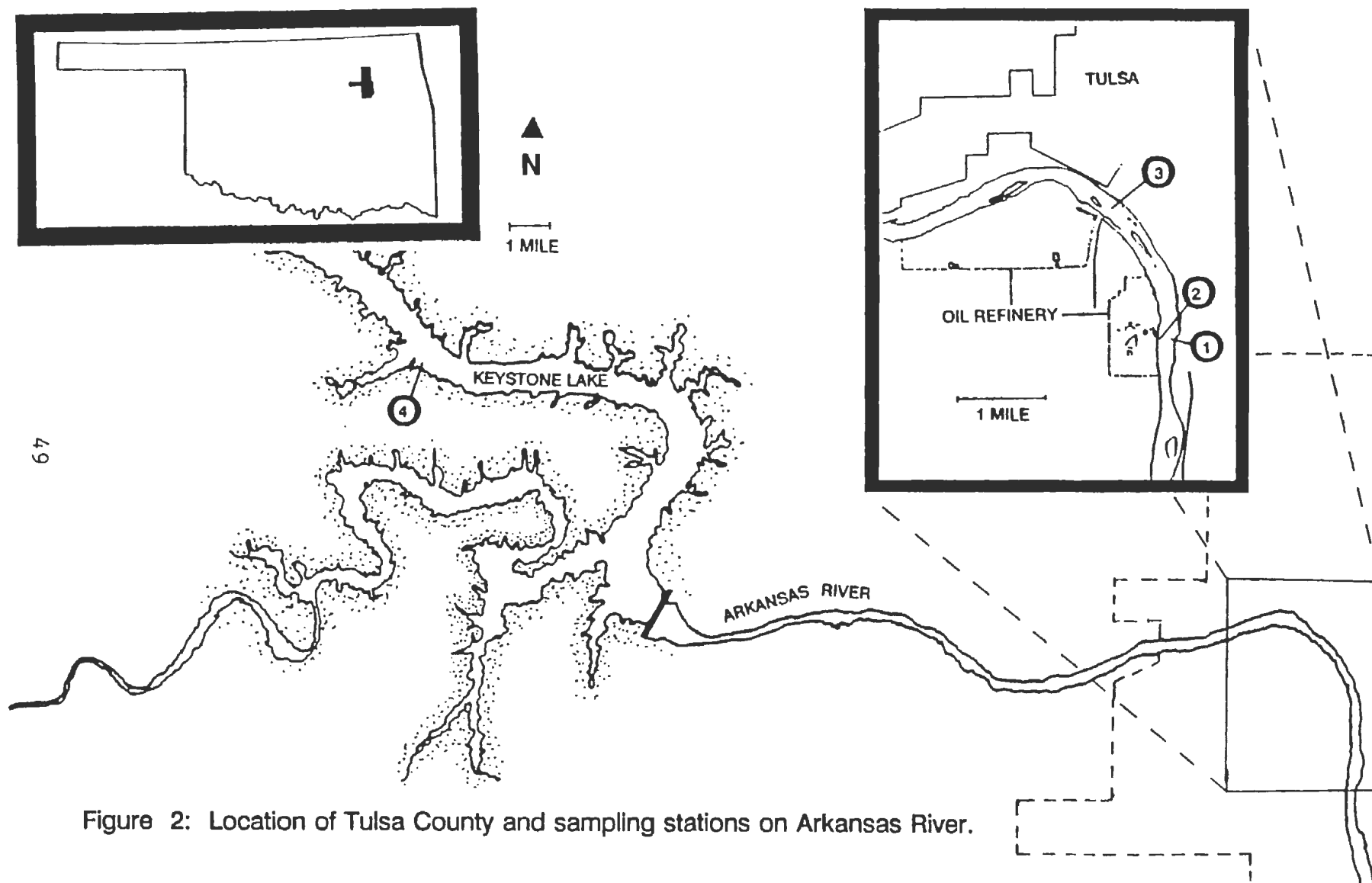
The focus of this study was channel catfish, Ictalurus punctatus. The channel catfish is both a tolerant benthic species with respect to environmental factors and also a much sought after food fish along the Arkansas River in Tulsa. The primary objectives were to: 1) identify what, if any, compounds were contaminating the Arkansas River in the Tulsa vicinity, 2) sample the channel catfish population and 3) determine by histological and morphological examination if lesions were present that were consistent with other known chemically-induced lesions and if fish from the suspected impacted stations were morphologically different from fish collected at a reference station.

Materials and Methods

Site Description

Tulsa is a large city in the northeastern corner of Oklahoma (Figure 2). There are two operating oil refineries located on the Arkansas River and also one that it closed which has been declared an Environmental Protection Agency

Superfund Site. The Sinclair Oil Company and the Sun Oil Company have crude oil processing capacities of 43,000 and 85,000 barrels per calendar day, respectively (Rock, 1991). These are located immediately adjacent to and slightly upstream to the study site. As is typical with this type of industry, effluents are discharged, after in-plant treatment, into a nearby waterway. The effluents are water that is used in the processing of the crude oil such as steam in the cracking towers. This water is chemically and biologically treated before release and must meet standards set by the EPA in NPDES (National Pollution Discharge Elimination System) permits issued to the refineries. Other major sources of contaminants are the city's stormwater drains. There are several large drains entering the river from the downtown area. Historically, stormwater run-off from large metropolitan areas has been shown to contain many contaminants such as those released by automobiles from the incomplete combustion of fossil fuel. The Arkansas River flows through Tulsa from the west with an average width of 0.5 kilometers and depth of 3 to 4 meters within the city limits. Sampling was done at four stations along the river (Figure 2). Stations 1 and 2 were at the discharge sites of the Sinclair refinery, S $\frac{1}{2}$ SW $\frac{1}{4}$ S13 T19N R12E, one on each bank of the river. Station 3 was between the Sinclair and Sun oil refineries beneath the 12th street bridge, N $\frac{1}{2}$ NW $\frac{1}{4}$ S13 T19N R12E, and Station 4 was several miles upstream, N $\frac{1}{2}$



SW $\frac{1}{4}$ S9 T20N R9E, in Keystone Lake which was the reference station.

Water Samples

Three samples of four liters each of water were taken. Water was collected in pre-cleaned, four-liter amber bottles. Samples were stored in the dark at 4°C until the time of extraction. Each four-liter sample was extracted on a Carbon-18 (C18) bonded solid phase extraction column (Bond Elut, Analytichem International, Product #607306). The columns were conditioned by passing two column volumes (12 ml) of methylene chloride through under a slight positive pressure, followed by two column volumes of reagent-grade water. The columns were not allowed to dry from this point on. The columns were then connected to a two-liter separatory funnel, and 4 liters of water were passed through under a slight vacuum. The columns were air dried under vacuum for 15 minutes to remove residual water prior to elution. Compounds were eluted with 40 ml of methylene chloride. This eluate was then passed through a 5 g column of sodium sulfate to remove excess water and rinsed twice with 2 ml of methylene chloride. The eluate was then concentrated by rotary evaporation in a 60°C water bath to 15 ml, transferred to a 15 ml concentrator tube, and finally concentrated to 1 ml in a 60°C water bath with nitrogen purge. The sample was then analyzed by gas

chromatography/mass spectrometry. A VG Analytical TS-250 mass spectrometer connected to a Hewlett-Packard 5890A gas chromatograph was used for the analysis. A 30-meter, 0.32-mm inner diameter capillary column with an SE-54 bonded phase was used in the chromatograph. One to three microliters, depending on concentration, was injected and the sample was subjected to a programmed temperature gradient which raised the temperature 10°/minute from 50° to 280° C. The spectra of each peak was compared to reference spectra contained in the NBS on-line library (Milne and Heller, 1978; Heller and Milne, 1980).

Sediment Samples

Sediment samples were collected at the same locations described above. Three replicate samples of one liter each were taken in pre-cleaned amber bottles with teflon lid liners (Scientific Specialties Service, Product #B71132). The samples were stored at -40°C until extraction. In pilot studies, two separate methods of sediment extraction were employed. One was used by Malins et. al. in studying Puget Sound sediments (1980) and the other by Fabacher et. al. in studying Black River sediments (1988). These methods were compared for their ability to resolve compounds and the better of the two, that which provided the best separation of our compounds (Fabacher et al., 1988), by our lab, was

used, with slight modifications described below for the remainder of the study.

Dry Sediment Extraction with
Fractionation on Neutral Alumina

The sediments were thawed and air dried under a hood for 24 hours prior to extraction. A 100 g aliquot of sediment was then powdered in a blender (Hamilton Beach, model #585-3). The sediment was extracted twice with 100 ml of a benzene:methanol (60:40) and twice with 100 ml of methylene chloride. At each step the slurry was shaken for two hours at 400 rpm on an orbital shaker. The sediment was allowed to settle out for 20 minutes, and the solvent was then decanted off the top into a 500 ml solvent rinsed bottle. The extracts were concentrated with a rotary evaporator in a 60°C water bath to about 10 ml. This solution was transferred to a 15 ml concentrator tube with two, two ml rinses of methylene chloride. Finally, the sample was concentrated at 60°C under a continuous nitrogen stream to one ml. The sample was then considered ready for fractionation on an alumina column.

An 11 x 250 mm glass chromatography column with a 200 ml solvent reservoir was fitted with a glass wool plug and filled with 9 g of neutral alumina activated at 200°C for 12 hours. Enough N-hexane was added to cover the alumina and the solution was shaken to remove any air bubbles. A small

layer of sand was placed on top of the alumina to prevent disturbance when adding sample or solvent. Samples were applied and eluted with 400 ml of N-hexane. This first fraction contained mostly aliphatic hydrocarbons. The column was then eluted with 1000 ml of benzene. This fraction contained mostly aromatic hydrocarbons. Finally, the column was eluted with 1550 ml of chloroform, and this final fraction contained mostly the nitrogen containing aromatic hydrocarbons. All three fractions were reduced by rotary evaporation to about 15 ml and resultant solutions were concentrated on a 60°C water bath under a nitrogen stream to one ml. The samples were then analyzed by gas chromatography/mass spectrometry. A VG Analytical TS-250 mass spectrometer was used with the same conditions as described earlier for the water analysis. One to three microliters, depending on concentration, was injected and the sample was subjected to a programmed temperature gradient (10°/minute from 50° to 280° C). The spectra of each peak was compared to reference spectra, contained in the NBS on-line library (Milne and Heller, 1978; Heller and Milne, 1980) for compound identification.

Sampling of fish populations

Two gill nets, 100 yards long by four foot deep with 3 to 4 inch mesh, were set out overnight, approximately 16-20 hours, at each station. Electroshocking was also used where

were amenable to electroshocking because of slow moving shallow water. A Smith-Root pulsed DC shocking unit was used. Sampling continued until 53 channel catfish had been captured at the impacted stations and 16 at the reference station. Sampling occurred for approximately eight net nights. Catfish were weighed, measured for total length, and grossly examined. Gross examination included looking for external lesions including fin erosion and clubbed barbels. Pectoral spines were removed for age analysis.

Relative weights (W_R) were calculated using standard weight regressions derived from Texas Parks and Wildlife Department age and growth data collected statewide (Childress, 1991). The relative weight is a percentage of the standard weight that is calculated on a logarithm (base-10) as the 75th-percentile of the weight on the log of the midpoint of the length-class. The equation is:

$$\text{Equation 1:} \quad \log_{10} W_s = a + b \log_{10} L$$

where W_s is the standard weight, a is the regression intercept, b is the regression coefficient, and L is the total length. The intercept and regression coefficient calculated by Childress were -6.019 and 3.390 respectively.

Lengths, weights, and relative weight differences were analyzed using a general linear model (GLM) procedure in the Statistical Analysis System (SAS, 1985) software. The GLM

Statistical Analysis System (SAS, 1985) software. The GLM procedure is an analysis of variance modified for unbalanced data. The measurements were compared between fish from the impacted stations and fish from the reference station and combining all sampling dates.

Livers were removed, examined, and processed for histopathological examination by sectioning into five millimeter thicknesses and preserving overnight in ten percent neutral buffered formalin followed by overnight rinsing in gently running tapwater. Sections were then embedded in paraffin and stained with hematoxylin and eosin and one fullface section of 5 μ M was microscopically examined. The Gulf Coast Research Lab in Ocean Springs, Mississippi, under the direction of Dr. William Hawkins, performed all evaluation of liver samples. The statistical analysis was performed using the GLM procedure in SAS. The occurrences of lesions were compared between fish from the impacted stations and from the reference station.

Results

Water and Sediment Sampling Results

A total of 17 compounds were identified in the water and sediment samples (Table III). Reconstructed ion chromatograms and spectra for each compound identified are shown in Appendix D. Water from Station 1 contained three compounds: 4,5-dimethylnonane, 2-methyl-1-(1,1-dimethyl-

contained one compound: α , β -dimethylbenzeneethanol.

Water from Station 2 contained two compounds: 2-methyl-1-(1,1-dimethylethyl)propanoic acid, and diethyl phthalate. Sediment contained 6 compounds: 1-methylnaphthalene, 1,5-dimethyl-naphthalene, 2-methylnaphthalene, anthracene, 9-octadecen-1-ol, pyrene, and 2,6,10-trimethyldodecane.

Water from Station 3 contained two compounds: 2-methyl-1-(1,1-dimethylethyl)propanoic acid, and decamethylpentasiloxane which is suspected to be an artifact from the silicone stopcock grease used on the glassware. Sediment contained 7 compounds: 1-methylnaphthalene, pyrene, 2,6,10-trimethyl-dodecane, phenanthrene, benzo[a]pyrene, and methylbenzene.

Water from Station 4 contained no compounds above detection limits. Sediment contained two compounds: 12-(acetyloxy)-methyl ester 9-octadecenoate, and (1-methylethyl)-benzene.

Appendix H contains a list of all compounds identified and the rating of the compound spectra compared to the library match.

TABLE III

WATER(W) AND SEDIMENT(S) ANALYSIS

Tulsa

COMPOUND	STATION			
	1(I)	2(I)	3(I)	4(R)
4,5-Dimethylnonane	W			
2-Methyl-2,2-dimethyl-1(1-methyl-ethyl)-1,3-propanediyl ester propanoic acid	W	W	W	
Diethyl phthalate	W	W		
α , β -Dimethylbenzeneethanol	S			
1-Methylnaphthalene		S	S	
1,5-Dimethylnaphthalene		S		
2-Methylnaphthalene		S		
Anthracene		S		
9-Octadecen-1-ol		S		
Pyrene		S	S	
2,6,10-Trimethyldodecane		S	S	
Phenanthrene			S	
Benzo[a]pyrene			S	
Methylbenzene			S	
12-(Acetyloxy)-methyl ester 9-octadecenoate				S

TABLE III Continued

COMPOUND	STATION			
	1(I)	2(I)	3(I)	4(R)
(1-Methylethyl)benzene				S

(I = Impacted, R = Reference)

Fish Sampling Results

A total of 69 channel catfish were sampled. A general linear model variation of an analysis of variance was applied to the weights, lengths, and relative weights comparing the impacted stations and the control station. There was a significant difference in all three measurements (Table IV). The fish from the reference station were significantly heavier (reference = 482 g, impacted = 463 g, $P = 0.0001$), longer (reference = 1343 mm, impacted = 880 mm, $P = 0.0356$), and the relative weights were higher (reference = 102%, impacted = 81%, $P = 0.0001$) than fish collected at the reference station. There were no differences between the relative weights of the catfish comparing between age classes within each station ($P = 0.1198$). Morphological deformities were noted in the forms of clubbed, split, and missing barbels. Twenty of the 53 fish from the impacted stations exhibited barbel deformities.

Results of histopathological analysis are shown in Table V. Liver sections from the impacted stations had some mottling and showed early signs of cellular alterations. There were significant differences between the impacted stations and the reference station in the numbers of fish with toxic changes. There were 15 fish from the impacted stations with toxic changes and none from the reference station ($P = 0.01$). The toxic changes were identified as cellular changes consistent with those observed in similar

chemical exposures. There was also a significant difference in the number of fish containing parasitic cysts. There were 24 from the impacted stations and only 3 from the reference station ($P = 0.01$). A significant difference was also observed in the numbers of livers with cells exhibiting reactive/degenerative focus. There were two at the reference station and none at the impacted stations ($P = 0.008$).

TABLE IV
MORPHOLOGIC ANALYSIS of CHANNEL CATFISH

Dependent Variable	F Value	Probability > F
Length	4.61	0.0356
Weight	19.23	0.0001
Relative Weight	36.57	0.0001
Age (Relative Weight)	2.02	0.1198

TABLE V

RESULTS OF HISTOPATHOLOGIC EXAMINATION
CHANNEL CATFISH

	No. Lesions observed (Percentage)	
Lesion Type	Impacted	Reference
1	32 (60)	12 (75)
2	15 (28)	0 (0)
3	8 (15)	1 (6)
4	24 (45)	3 (18)
5	3 (6)	0 (0)
6	3 (6)	1 (6)
7	3 (6)	0 (0)
8	0 (0)	2 (13)

Description of Lesions

- 1 No visible lesions
- 2 Toxic change
- 3 Pigment Deposits
- 4 Parasitic Cysts
- 5 Focal vacuolated hepatocytes
- 6 Pericholangiolar fibrosis and other biliary lesions
- 7 Focal lymphocytic infiltration
- 8 Reactive/degenerative focus

Discussion

There were differences in the number and classes of compounds found in the water and sediments at the impacted stations compared to the reference station. Five times as many compounds were present at the impacted stations when compared to the reference station.

Compounds such as the phthalate esters and pyrenes are common to aquatic environments associated with this type of contamination sources (U. S. Department of Energy (DOE) and U. S. Environmental Protection Agency (EPA), 1978). The phthalate esters are plasticizers in many plastics and are released into the environment as the plastics breakdown. The cyclic hydrocarbons are by-products formed in the refining of crude petroleum and during the incomplete combustion of fossil fuels.

Histopathological examination showed some trends of differences in liver conditions between the two populations with some statistically significant differences. The fish from impacted stations had fifteen occurrences of toxic changes. A toxic change was defined as a cellular alteration consistent with those seen in similar cases of chemical exposures. This was 28% of the fish that we sampled. No fish from the reference station exhibited toxic changes in the liver. Also a significantly higher number of fish at the impacted stations had parasitic cysts in the liver: 45% at impacted stations, 18% at the reference

station. Generally the parasites are not lethal to the fish but in high densities can be harmful. Parasites have been reported to be indicators of pollution in aquatic systems (Kuperman, 1992).

The weights, lengths, and relative weights were all significantly different between fish populations from the impacted stations and the reference station. Morphological indexes were used by Burke and colleagues (1993) to evaluate pollution exposure of the atlantic croaker. Similar results were reported by Grady and colleagues (1992) in brown bullhead that were fed a synthetic PAH. The fish that received the chemical were shorter and weighed less. The relative weight, which is an indicator of overall animal health (Wege and Anderson, 1991), was smaller in fish collected from the impacted stations than that of those collected at the reference station. This could be caused by direct, indirect, or a combination of direct and indirect effects. An indirect effect would be that of a reduced forage base which could be investigated by extensive sampling of the river. With a reduced forage base the catfish population would possibly have a nutritional imbalance in their diet. A direct effect would be that of the contaminants effecting the sensory and metabolic pathways of the catfish. Exposure to these types of compounds commonly results in narcosis (Amdur et al., 1993).

An equally important effect, mentioned previously, is

that of reproduction. The average age of fish from the reference station was 4.25 years and those from the impacted stations averaged 3.69 years. Although these were significantly different statistically, they may not be biologically significant. The average age of the population from the impacted stations may be decreasing because the older individuals are dying from some of the effects described above. Conversely, the population from the impacted stations could just be receiving more fishing pressure which tends to be selective for the older individuals (Nielsen and Johnson, 1984).

The effects that we have reported may be caused by a number of contaminant sources including, but not limited to, the chemicals that we have identified as being present in the water and sediments. More sampling of the catfish and fish population as a whole would facilitate a better understanding of what is occurring in the Arkansas River in Tulsa.

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APPENDICES

APPENDIX C

MORPHOMETRIC MEASUREMENTS

Channel Catfish weights, lengths, standard
weights(W_s)*, relative weights(W_R)*,
and age
Arkansas River, Tulsa, Oklahoma

Impacted Stations (Stations 1,2 & 3)

ACCESSION #	WEIGHT (g)	LENGTH (mm)	W_s	W_R	AGE
1	567	405	661.11	85.77	2
2	653	398	623.17	104.79	2
3	710	445	909.79	78.04	3
4	340	380	532.68	63.83	3
5	505	430	809.95	62.35	5
6	930	480	1176.00	79.08	4
7	750	450	944.91	79.37	3
8	240	330	330.19	72.69	4
9	460	400	633.85	72.57	4
10	635	470	1094.99	57.99	4
11	550	450	944.91	58.21	5
12	470	390	581.71	80.80	2
13	1130	580	2233.68	50.59	4
14	2156	590	2366.95	91.09	4
15	802	440	875.60	91.59	3
16	1305	530	1645.49	79.31	5
17	1305	490	1261.14	103.48	3
18	1162	491	1269.89	91.50	4
19	1276	505	1396.88	91.35	4
20	1135	508	1425.21	79.64	4
21	1108	501	1359.72	81.49	4
22	1249	505	1396.88	89.41	4
23	1503	515	1492.89	100.68	4
24	1362	516	1502.74	90.63	4
25	1081	504	1387.52	77.91	4
26	1108	471	1102.91	100.46	4
27	568	400	633.85	89.61	3
28	794	444	902.88	87.94	4
29	740	441	882.36	83.87	4
30	454	381	537.45	84.47	2
31	794	448	930.75	85.31	3
32	854	462	1033.09	82.66	3
33	740	424	772.27	95.82	3
34	708	456	988.30	71.64	4

Impacted Stations (Stations 1,2 & 3)

ACCESSION #	WEIGHT (g)	LENGTH (mm)	W _s	W _R	AGE
35	708	434	835.78	84.71	3
36	654	426	784.69	83.34	3
37	681	436	848.91	80.22	3
38	707	436	848.91	83.28	3
39	681	445	909.79	74.85	4
40	740	465	1056.00	70.08	4
41	1049	480	1176.00	89.20	4
42	854	460	1018.00	83.89	4
43	967	465	1056.00	91.57	4
44	1135	520	1542.59	73.58	5
45	881	440	875.60	100.62	3
46	1049	495	1305.30	80.36	3
47	935	530	1645.49	56.82	5
48	1108	505	1396.88	79.32	5
49	767	455	980.98	78.19	4
50	740	460	1018.00	72.69	**
51	854	465	1056.00	80.87	4
52	994	500	1350.54	73.60	5
53	994	485	1218.05	81.61	4
Mean	880.04	462.87	1086.14	81.41	

Reference Station (Station 4)

ACCESSION #	WEIGHT (g)	LENGTH (mm)	W _s	W _R	AGE
1	1535	530	1645.49	93.29	5
2	1988	510	1444.32	137.64	4
3	908	450	944.91	96.09	4
4	1194	490	1261.14	94.68	4
5	1930	540	1753.13	110.09	5
6	2129	550	1865.65	114.12	5
7	795	435	842.32	94.38	5
8	740	430	809.95	91.36	4
9	1308	500	1350.54	96.85	3
10	627	410	689.19	90.98	4
11	1162	485	1218.05	95.40	4
12	908	455	980.98	92.56	3
13	2329	580	2233.68	104.27	5
14	1108	465	1056.00	104.92	5
15	1816	540	1753.13	103.59	5
16	1022	455	980.98	104.18	3
Mean	1343.69	489.06	1301.84	101.52	

*(Based on standard-weight curves from Childress, 1991)

** = Missing data

APPENDIX D

CHROMATOGRAMS AND MASS SPECTRA

The following are chromatograms and mass spectra for water and sediment samples. The accession labels and pages are as follows:

Accession Label	Page
TW-1 = Water, Station 1	77-80
TW-2 = Water, Station 2	81-83
TW-3 = Water, Station 3	84-85
TW-4 = Water, Station 4	86
TS-1-1 = Sediment, Station 1, fraction #1	87
TS-1-2 = Sediment, Station 1, fraction #2	88
TS-1-3 = Sediment, Station 1, fraction #3	89-90
TS-2-1 = Sediment, Station 2, fraction #1	91-97
TS-2-2 = Sediment, Station 2, fraction #2	98-99
TS-2-3 = Sediment, Station 2, fraction #3	100
TS-3-1 = Sediment, Station 3, fraction #1	101-106
TS-3-2 = Sediment, Station 3, fraction #2	107-108
TS-3-3 = Sediment, Station 3, fraction #3	109
TS-4-1 = Sediment, Station 4, fraction #1	110-111
TS-4-2 = Sediment, Station 4, fraction #2	112-113
TS-4-3 = Sediment, Station 4, fraction #3	114

The mass spectra are presented as two spectra. The top spectra is that of the sample with the peak number in the upper right corner. The bottom spectra is that of the library match corresponding to the sample. The name and molecular formula of the compound are given at the top. Also, the CAS

registry number is listed, if available, at the top right following the label "RFN". In the interest of being concise, spectra were only included once per occurrence.

IHP
A: 2483

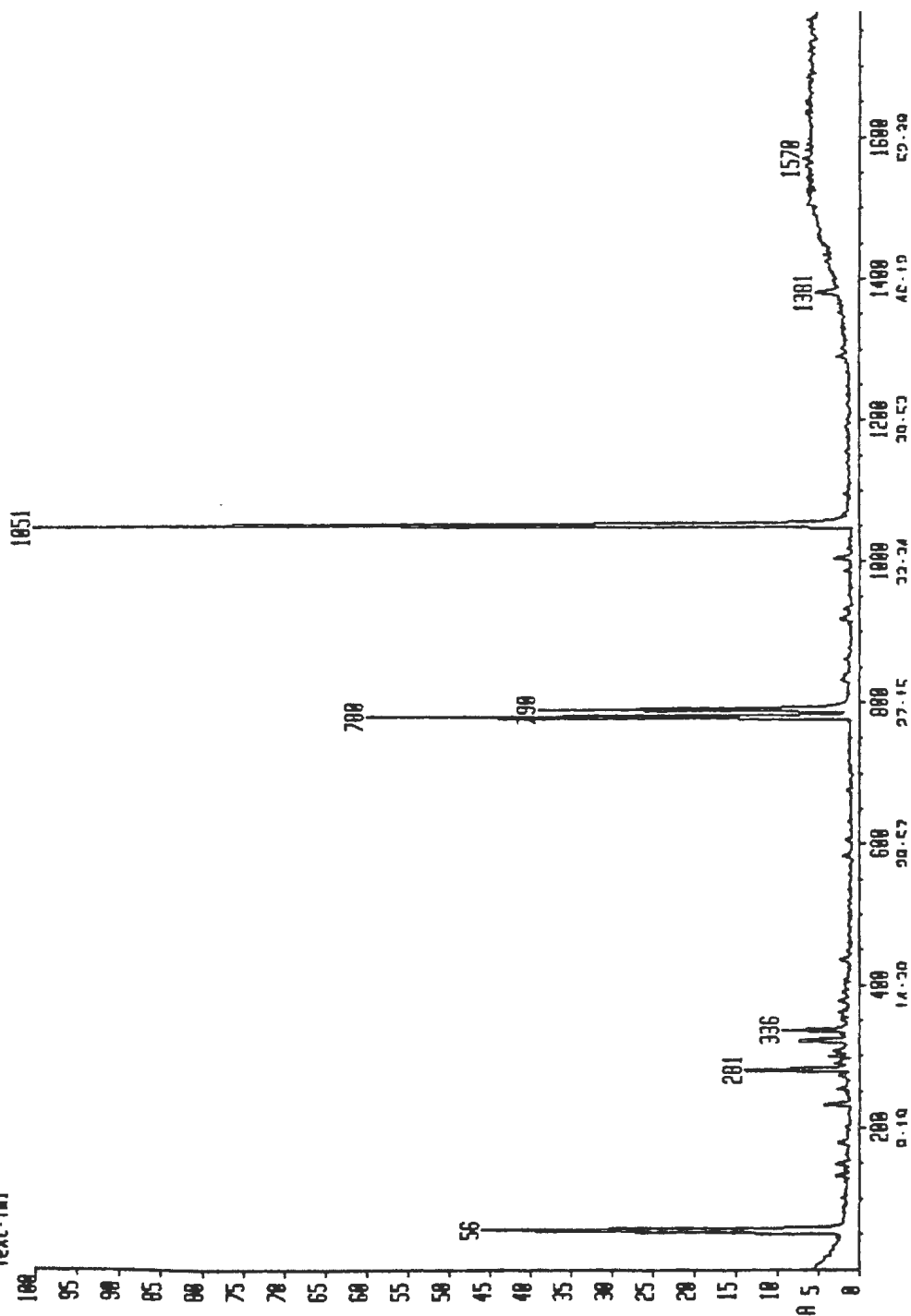
Sys: DEFAULTS

(E1+)

12007204 81-1775 20-JUL-92 13:16 TS250

Chromatogram Identifiers: A: ATIC

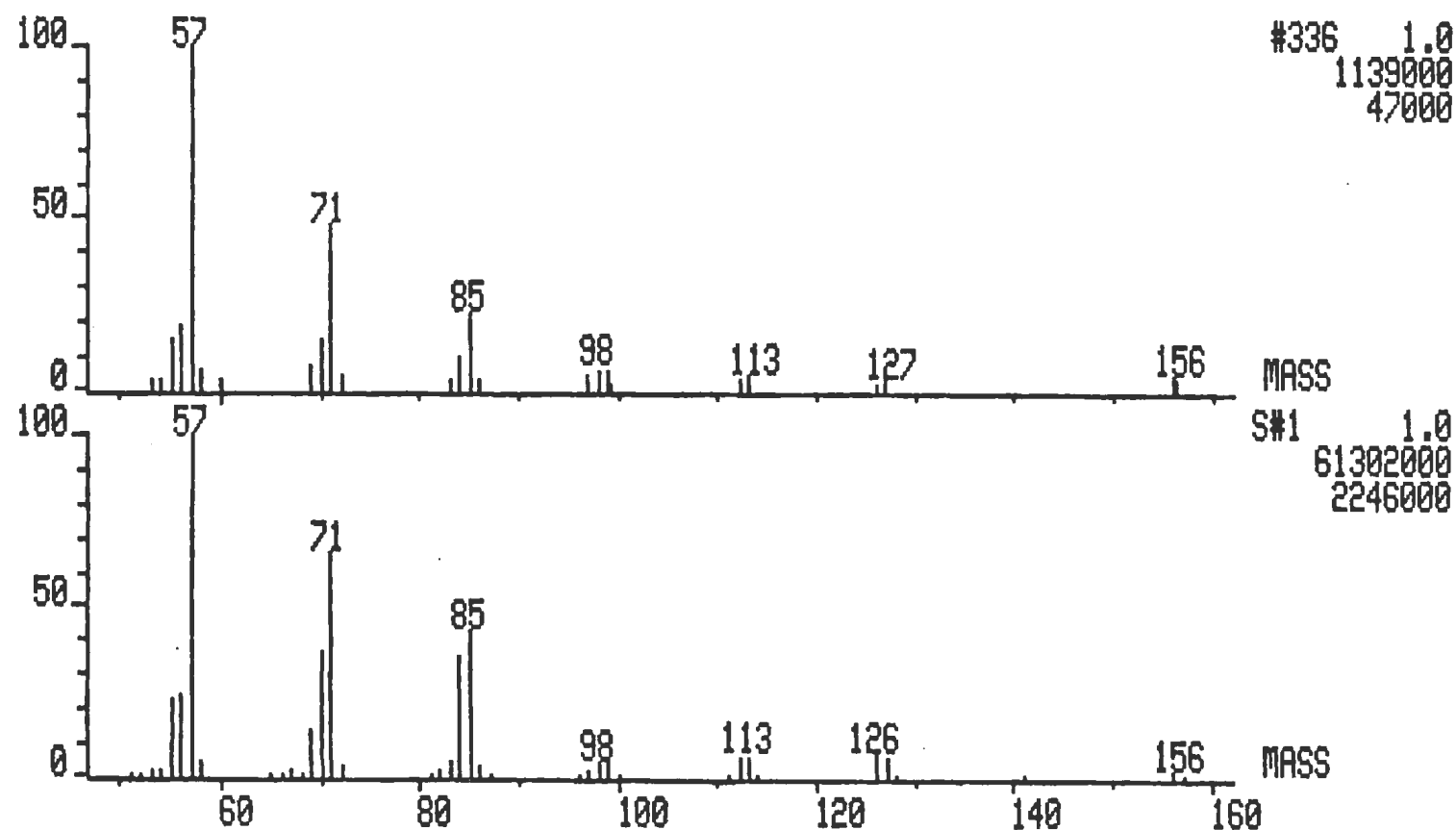
Text: TMI



LIBFITS2#1* x1 Bgd=336 T2007204
NONANE, 4,5-DIMETHYL-
C11.H24.

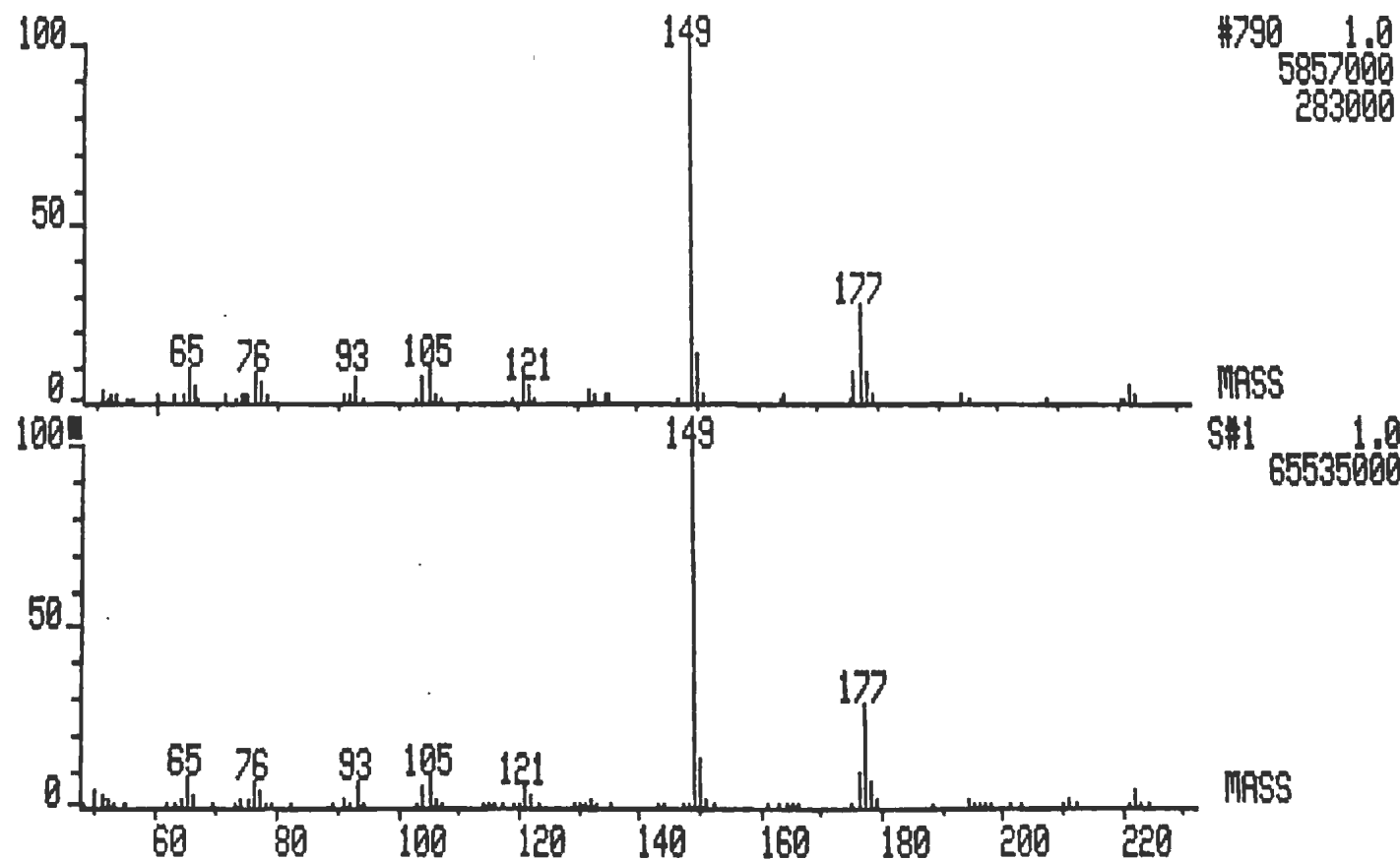
+0:00:00

Lib:NBS p585 M752 r709 RFN:17302-23-7
8728 Bpk: 43 Mwt: 156

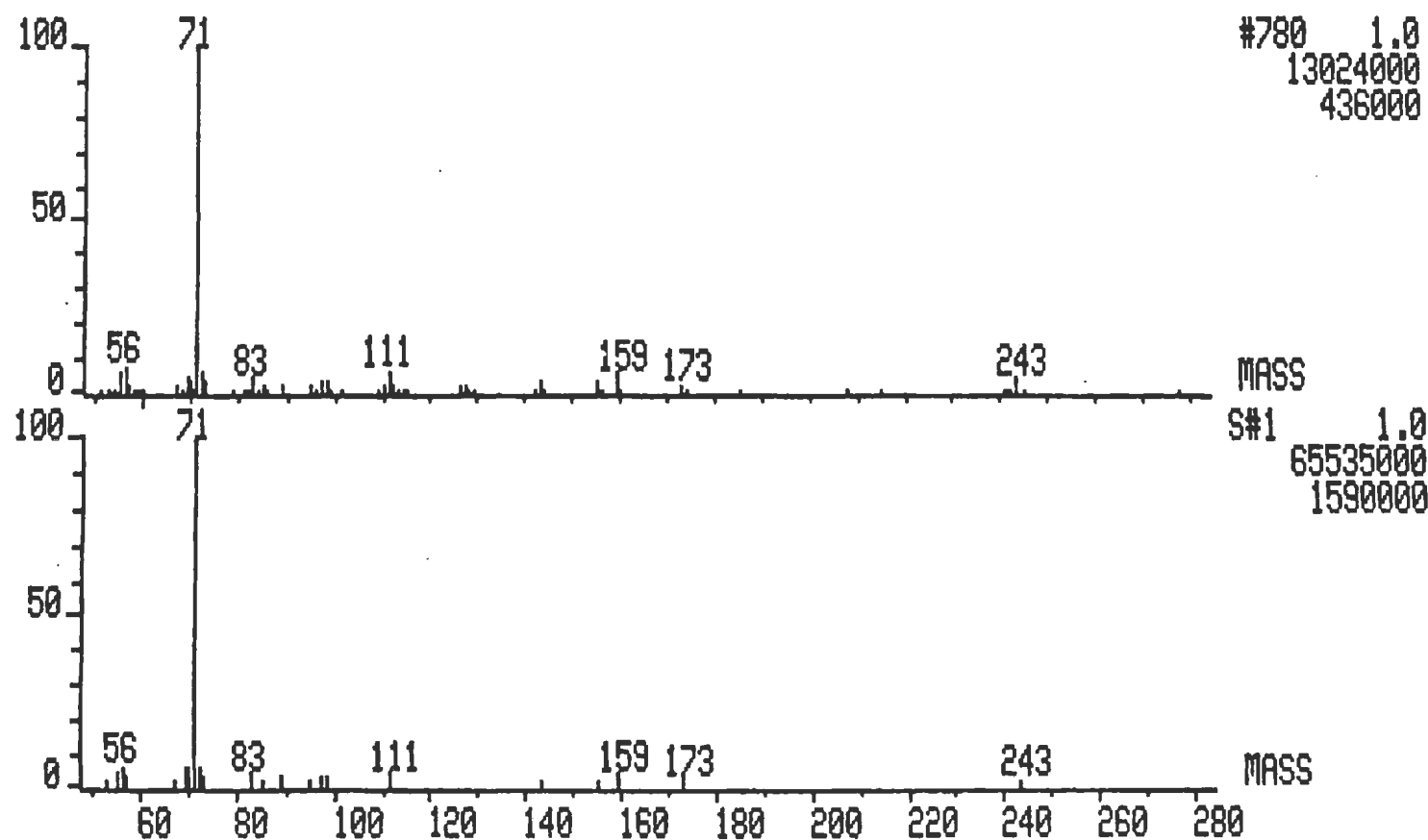


LIBFITS2#1* x1 Bgd=790 T2007204 +0:00:00
DIETHYL PHTHALATE
C12.H14.O4.

Lib:NBS p900 M942 r947 RFN:84-66-2
19827 Bpk: 149 Mwt: 222



LIBFITS1#1* x1 Bgd=780 T2007204 +0:00:00
 PROPANOIC ACID, 2-METHYL-, 1-(1,1-DIMETHYLETHYL)- p791 M909 r855 RFN:0-00-0
 C16.H30.O4. Lib:NBS 27805 Bpk: 71 Mwt: 286



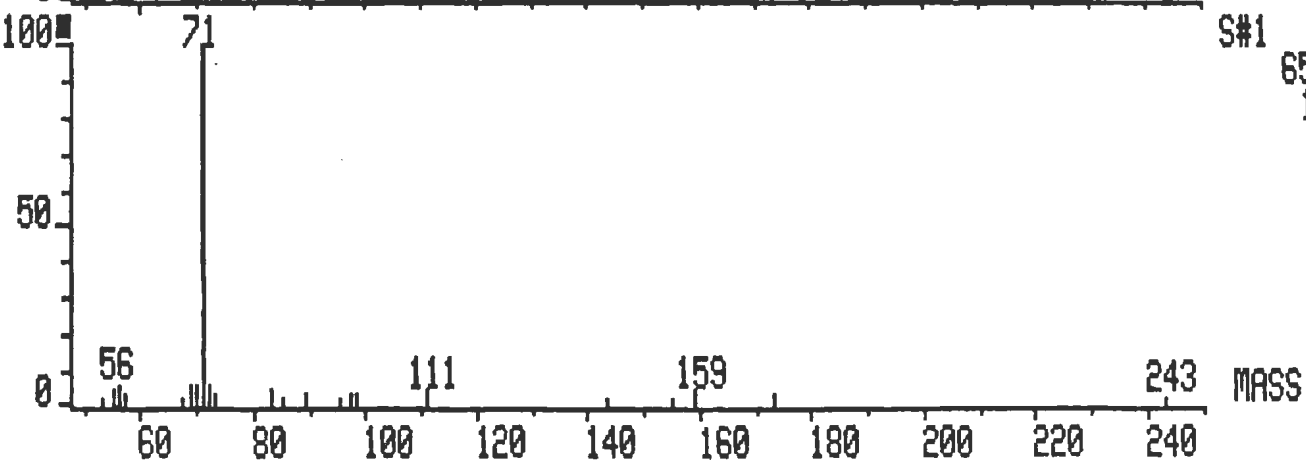
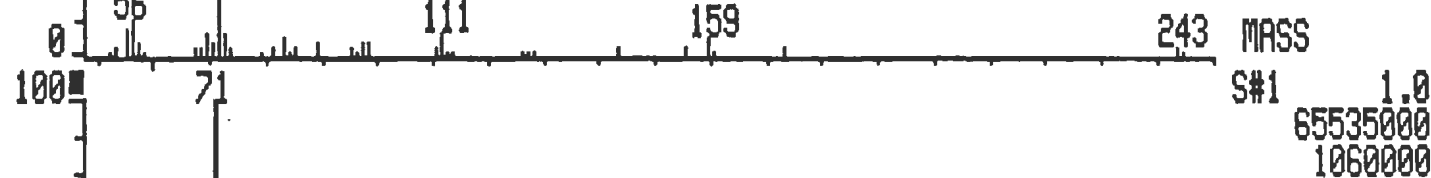
3



LIBFITS1#1* x1 Bgd=780 T1407206 +0:00:00

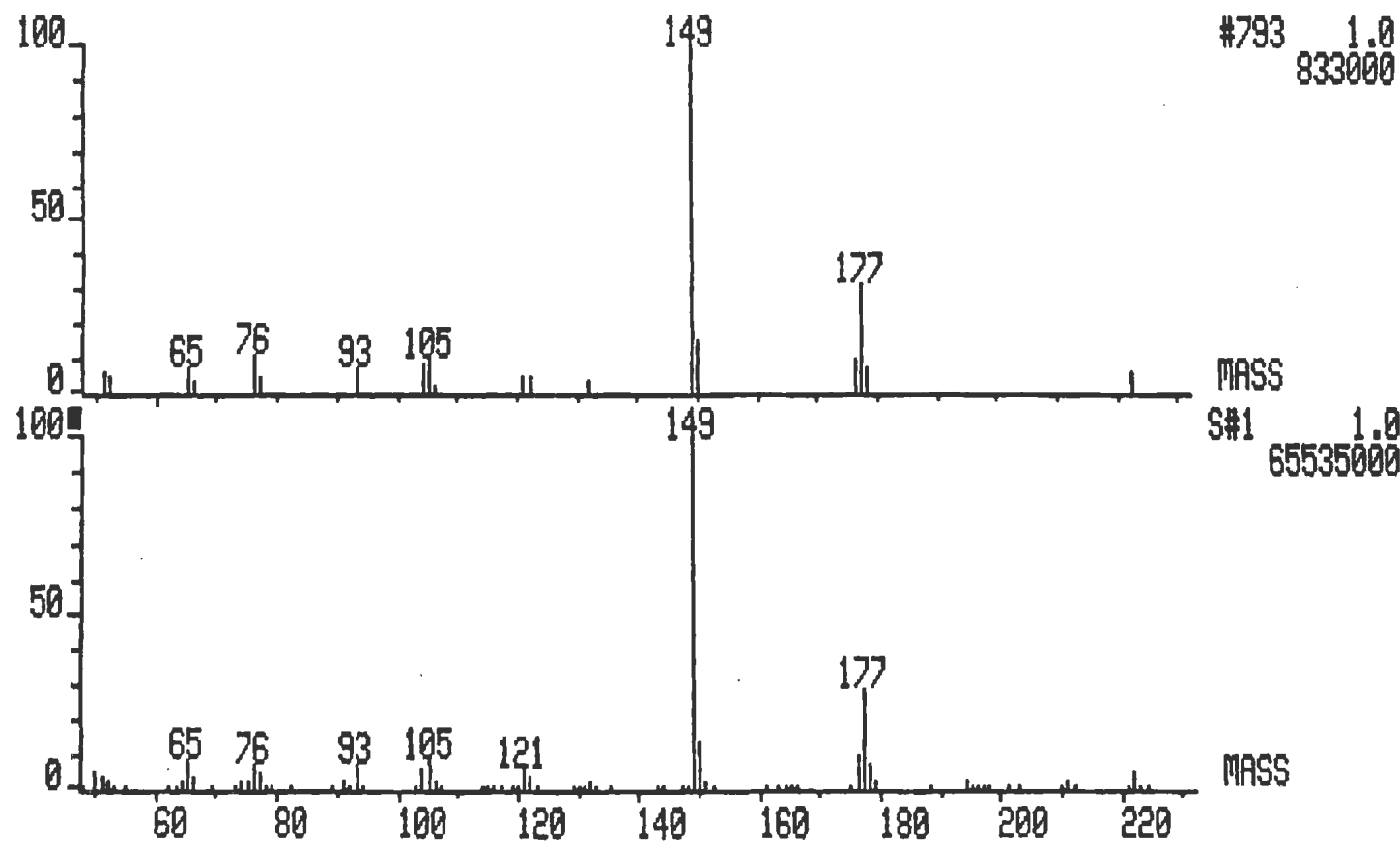
PROPANOIC ACID, 2-METHYL-, 1-(1,1-DIMETHYLETHYL)- p863 M915 r933 RFN:0-00-0

C16.H30.04. Lib:NBS 27805 Bpk: 71 Mwt: 286



LIBFITS2#1* x1 Bgd=793 T1407206
DIETHYL PHTHALATE
C12.H14.O4.

+0:00:00
p896 M896 r994 RFN:84-66-2
Lib:NBS 19827 Bpk: 149 Mwt: 222

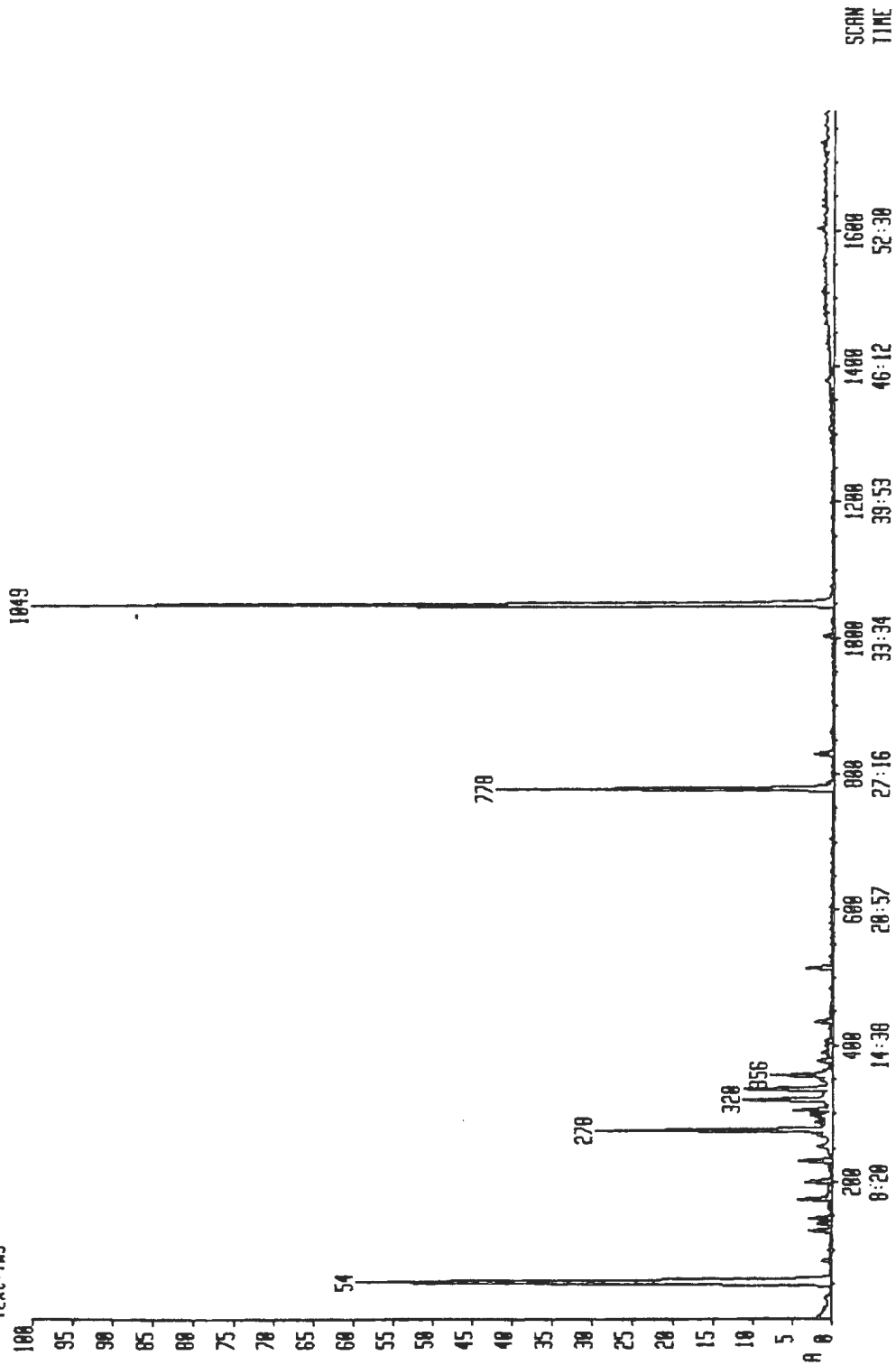


1HP
R: 1496

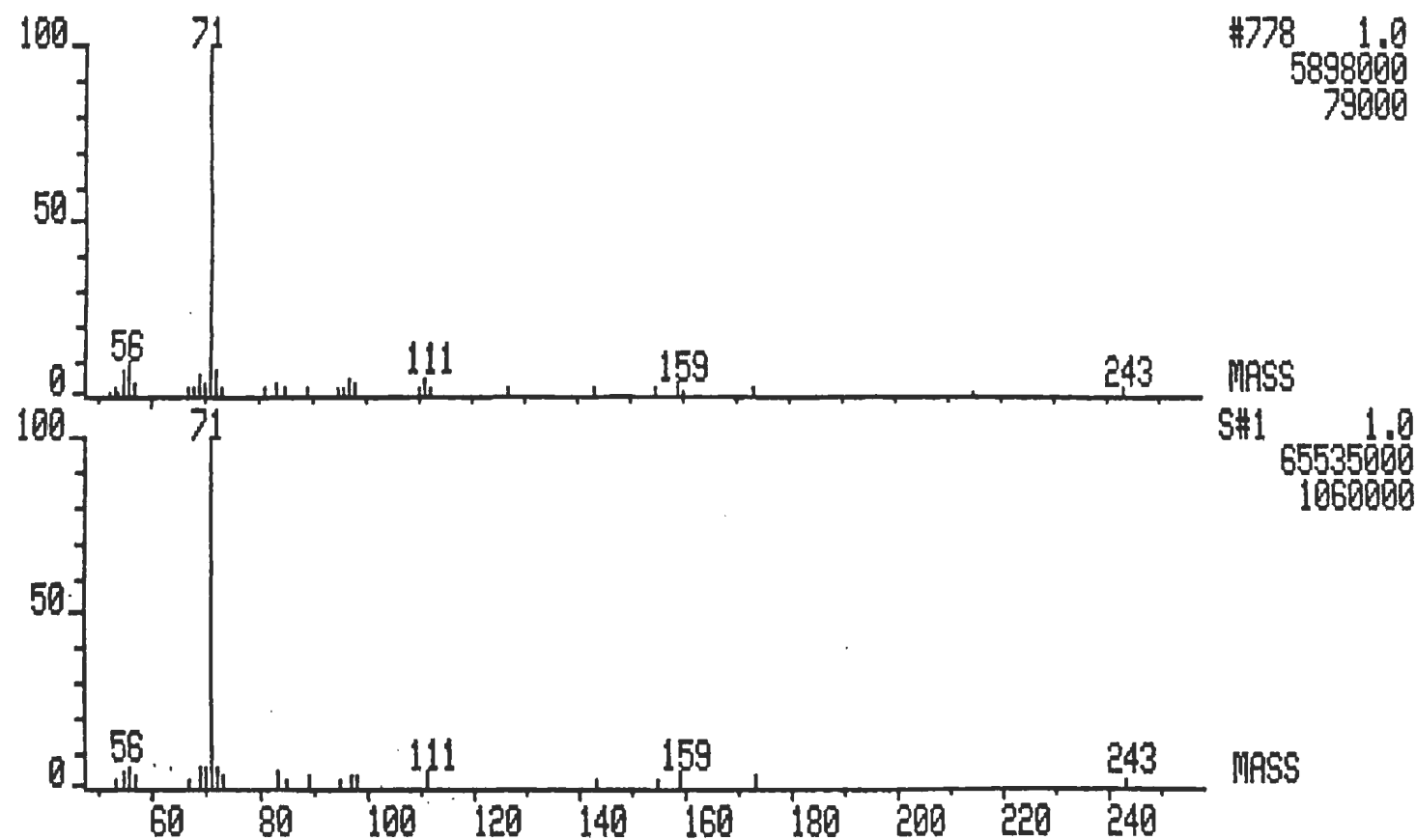
Sys:DEFAULTS

(E1+)

T1507202 #1-1775 15-JUL-92 09:58 TS250
Chromatogram Identifiers : A :ATTC
Text:TW3



LIBFITS2#1* x1 Bgd=778 T1507202 +0:00:00
 PROPANOIC ACID, 2-METHYL-, 1-(1,1-DIMETHYLETHYL)- p865 M914 r935 RFN:0-00-0
 C16.H30.04. Lib:NBS 27805 Bpk: 71 Mwt: 286



1HP
A: 2203

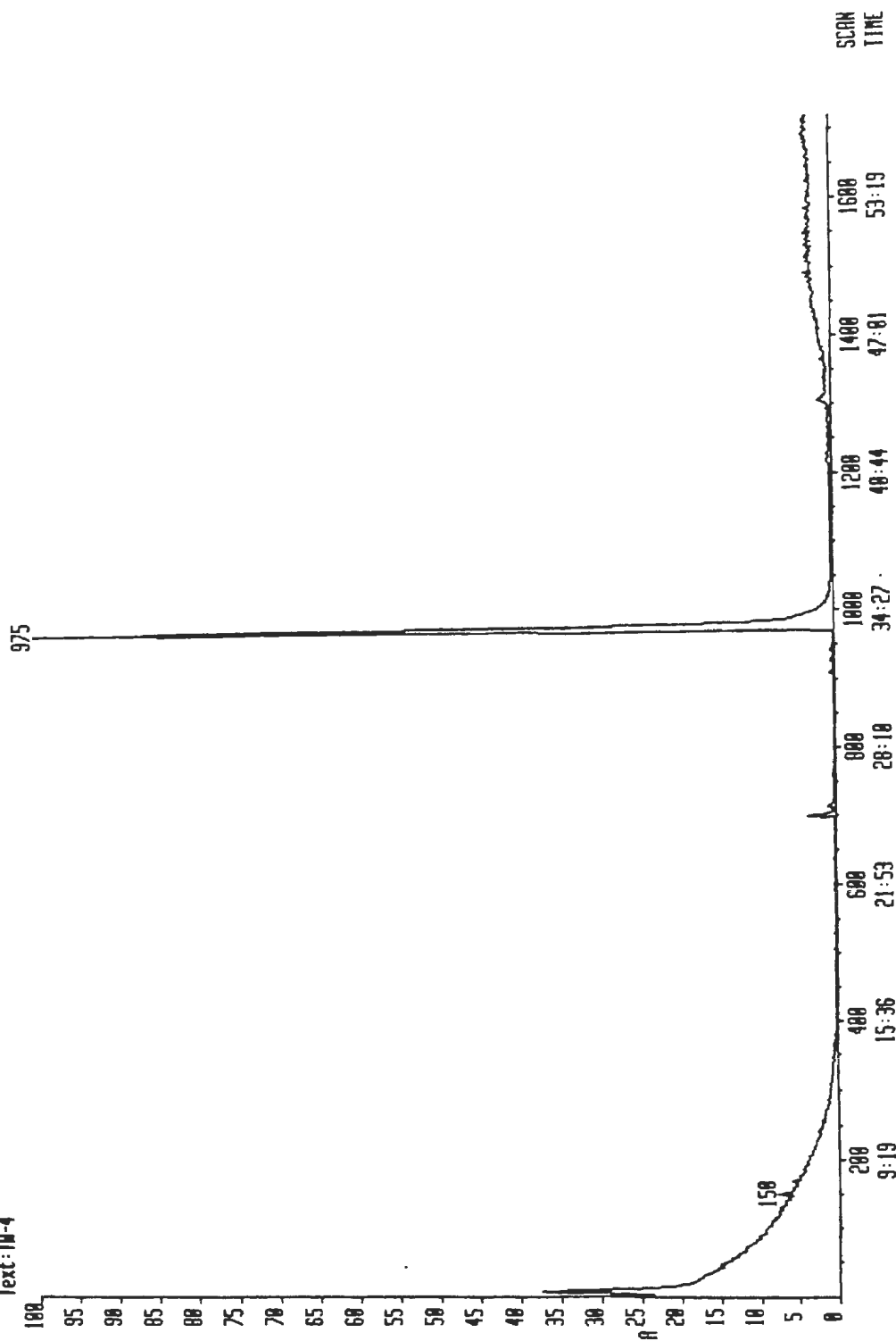
Sys: DEFAULTS

(E1+)

T1002301 01-1719 10-FEB-93 10:49 15250

Chromatogram Identifiers : A : ATIC

Text: TM-4



T2107204 11-1505 21-JUL-92 15:57 TS250

(E1+)

Sys:DEFAULTS

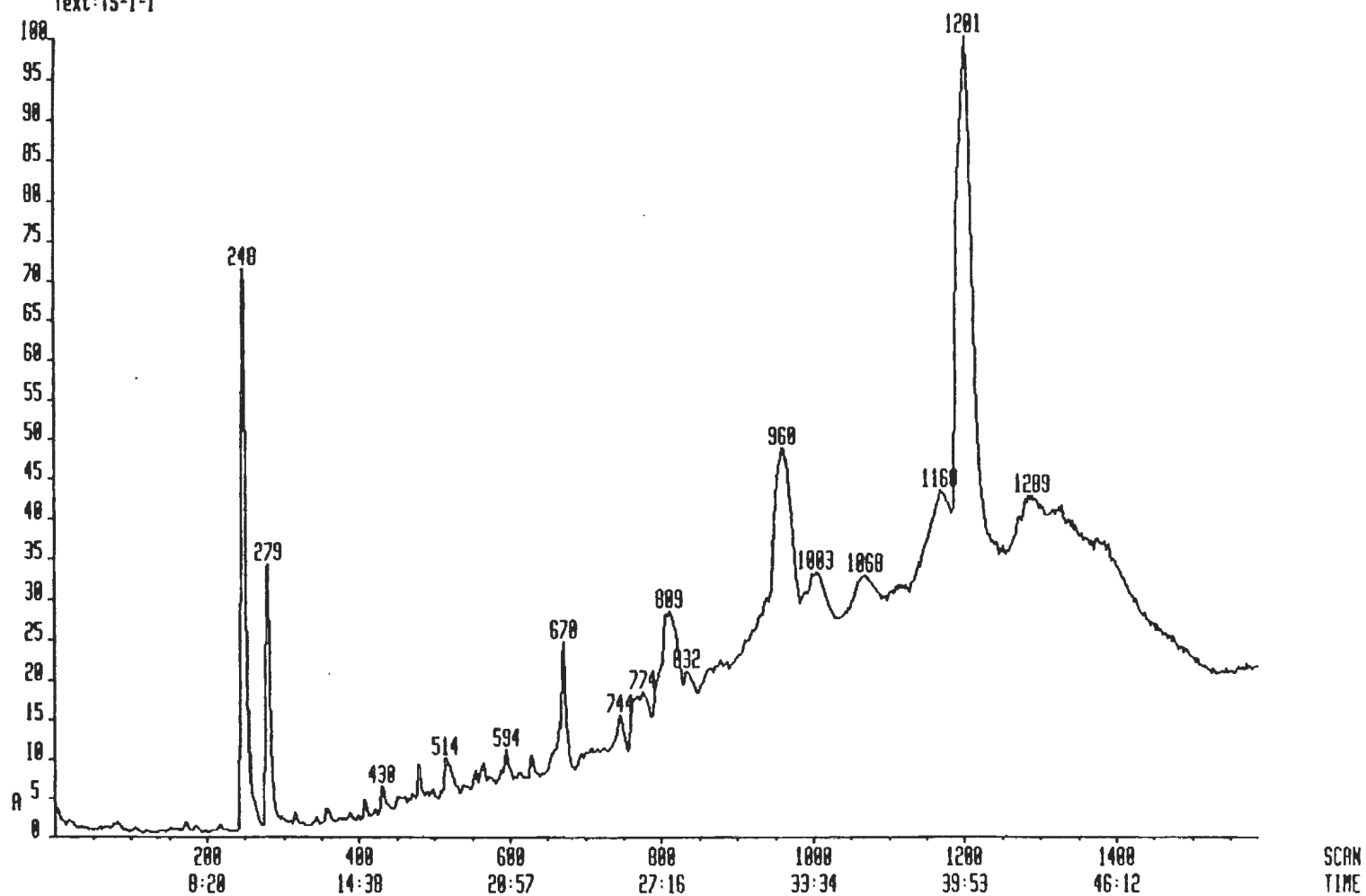
IHP

Chromatogram Identifiers : A:ATIC

A:

12631

Text:TS-1-1



T1507204 #1-1775

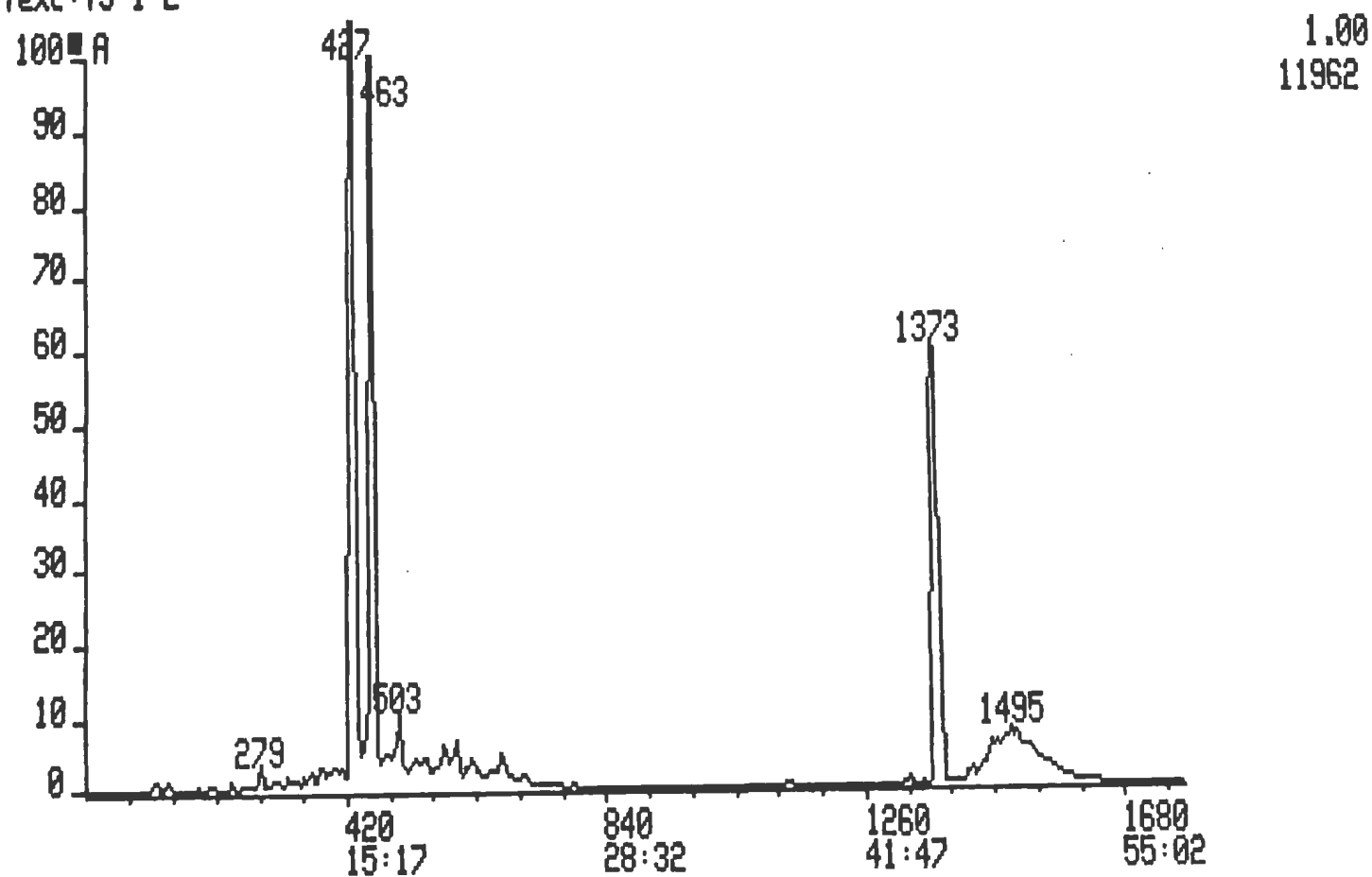
15-JUL-92 13:36 TS250

(EI+)

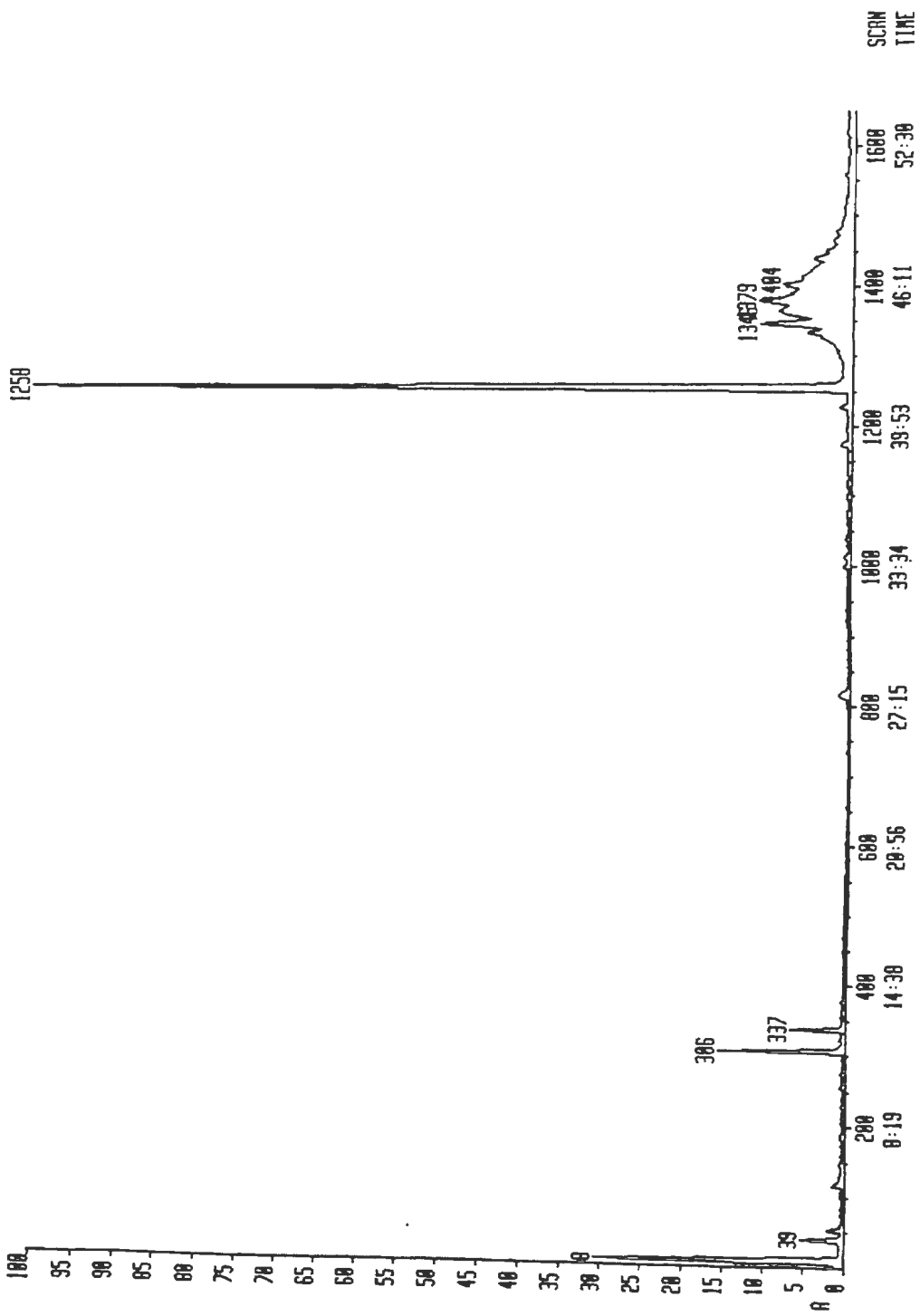
Sys:DEFAULTS

A:ATIC

Text:TS-1-2



12007207 01-1649 20-JUL-92 16:44 19250
 Chromatogram Identifiers : A : ATIC
 Text: TS-1-3
 Sys: DEFAULTS
 IHP
 A: 26311



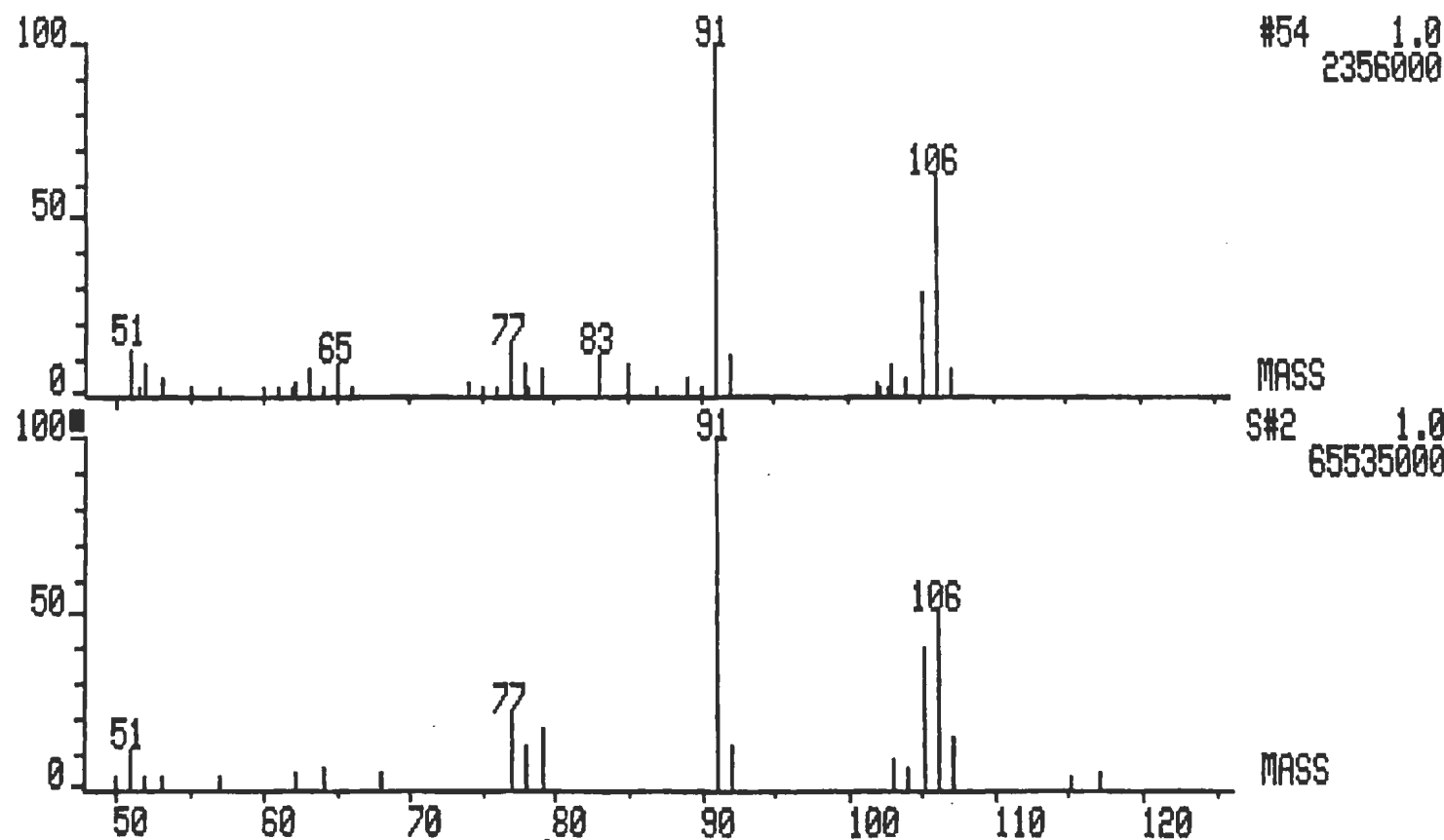
L19FITS2*2* x1 Bgd=42 T2007207
 BENZENEETHANOL, .ALPHA.,.BETA.-DIMETHYL-
 C10.H14.O.

+0:00:00

p594 M834 r696 RFN:52089-32-4

Lib:NBS

7487 Bpk: 91 Mwt: 150



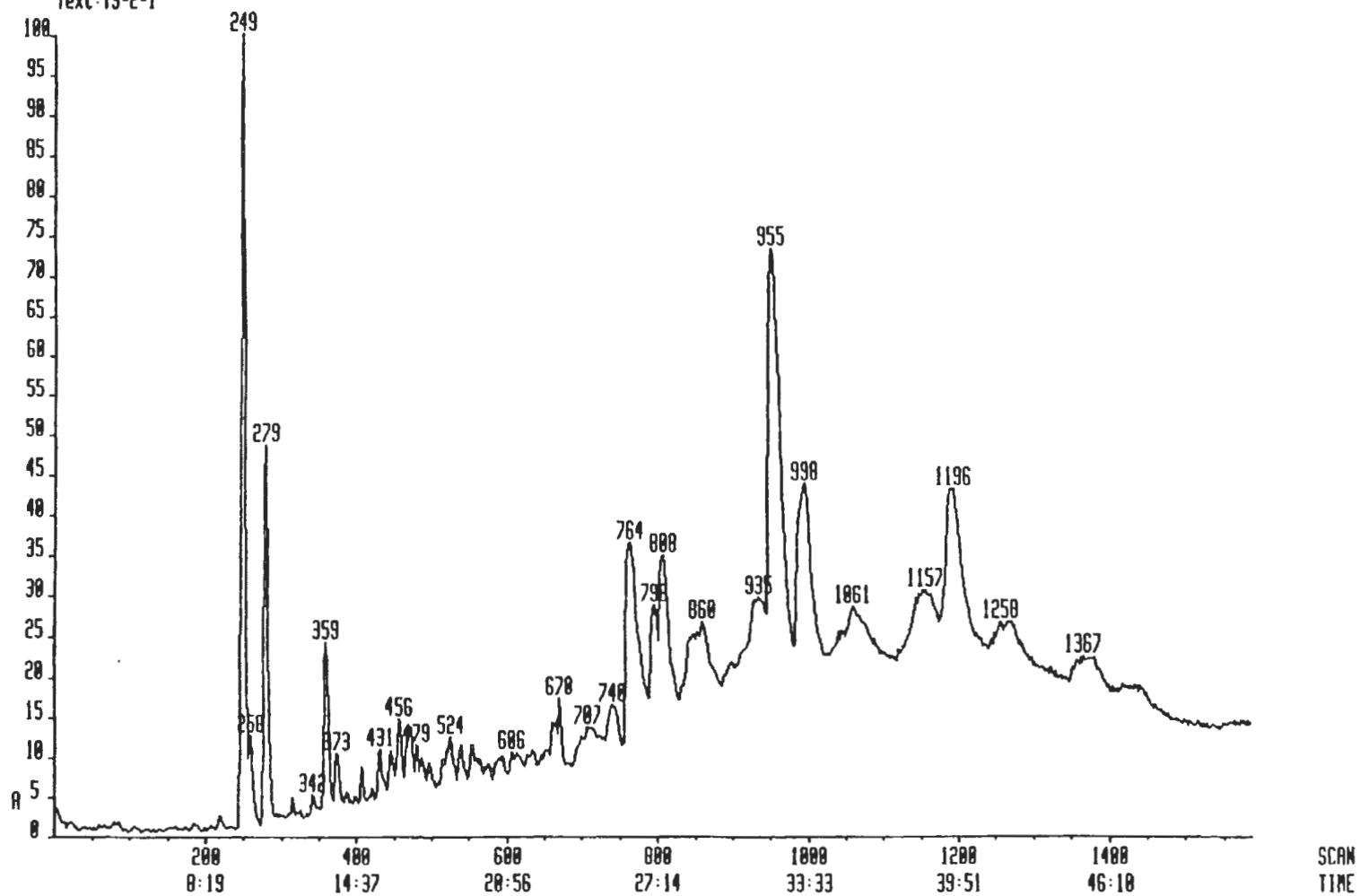
T2107203 01-1506 21-JUL-92 12:55 TS250
Chromatogram Identifiers : A : ATIC
Text: TS-2-1

(E1+)

Sys: DEFAULTS

IHP

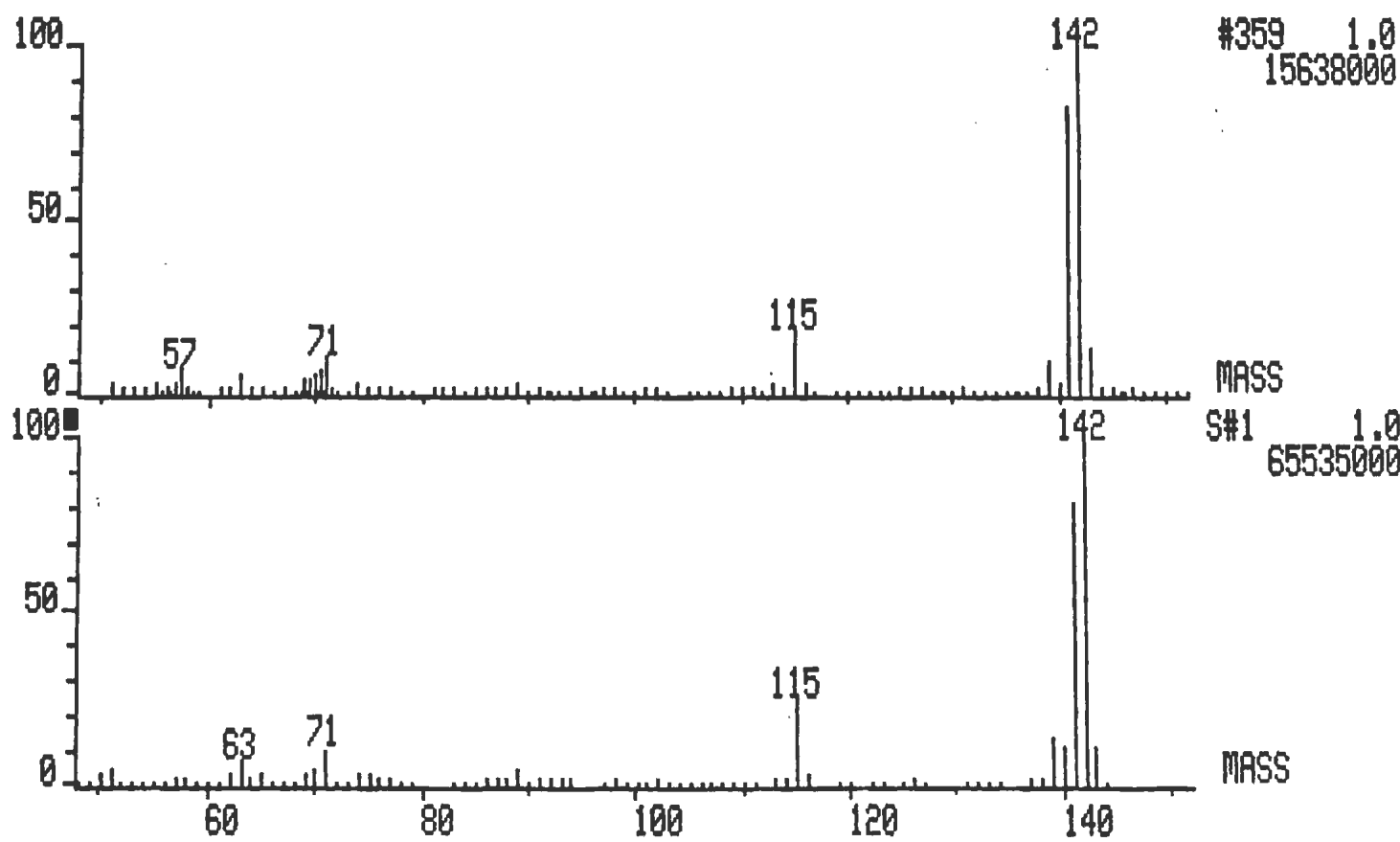
R: 14188



LIBFITS2#1* x1 Bgd=359 T2107203
NAPHTHALENE, 1-METHYL-
C11.H10.

+0:00:00

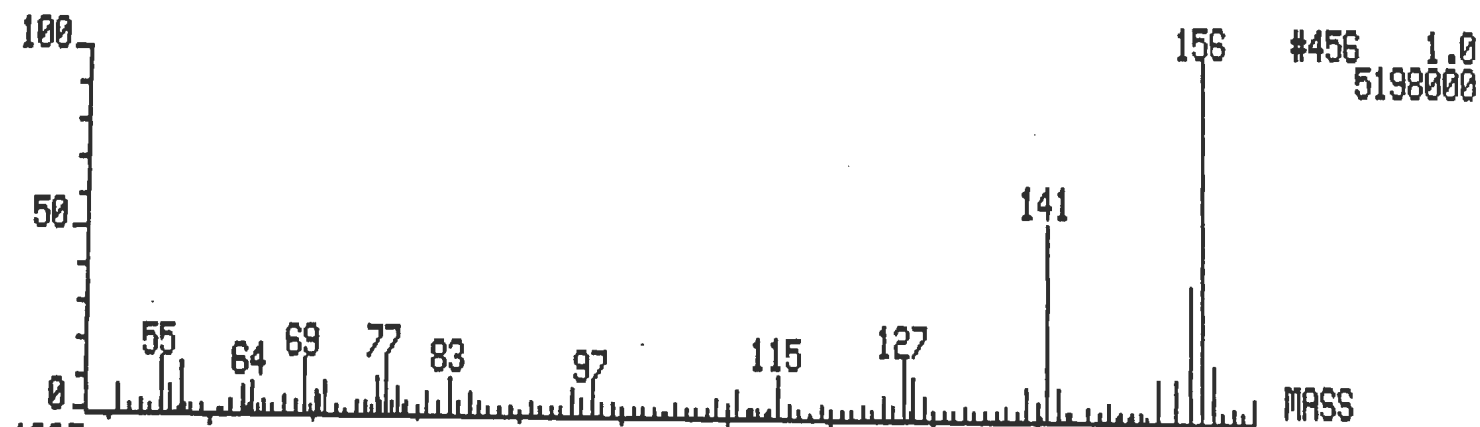
p897 M945 r905 RFN:90-12-0
Lib:NBS 6111 Bpk: 142 Mwt: 142



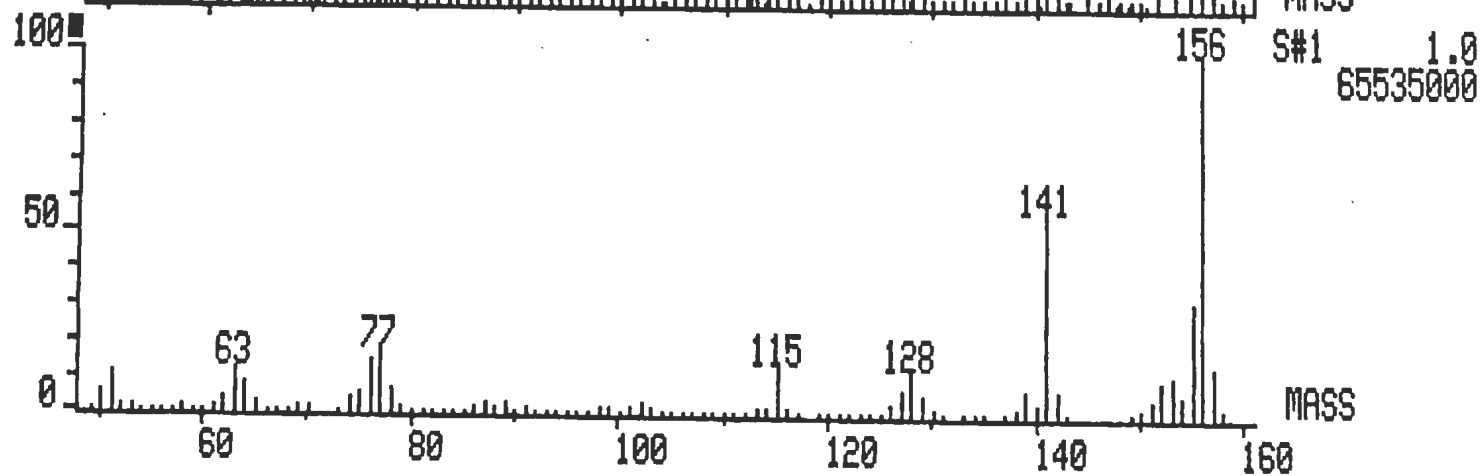
LIBFITS1#1* x1 Bgd=456 T2107203
NAPHTHALENE, 1,5-DIMETHYL-
C12.H12.

+0:00:00

p726 M835 r762 RFN:571-61-9
Lib:NBS 8745 Bpk: 156 Mwt: 156



#456 1.0
5198000



MASS S#1 1.0
65535000

MASS

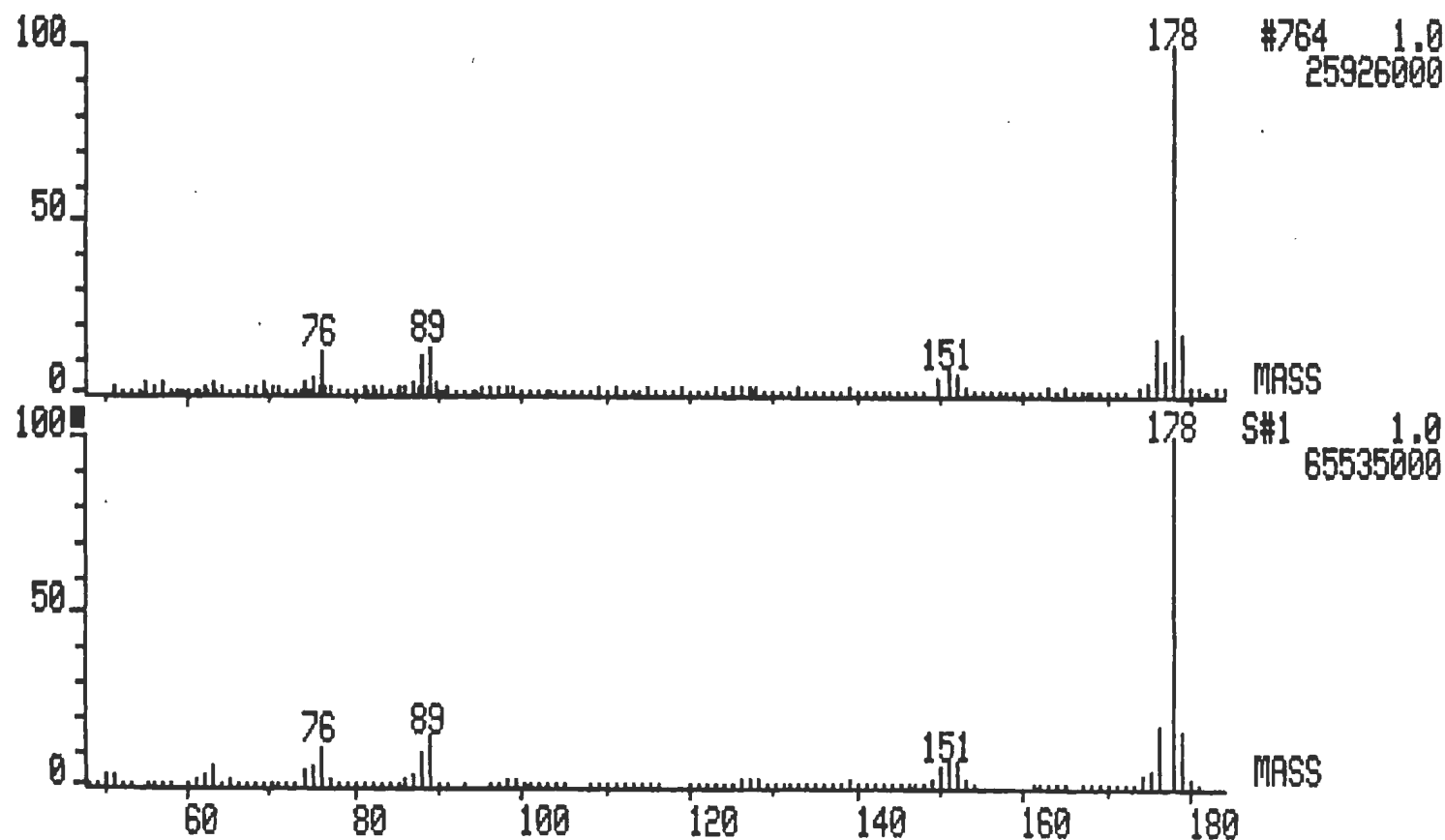
LIBFITS1#1* x1 Bgd=764 T2107203
ANTHRACENE
C14.H10.

+0:00:00

p771 M917 r785 RFN:120-12-7

Lib:NBS

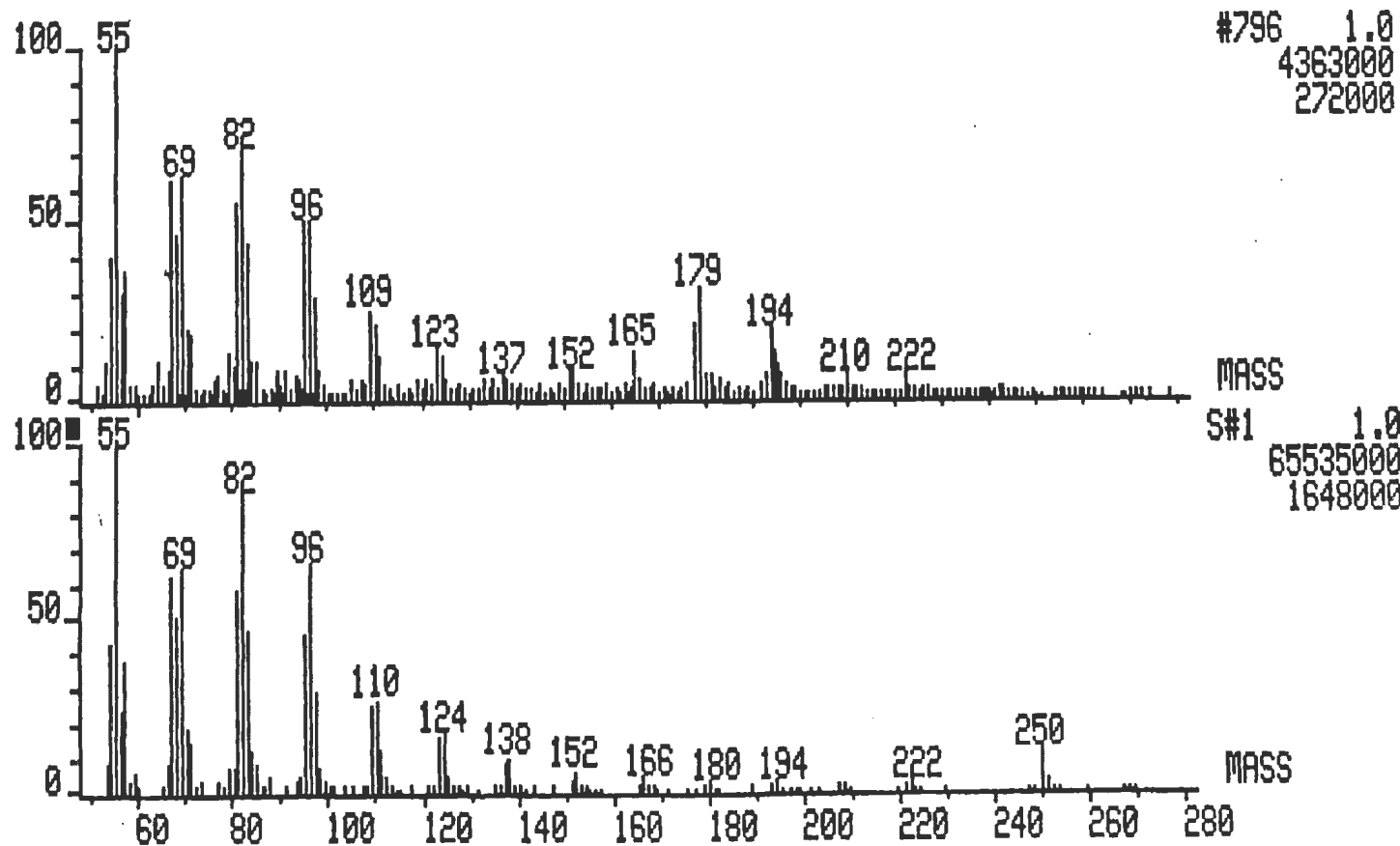
12788 Bpk: 178 Mwt: 178



LIBFITS2#1* x1 Bgd=796 T2107203
9-OCTADECEN-1-OL, (Z)-
C18.H36.O.

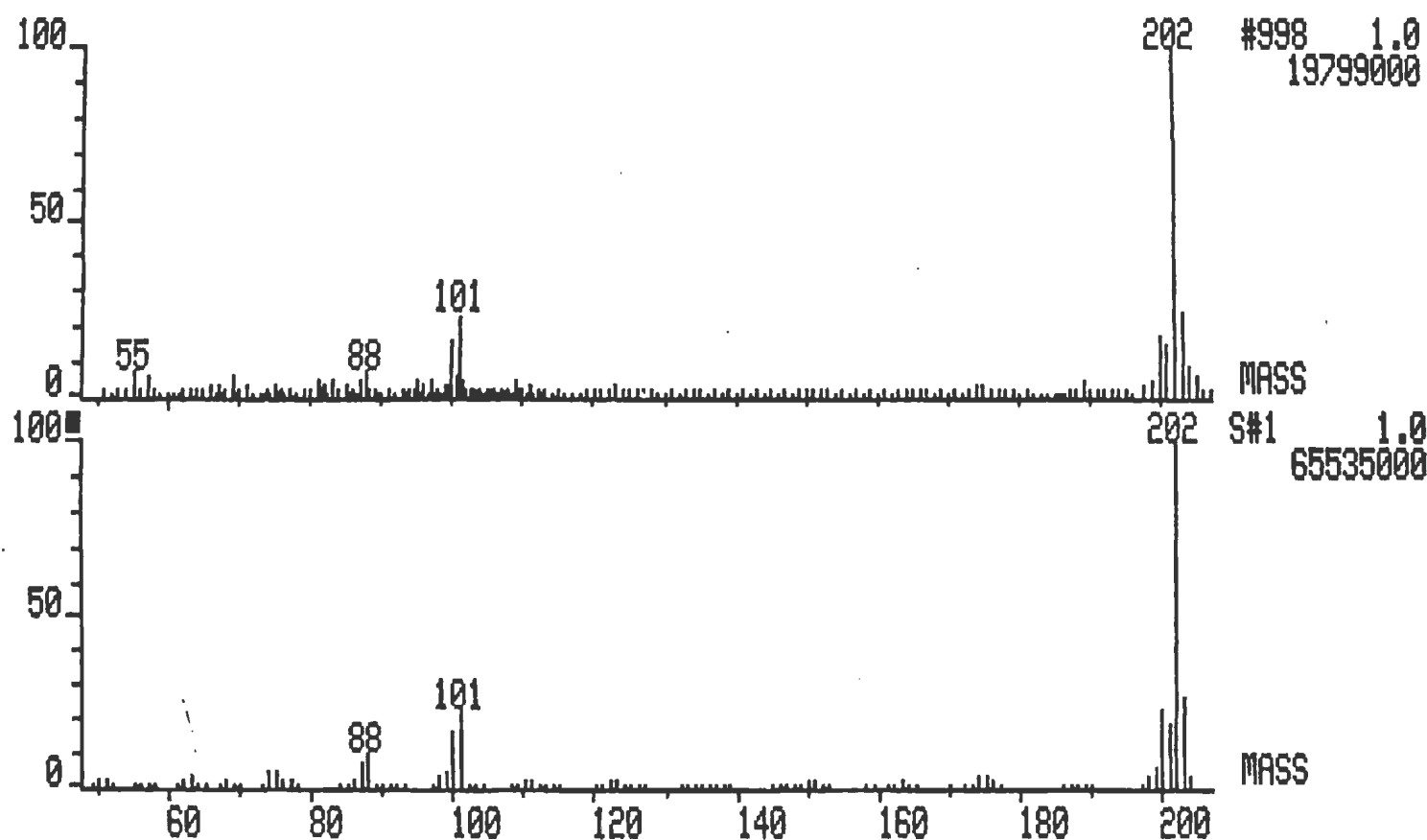
+0:00:00

p577 M781 r620 RFN:143-28-2
Lib:NBS 25864 Bpk: 55 Mwt: 268



LIBFITS2#1* x1 Bgd=955 T2107203 +0:00:00
PYRENE
C16.H10.

p481 M722 r485 RFN:129-00-0
Lib:NBS 16899 Bpk: 202 Mwt: 202



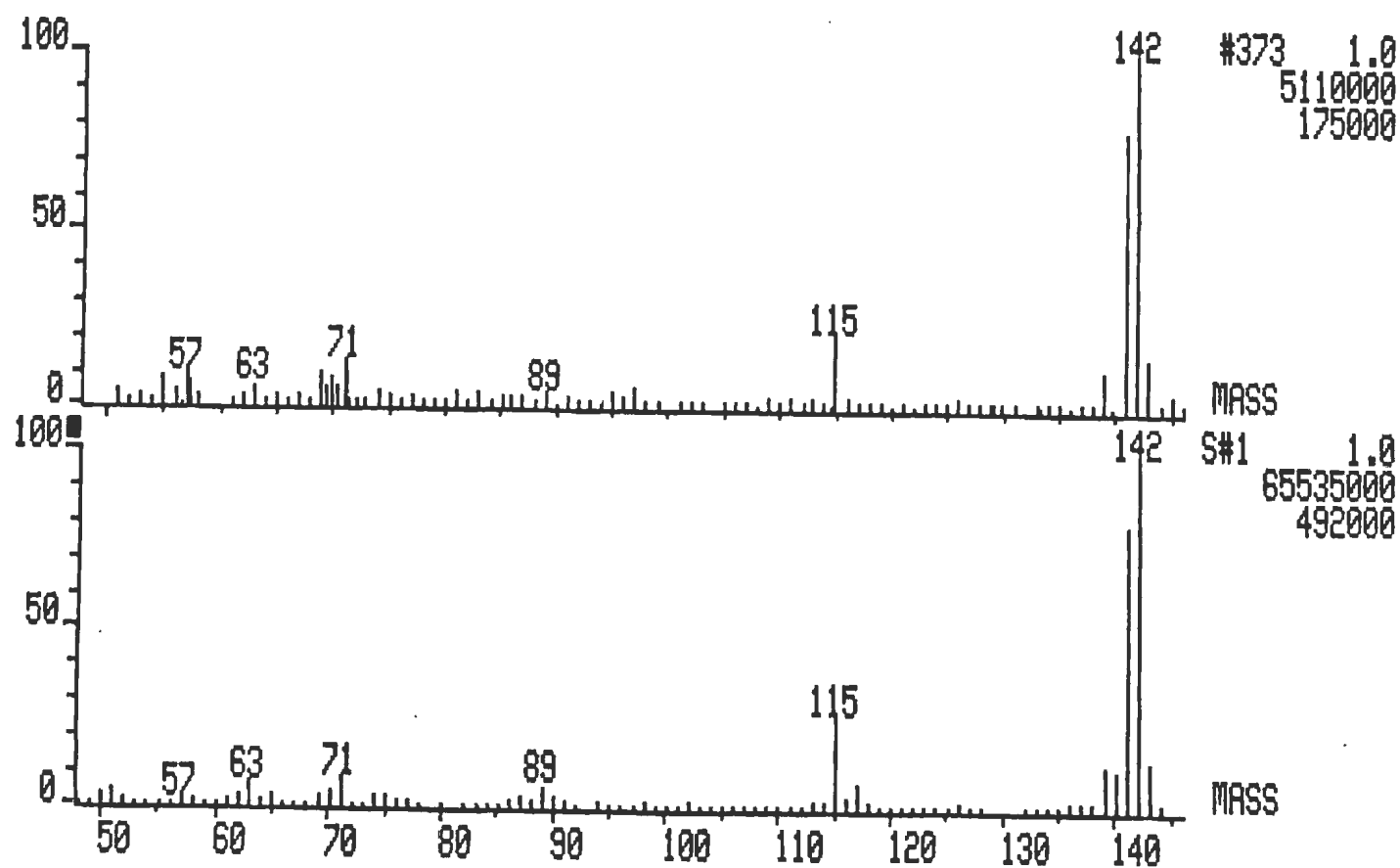
LIBFITS1#1* x1 Bgd=373 T2107203
NAPHTHALENE, 2-METHYL-
C11.H10.

+0:00:00

p772 M873 r781 RFN:91-57-6

Lib:NBS

6112 Bpk: 142 Mwt: 142



T1507205 #1-1029

15-JUL-92 15:10 TS250

(EI+)

Sys:DEFAULTS

A:ATIC

Text:TS-2-2

100% A

90

80

70

60

50

40

30

20

10

0

112

180

7:42

360

13:23

540

19:04

720

24:45

900

30:25

428

445

504

598

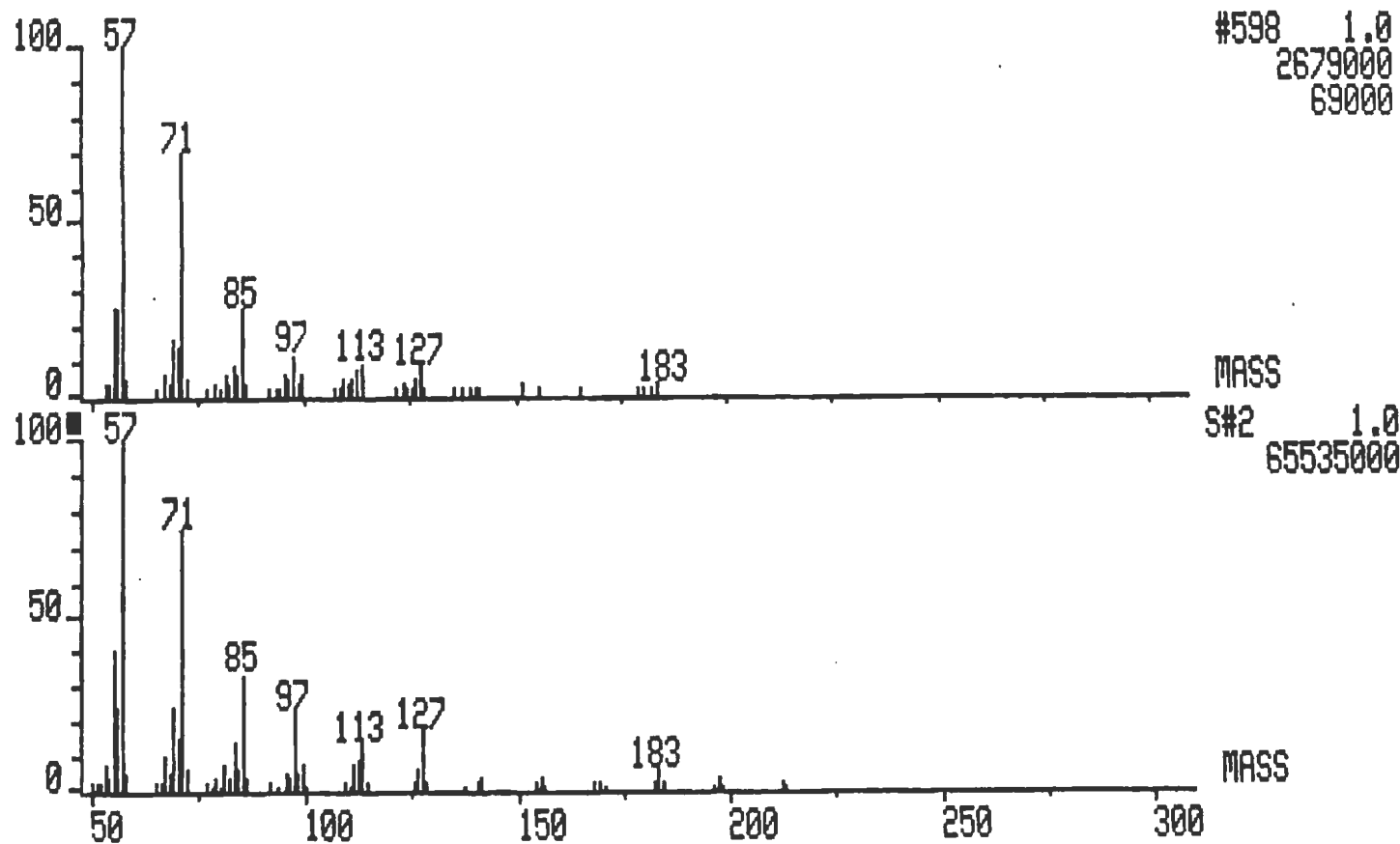
1.00

12551

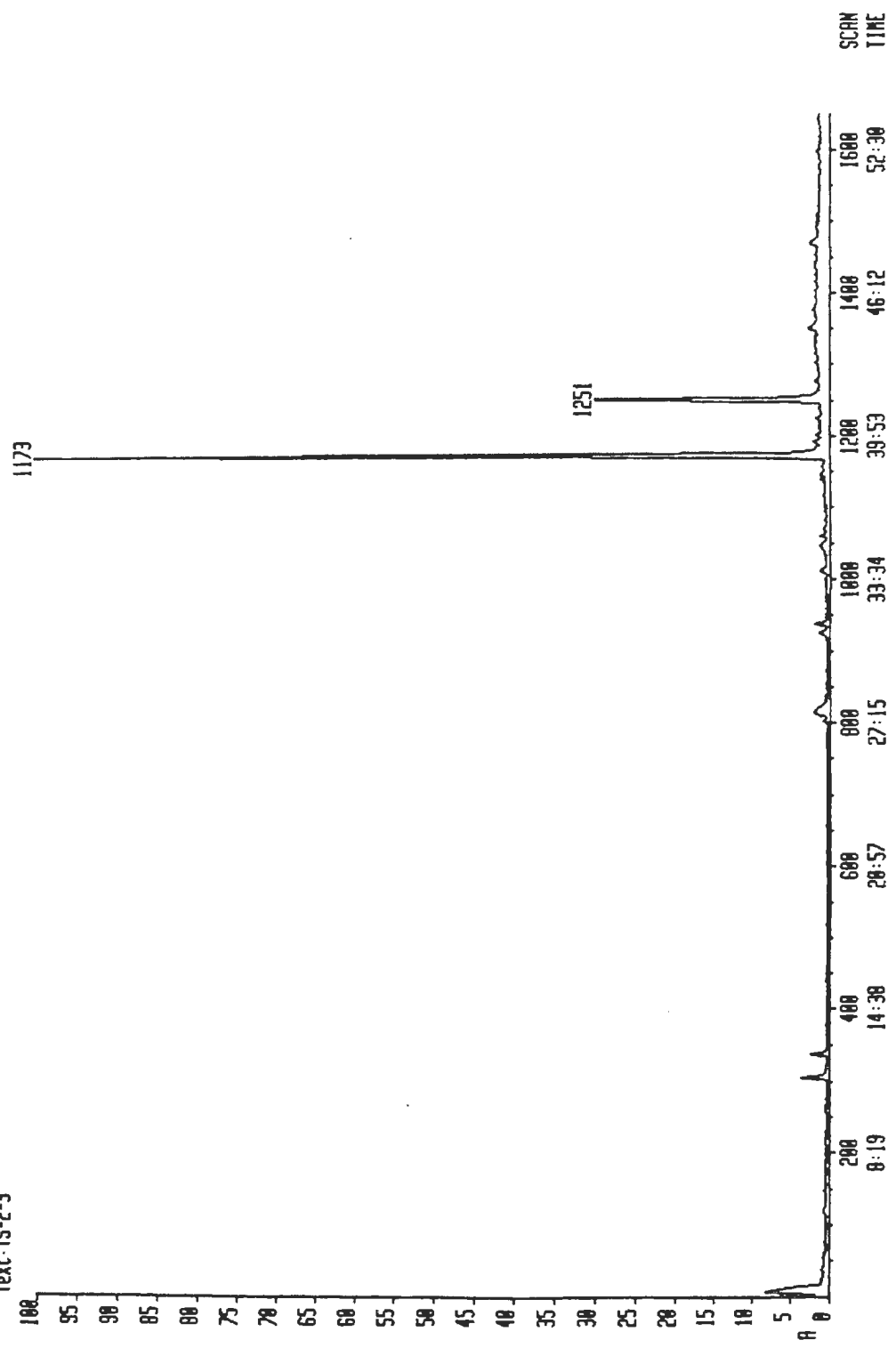
LIBFITS2#2* x1 Bgd=598 T1507205
DODECANE, 2,6,10-TRIMETHYL-
C15.H32.

+0:00:00

p707 M761 r900 RFN:3891-98-3
Lib:NBS 18545 Bpk: 57 Mwt: 212



12007206 11-1649 20-JUL-92 15:45 TS250 (E1+) Sys:DEFAULTS IHP 12151
 Chromatogram Identifiers : A :ATTC
 Text:TS-2-3



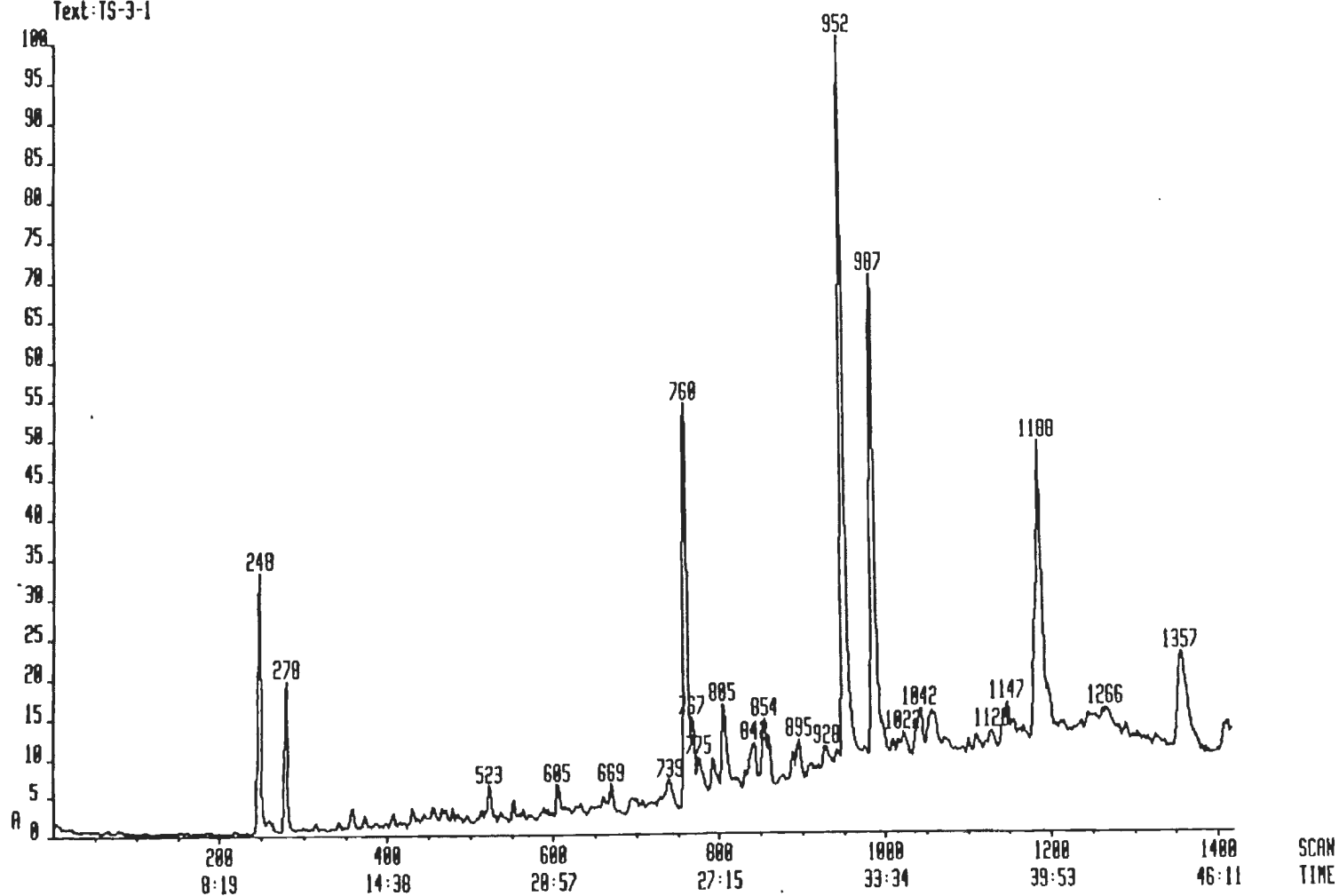
T2107202 01-1417 21-JUL-92 10:56 TS250
Chromatogram Identifiers : A :ATIC
Text:TS-3-1

(EI+)

Sys:DEFAULTS

IHP
R:

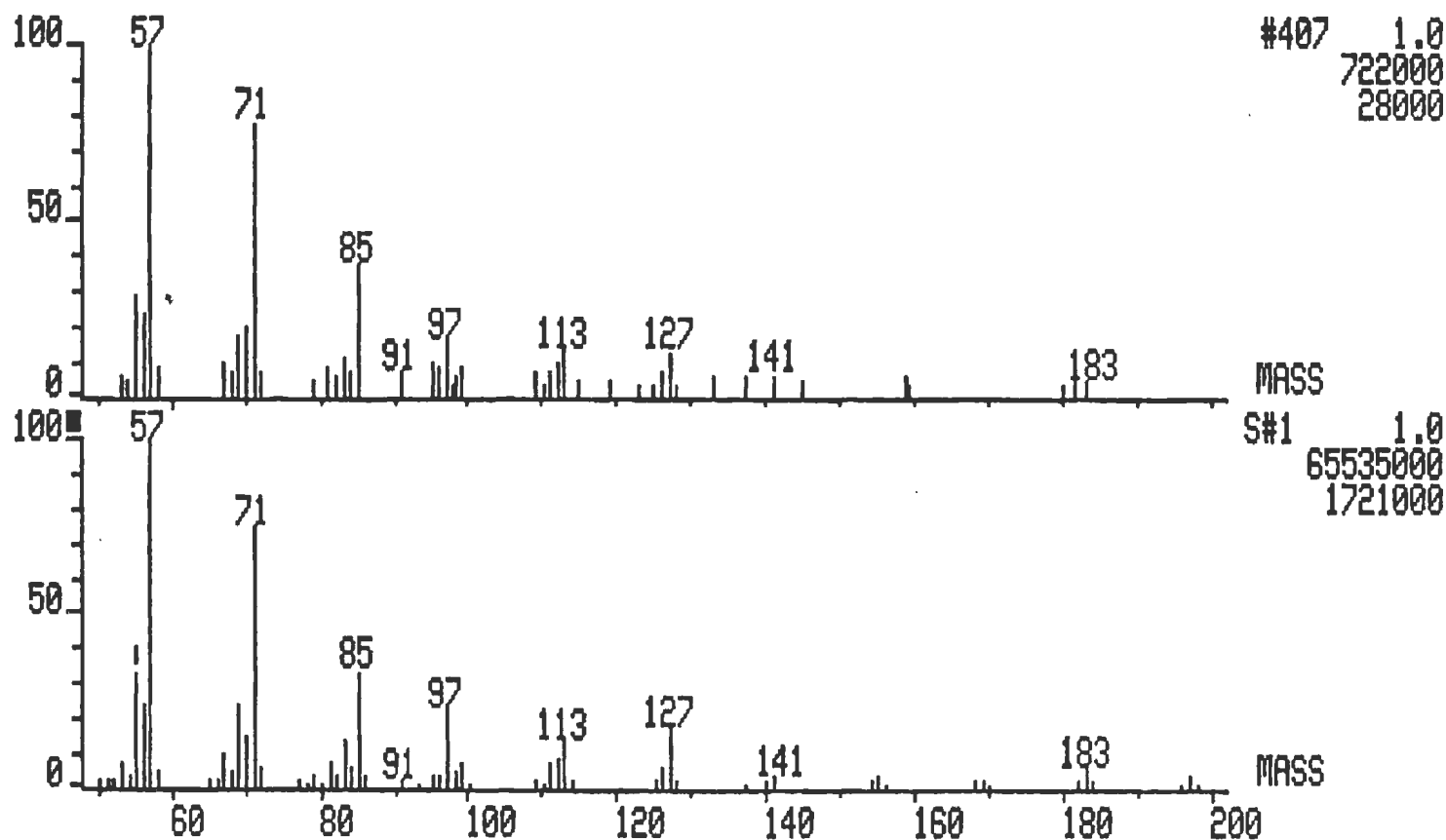
9433



LIBFITS1#1* x1 Bgd=407 T2107202
DODECANE, 2,6,10-TRIMETHYL-
C15.H32.

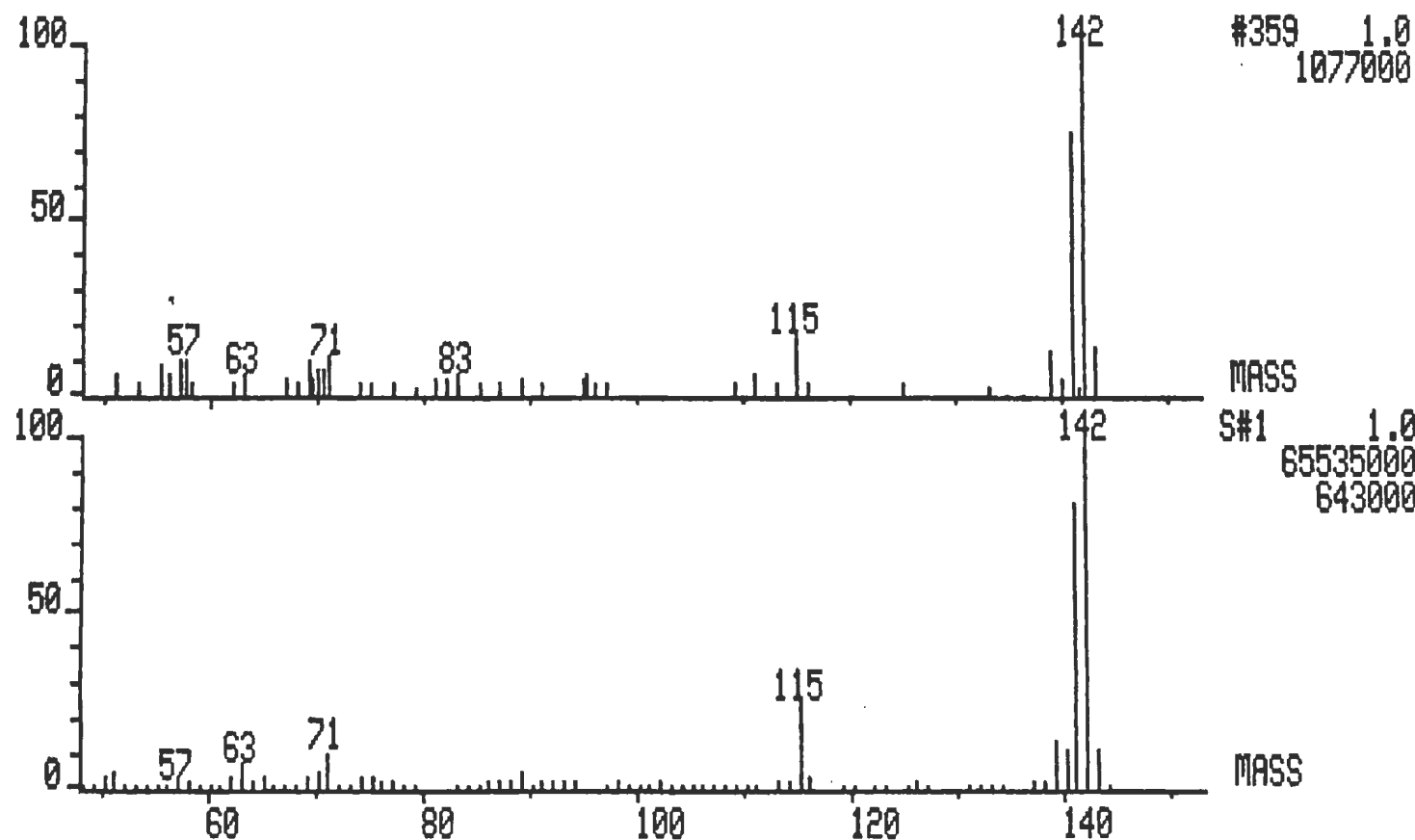
+0:00:00

p645 M714 r847 RFN:3891-98-3
Lib:NBS 18545 Bpk: 57 Mwt: 212



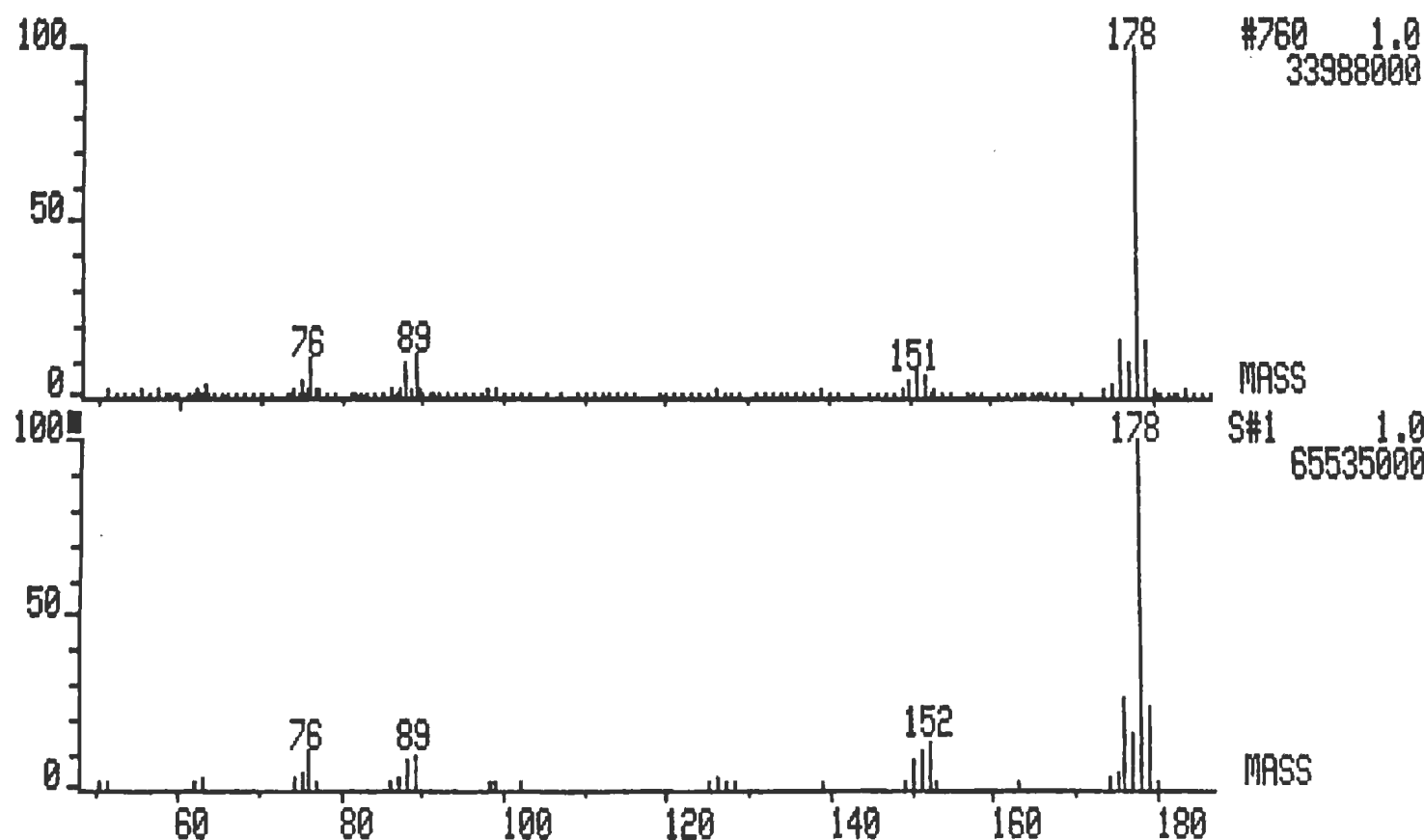
LIBFITS2#1* x1 Bgd=359 T2107202
NAPHTHALENE, 1-METHYL-
C11.H10.

+0:00:00
p777 M838 r831 RFN:90-12-0
Lib:NBS 6111 Bpk: 142 Mwt: 142



LIBFITS2#1* x1 Bgd=760 T2107202
PHENANTHRENE
C14.H10.

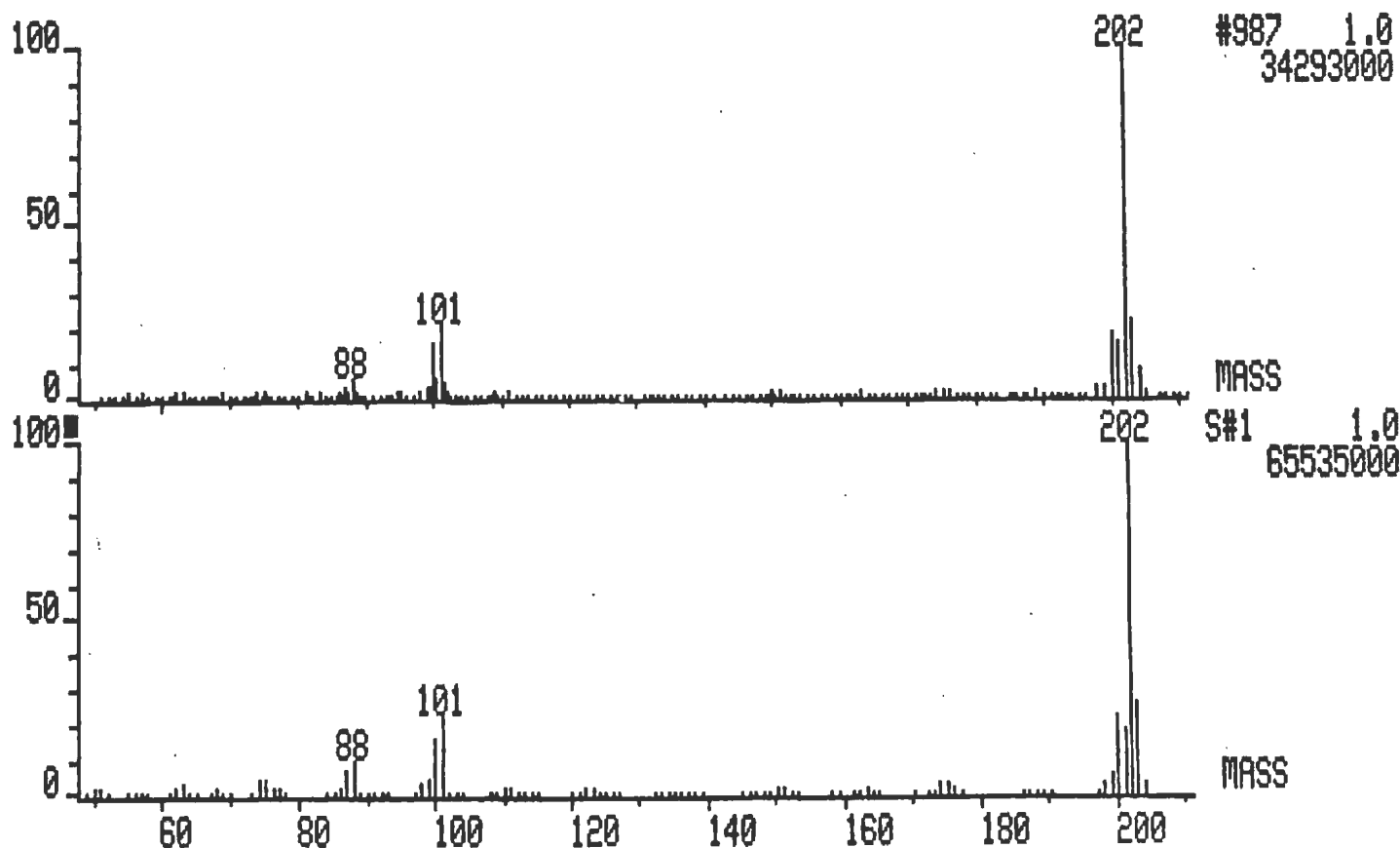
+0:00:00
p903 M974 r905 RFN:85-01-8
Lib:NBS 12787 Bpk: 178 Mwt: 178



LIBFITS2#1* x1 Bgd=987 T2107202
PYRENE
C16.H10.

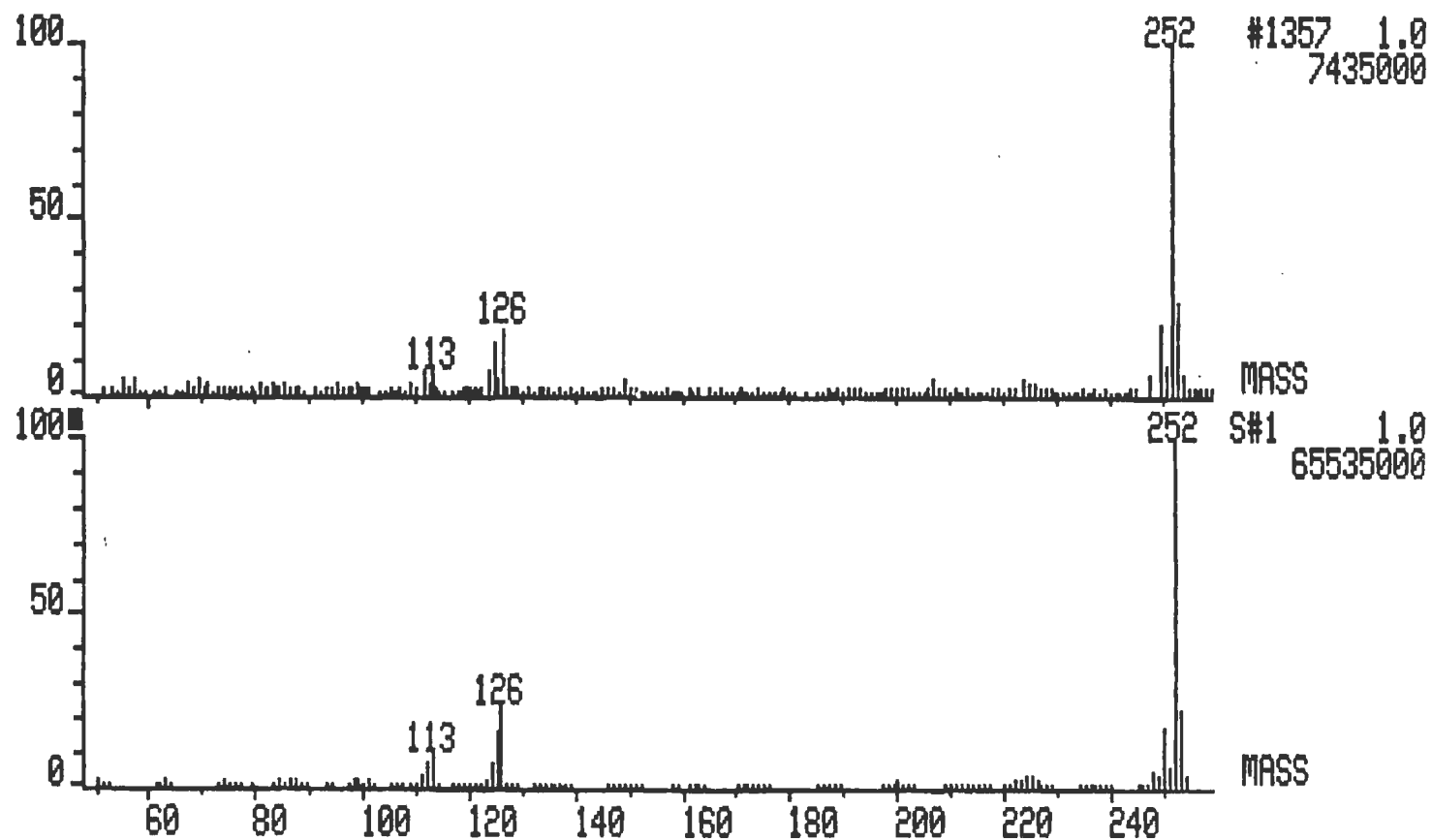
+0:00:00

p843 M964 r848 RFN:129-00-0
Lib:NBS 16899 Bpk: 202 Mwt: 202



LIBFITS2#1* x1 Bgd=1357 T2107202
BENZO A PYRENE
C20.H12.

+0:00:00
p616 M843 r652 RFN:50-32-8
Lib:NBS 23976 Bpk: 252 Mwt: 252



T1507206 #1-937

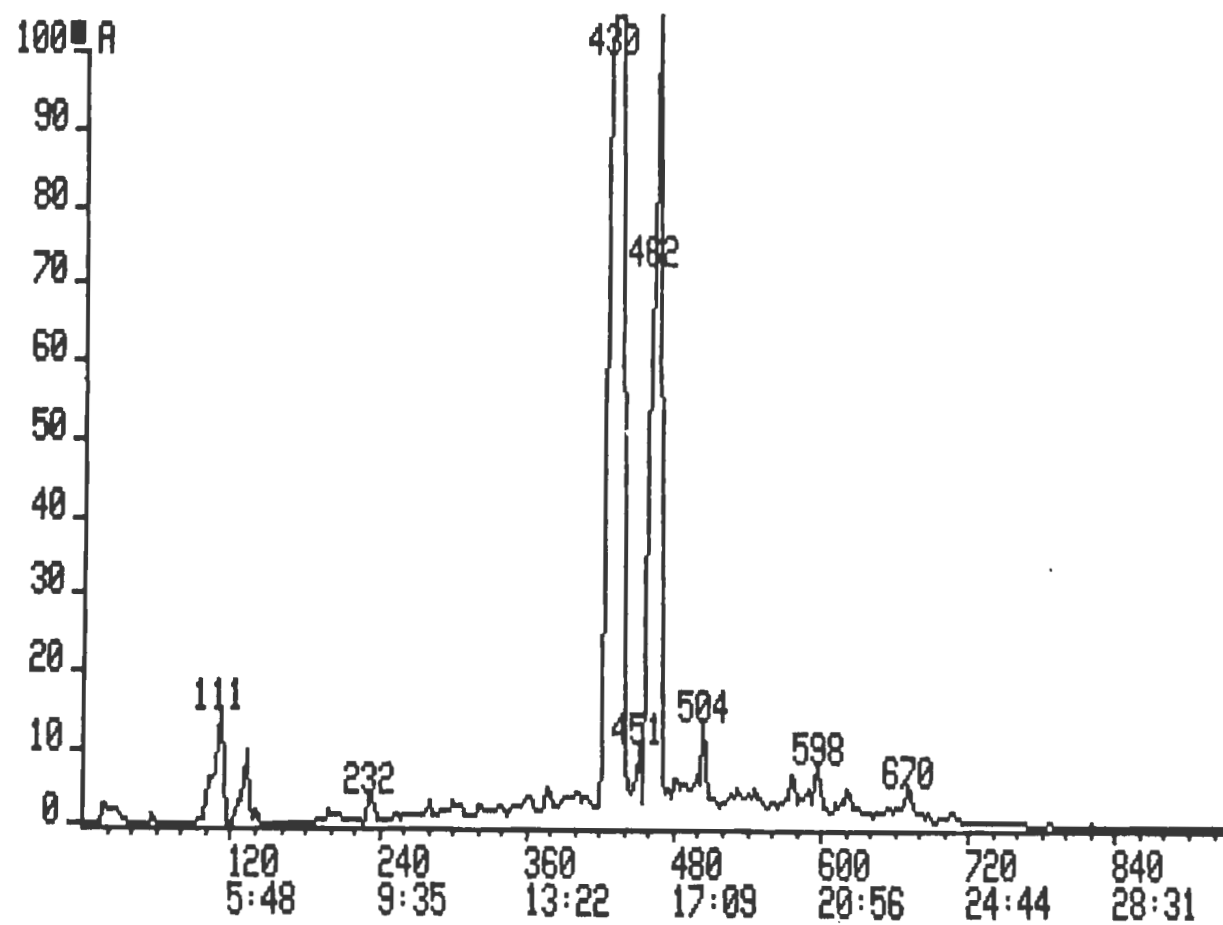
15-JUL-92 16:18 TS250

(EI+)

Sys:DEFAULTS

A:ATIC

Text:TS-3-2

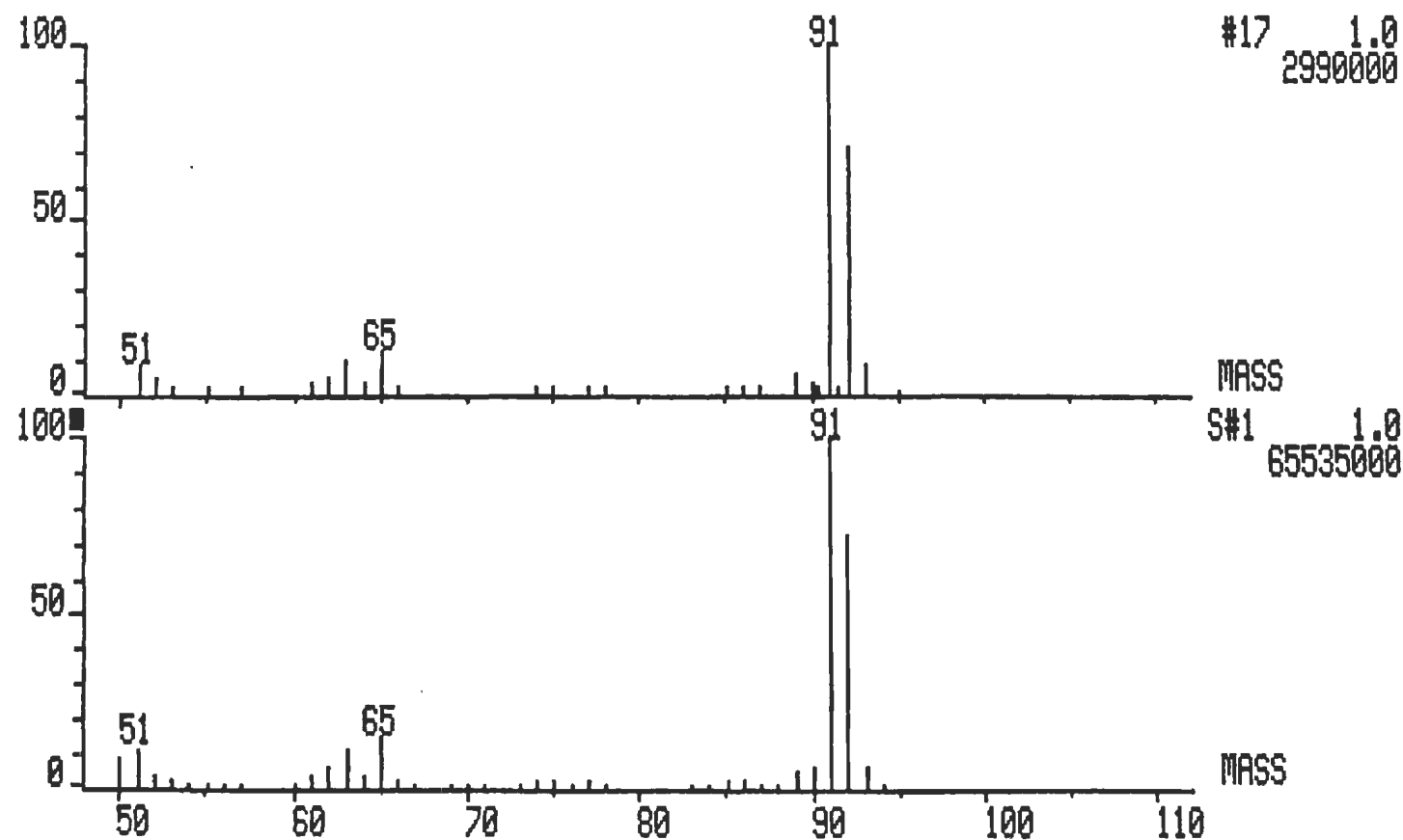


1.00
17282

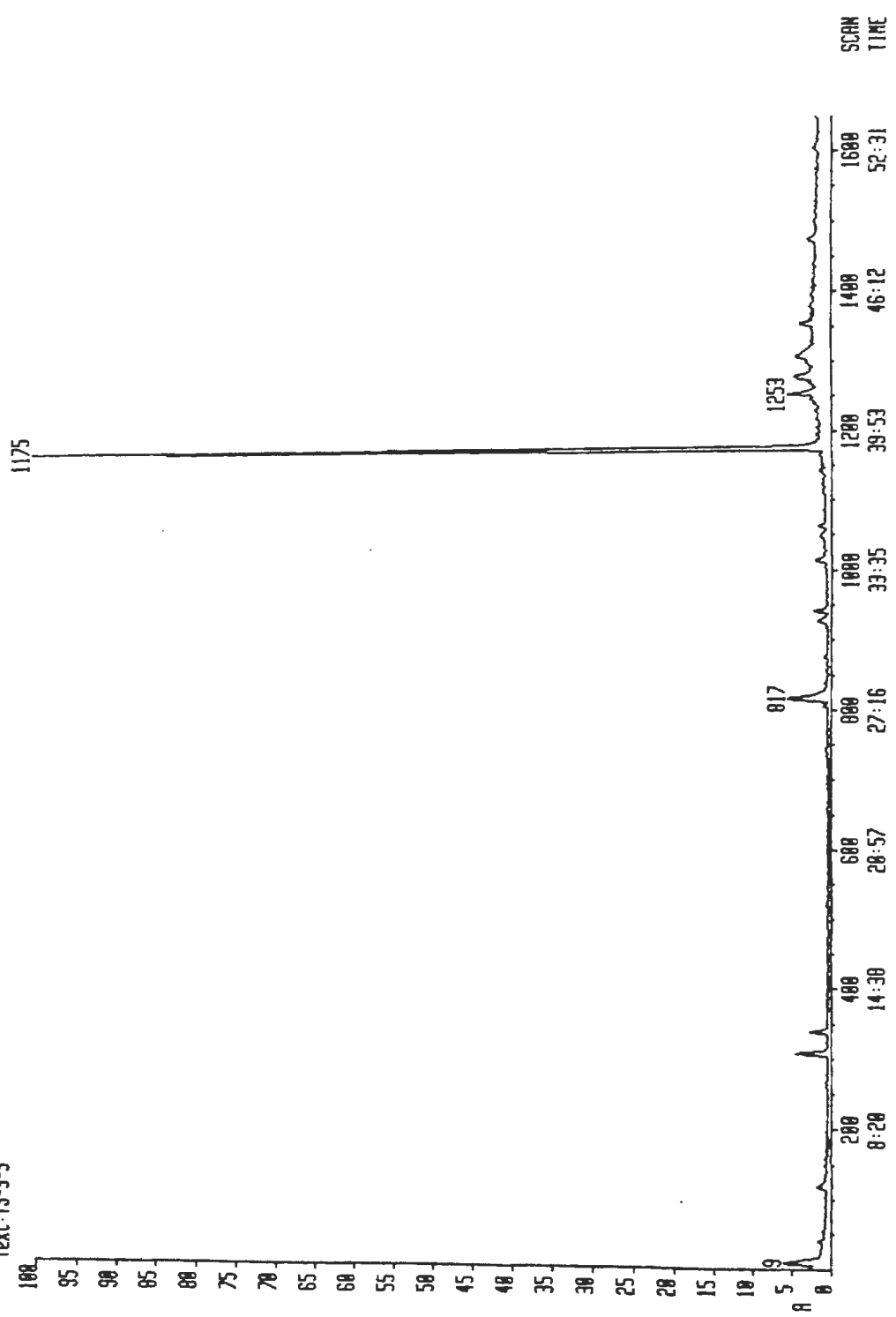
LIBFITS1#1* x1 Bgd=17 T1507206
BENZENE, METHYL-
C7.H8.

+0:00:00

p900 M902 r990 RFN:108-88-3
Lib:NBS 854 Bpk: 91 Mwt: 92



12887205 11-1648 28-JUL-92 14:34 TS250 (EI+) Sys:DEFAULTS IHP 8441
Chromatogram Identifiers : A :RTIC
Text:TS-3-3



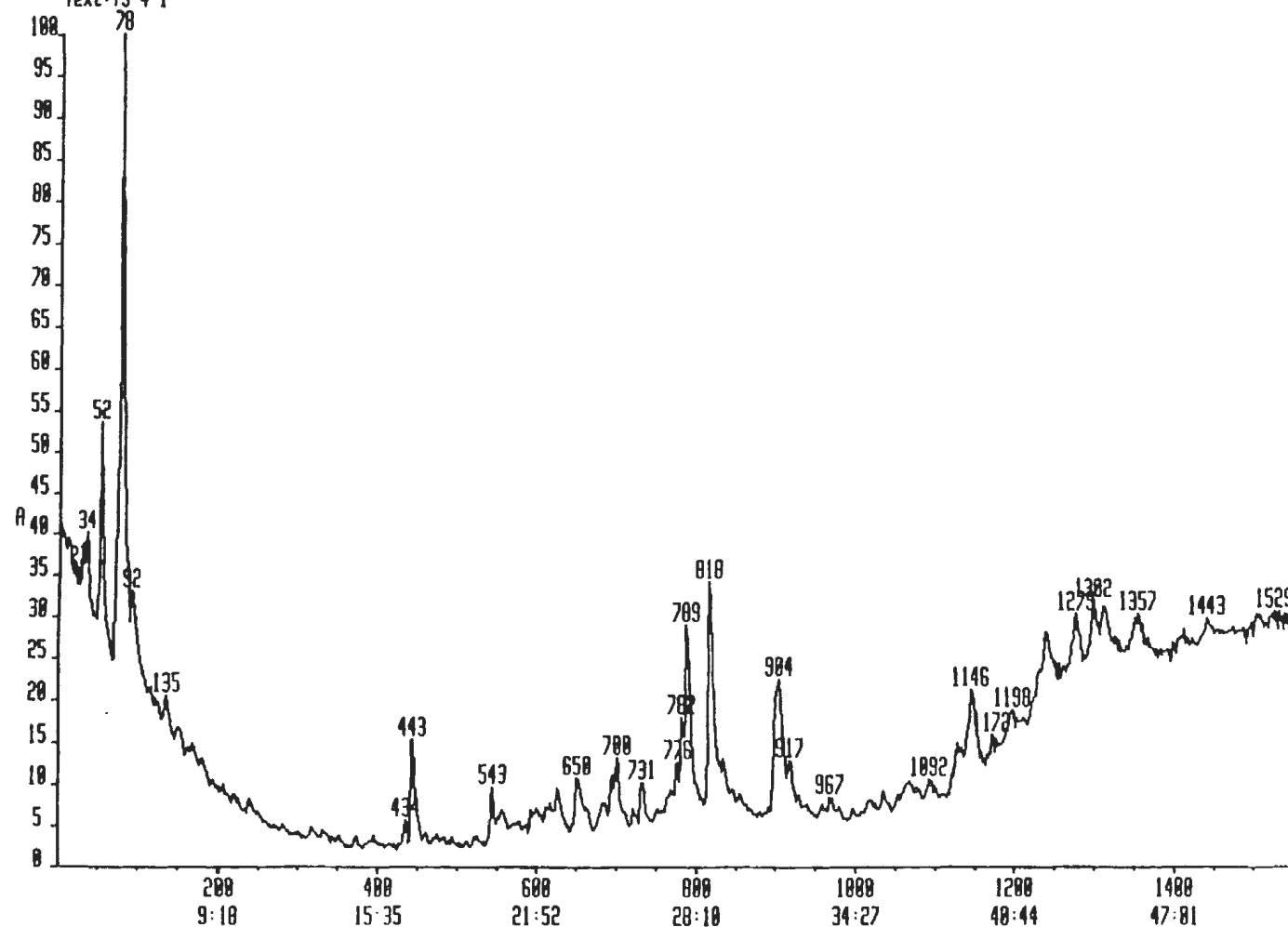
T1902303 01-1560 19-FEB-93 13:43 TS250
Chromatogram Identifiers : A : ATIC
Text:TS-4-1

(EI+)

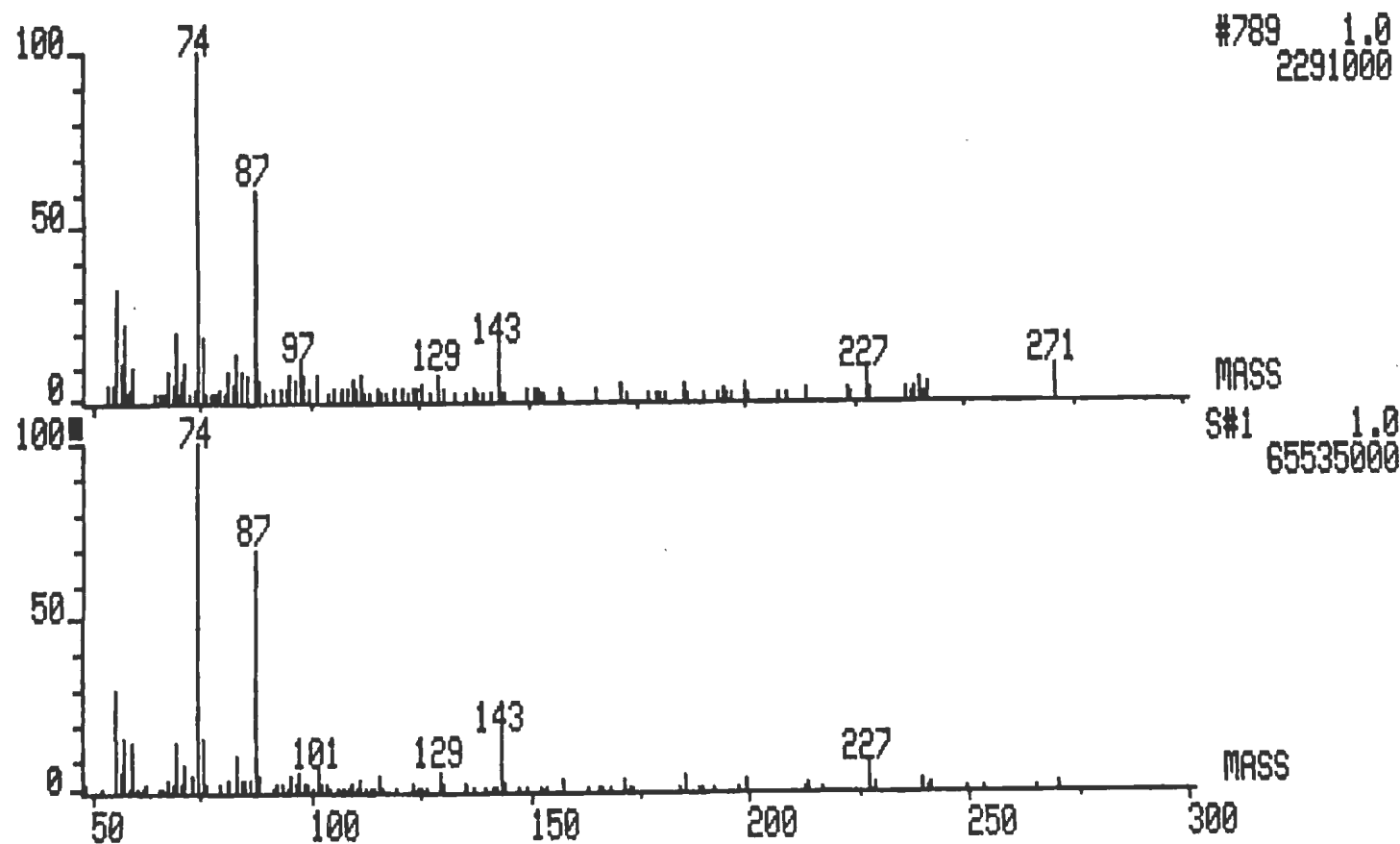
Sys:DEFAULTS

IHP

R: 3041



LIBFITS1#1* x1 Bgd=789 T1902303 +0:00:00
 9-OCTADECENOIC ACID, 12-(ACETOXY)-, METHYL ESTE p644 M794 r734 RFN:140-03-4
 C21.H38.O4. Lib:NBS 33679 Bpk: 74 Mwt: 354



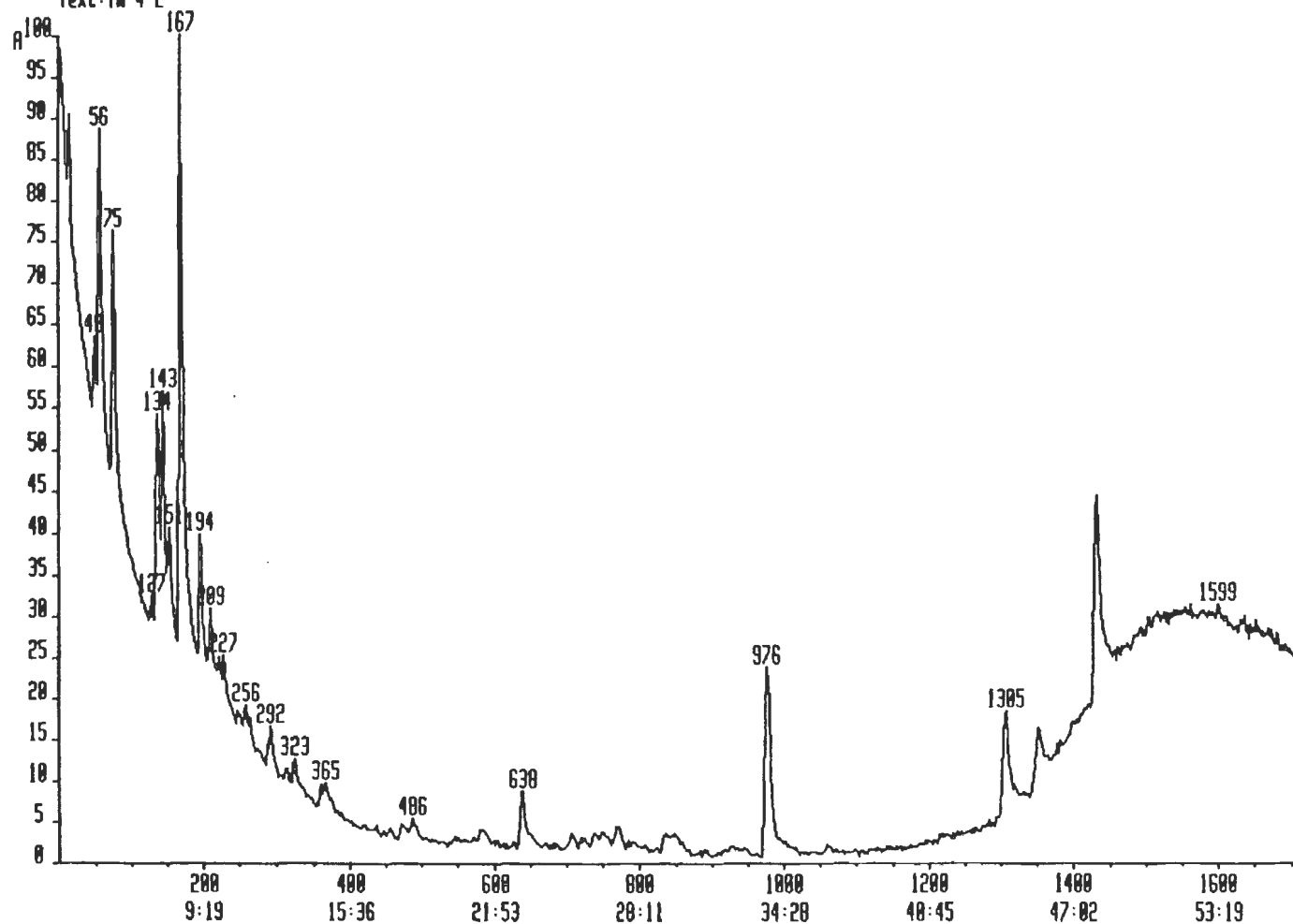
T1902301 #1-1710 19-FEB-93 11:37 TS250
Chromatogram Identifiers : A : ATIC
Text: TW-4-2

(E1+)

Sys: DEFAULTS

IHP

R: 2434

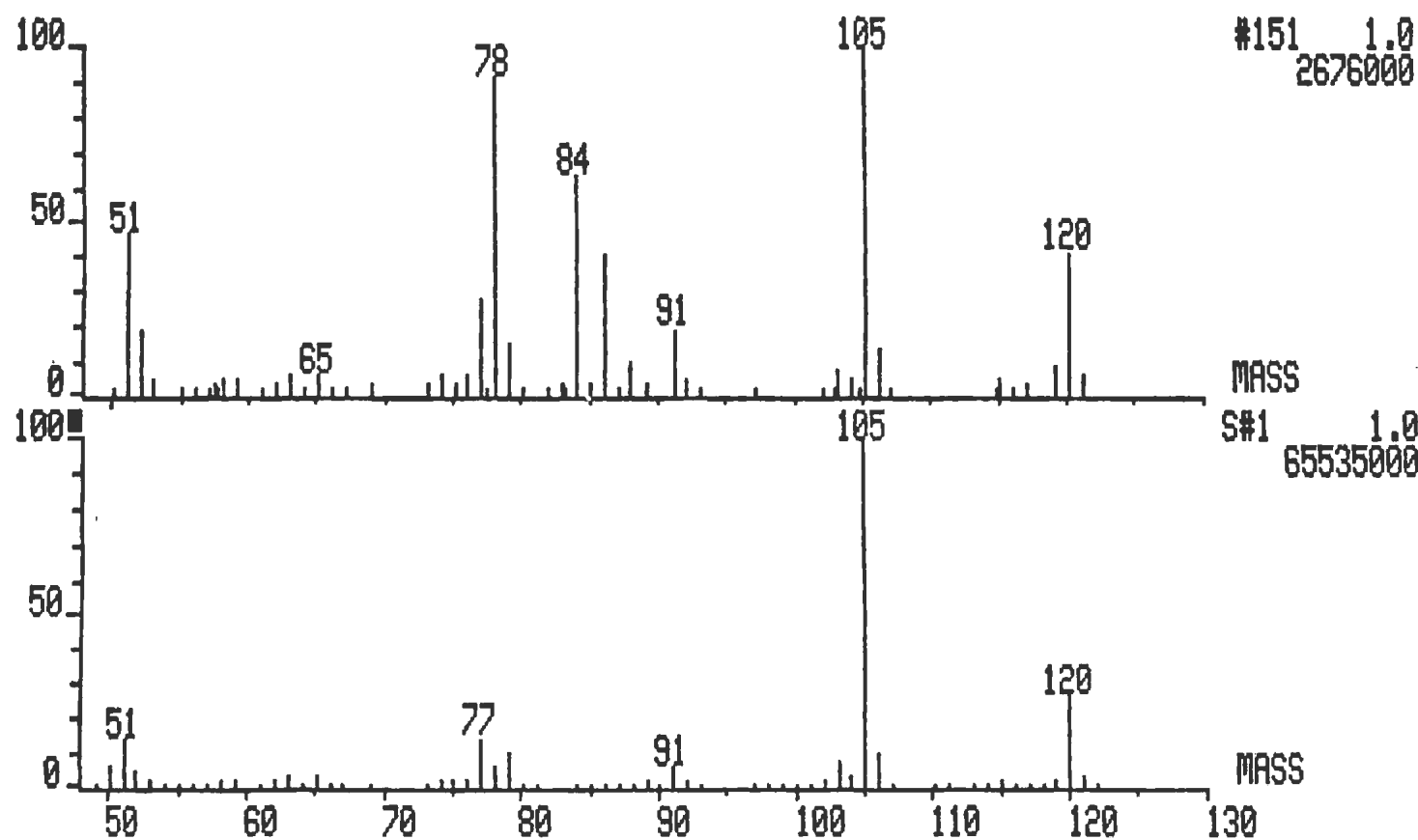


SCAN
TIME

LIBFITS1#1* x1 Bgd=151 T1902301
BENZENE, (1-METHYLETHYL)-
C9.H12.

+0:00:00

p673 M679 r704 RFN:98-82-8
Lib:NBS 3096 Bpk: 105 Mwt: 120



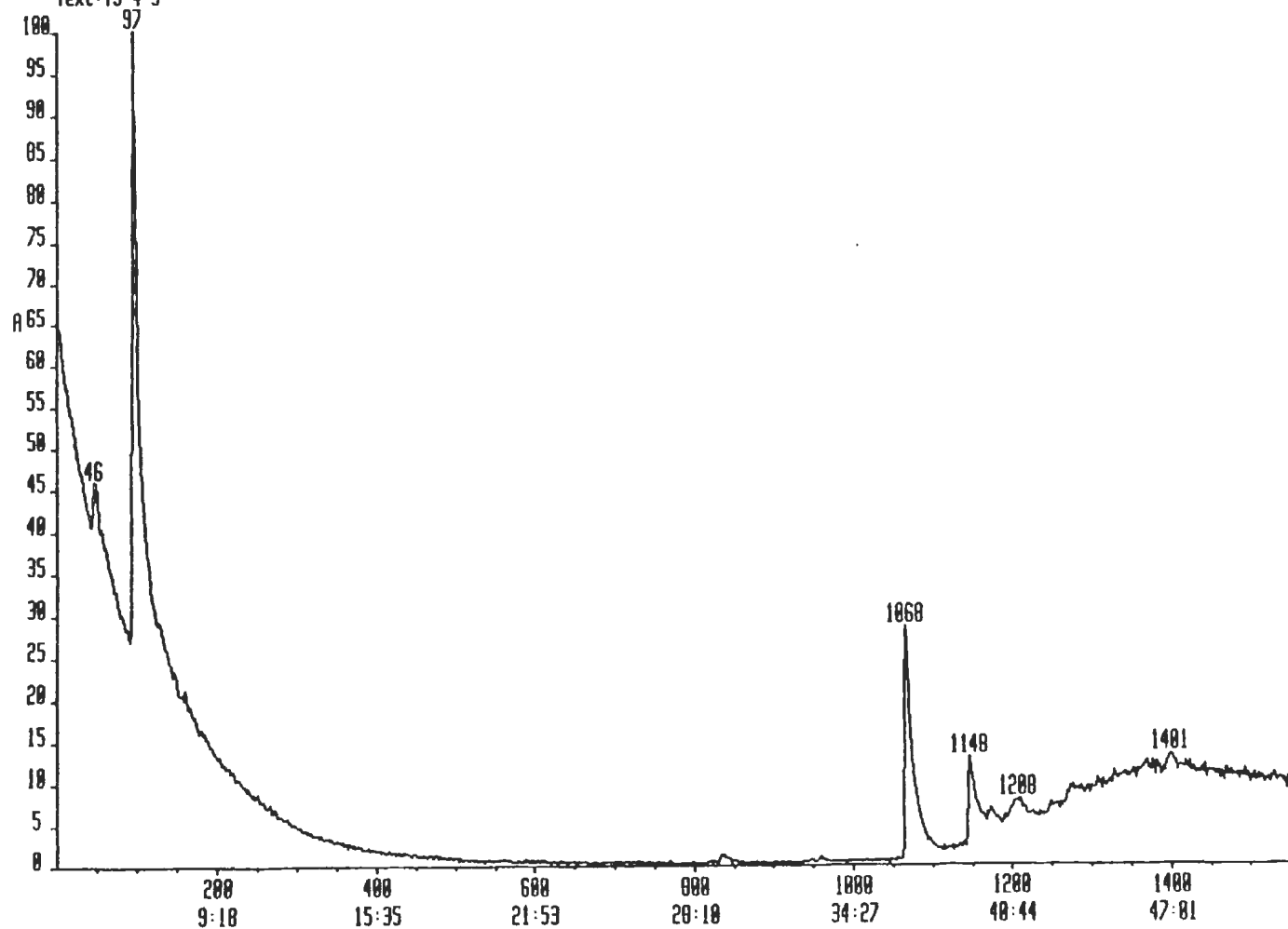
T1982382 01-1560 19-FEB-93 12:42 TS250
Chromatogram Identifiers : A : ATIC
Text: TS-4-3

(EI+)

Sys: DEFAULTS

IHP

R: 3195



SCAN
TIME

CHAPTER IV

ANTHROPOGENIC CONTAMINANTS AND FISH
HEALTH NEAR THREE OIL REFINERIES

Introduction

Oil refineries have long been studied for their effects on biological communities (e.g. Wilhm and Dorris, 1968; Snow and Rosenberg, 1975; Lock et al., 1981). Hundreds of different compounds can be identified in the wastewaters of a typical oil refinery (U. S. Dept. of Energy (DOE) and U. S. Environmental Protection Agency (EPA), 1978). Many of these compounds are considered carcinogenic not only to humans, but also to other mammals and lower vertebrates.

Chemical compounds such as polycyclic aromatic hydrocarbons (PAHs) and chlorinated hydrocarbons have been associated with many types of lesions and morphologic abnormalities in aquatic organisms. These classes of compounds are found in the effluents of oil refineries. Recent studies have focused on a number of different biologically important factors to determine the effects that these chemicals are having on the aquatic community. One is to conduct laboratory exposures, or bioassays, of indicator organisms such as daphnia (Daphnia magna), fathead minnows

(Pimephales promelas), and medaka (Oryzias latipes) to water, sediments, and effluents from sites near the oil refineries. Another method is to look at the species diversity and other measurements of community dispersion, or ecological surveys, of fish and macrobenthos at impacted and reference sites near oil refineries.

Bioassays have been used for many years as a method of determining the effects of contaminants on aquatic organisms. Organisms such as algae, bacteria, protozoa, zooplankton, macroinvertebrates, and fish are the major groups used in bioassays (Wilhm, 1975). Generally, acute or chronic tests are performed. Acute tests evaluate the lethality to a group of organisms of the exposure to a specific contaminant. The tests range from 24 to 96 hours in length. Chronic tests measure lethal and non-lethal effects on the overall life cycle of an organism following a long term exposure. Chronic tests generally evaluate reproduction, feeding behavior, growth, mutagenicity, teratogenicity, enzyme activity, excretion, and morphological changes.

Ecological surveys have also been used for many years to evaluate effects of contaminant exposures on aquatic organisms. Forbes (1910) used historical data and data from subsequent sampling of the Illinois River in Chicago to evaluate the effects of the introduction of sewage effluent on the aquatic communities. Diversity indices have been

widely used to express differences in two communities of organisms. Measures of species diversity and community similarity have been controversial in the biological community.

Two measures of species diversity have come to be widely used by ecologists today. The Simpson's diversity index (1949) was one of the earliest indices that included both the total number of species present and the relative abundance of each species. The equation of:

Equation 1:

$$D = \frac{\sum_{i=1}^s n_i(n_i-1)}{n(n-1)}$$

where D is the diversity, s is the total number of species, n_i is the number of individuals of the i^{th} species, and n is the total number of individuals, defines Simpson's diversity index. This was described by Krebs (1972) as being the probability of randomly picking two organisms of different species.

Shannon's diversity index is another commonly used index. It is based on information theory and is centered on the concept of uncertainty. If there are very few species present, we can be fairly sure of which species a randomly sampled individual will be. The equation is:

Equation 2:
$$H' = \frac{(N \log N - \sum n_i \log n_i)}{N}$$

where H' is the diversity, N is the total number of individuals, and n_i is the number of individuals of the i^{th} species. The Shannon diversity is most appropriately used where one is acquiring random samples from a larger community (Brower and Zar, 1977). Since the random sample probably does not contain representatives from all of the species present, the index is somewhat biased but not so much as to affect the diversity index.

Community similarity indices are a method of quantifying how two separate communities relate to one another. Two indices are the Percent Similarity and Morisita's index (1959). The Percent Similarity is a sum of the lowest percentages of the total number of individuals that a species represents. For example a species which comprises 50% of one community and 22% of the other community would account for 22% of the total 100% similarity possible for two communities. This is based on total numbers of individuals and total numbers of species present.

Morisita's index is based on Simpson's diversity index. It is the probability that two individuals drawn from two communities will be from the same species. The formula is:

Equation 3:
$$I_M = \frac{2 \sum x_i y_i}{(\lambda_1 + \lambda_2) N_1 N_2}$$

where x_i is the abundance of the i^{th} species in community one and y_i is the abundance of the i^{th} species in community two, λ_1 is the Simpson diversity of community one and λ_2 is the Simpson diversity of community two, and the N 's are the total number of individuals from the respective communities. The value of the index ranges from 0 to around 1, 1 being the most similar.

The petroleum industry produces many compounds of the classes cited previously (DOE and EPA 1978). Such chemicals may be inadvertently introduced into the streams and lakes. In addition, many non-point sources can contribute contaminants to the water. For example, run-off from farm lands treated with pesticides and herbicides can be a source of contaminants (Neary et al., 1993). Today, many species of fish world-wide are known to exhibit neoplasms. The species with liver neoplasms are all bottom feeders (Harshbarger and Clark, 1990). The potentially carcinogenic hydrocarbons tend to collect in the sediments because of their chemical nature, very non-polar, non water-soluble, hydrophobic, and non-ionic. Polycyclic aromatic hydrocarbons generally are found at concentrations 1000-fold greater in sediments than in water (Malins and Ostrander, 1991). Oklahoma has a large number of oil and gas refineries. Therefore, the fish populations near these areas may be exposed to high concentrations of pollutants. Bottom dwelling families in the midwest include the

Cyprinidae, Catastomidae, and Ictaluridae. These fishes are possibly exposed to high concentrations of pollutants because they tend to spend much of their time in close proximity to the sediments.

Three sites in Oklahoma were chosen for this study. All three have or had operating oil refineries with streams receiving effluents. The primary objectives of this study were to: 1) perform a chemical profile of the water and sediment at three locations in the stream, one upstream of the refinery, one near the refinery effluent discharge, and one downstream; 2) complete a preliminary survey of fish populations present at the three sampling locations; 3) determine if there are differences in the fish populations; and 4) conduct an in-depth histological examination of bottom-dwelling species found at these locations.

Materials and Methods

Ardmore

Ardmore is approximately 180 miles south of Oklahoma City in the south central part of the state in Love county (Figure 3). Total Oil Refinery is located here with effluent drainage flowing into Sand Creek and eventually reaching the Washita River. The most recent reports show that the refinery is capable of processing about 62,000 barrels of crude oil per calendar day (Rock, 1991). Sampling was conducted downstream from the refinery

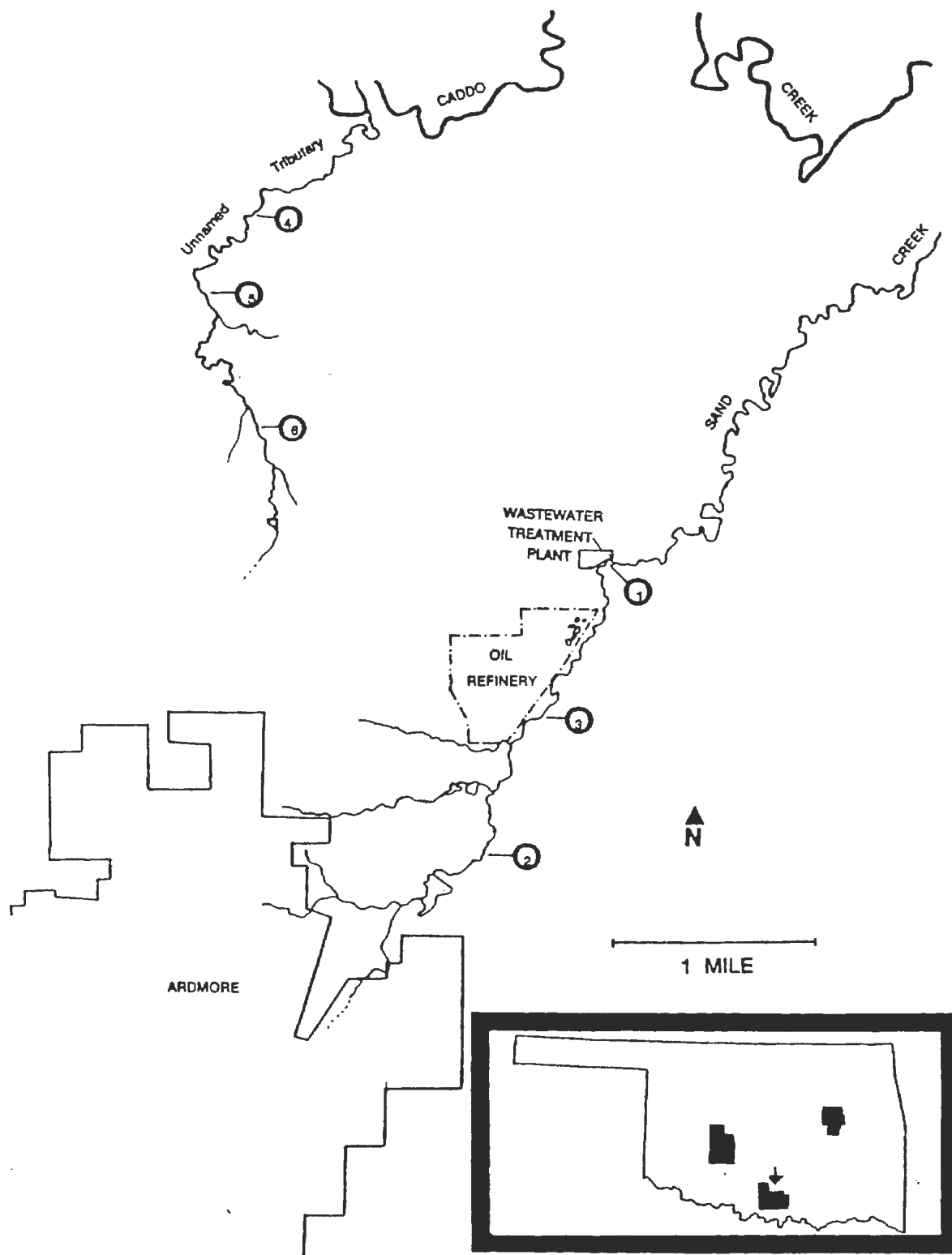


Figure 3: Location of Carter County and sampling stations on Sand Creek.

discharge (Station 1), upstream from the refinery discharge (Station 2), and at the point of discharge (Station 3) (Figure 3). Station 1 was located in SE $\frac{1}{4}$ SW $\frac{1}{4}$ NE $\frac{1}{4}$ S16 T4S R2E. This was approximately 0.5 miles north of the southern boundary of the refinery. Station 2 was in SW $\frac{1}{4}$ SW $\frac{1}{4}$ S21 T4S R2E. This was approximately 0.5 miles south of the southern boundary of the refinery. Station 3 was approximately 0.5 miles north of station two in NE $\frac{1}{4}$ NW $\frac{1}{4}$ S21 T4S R2E.

An alternate reference stream was also sampled to make comparisons to a stream of similar elevation and flow. The reference stream was an unnamed tributary to Caddo Creek approximately 1.5 miles west and 1.5 miles north of the refinery.

Cyril

Cyril, Oklahoma is located in Caddo county (Figure 4) in the southwestern part of the state approximately 90 miles southwest of Oklahoma City. This was the location of the Oklahoma Refining Corporation oil refinery until its close in the mid 1980's at which time they were capable of processing about 15,000 barrels of crude oil per calendar day (Kinney, 1983). Our focus at this site was Gladys Creek. It flows from the north, along the eastern boundary of an oil refinery and continues on to the south where it eventually reaches the Little Washita River. The sampling stations are shown on the map (Figure 4) as: Station 1,

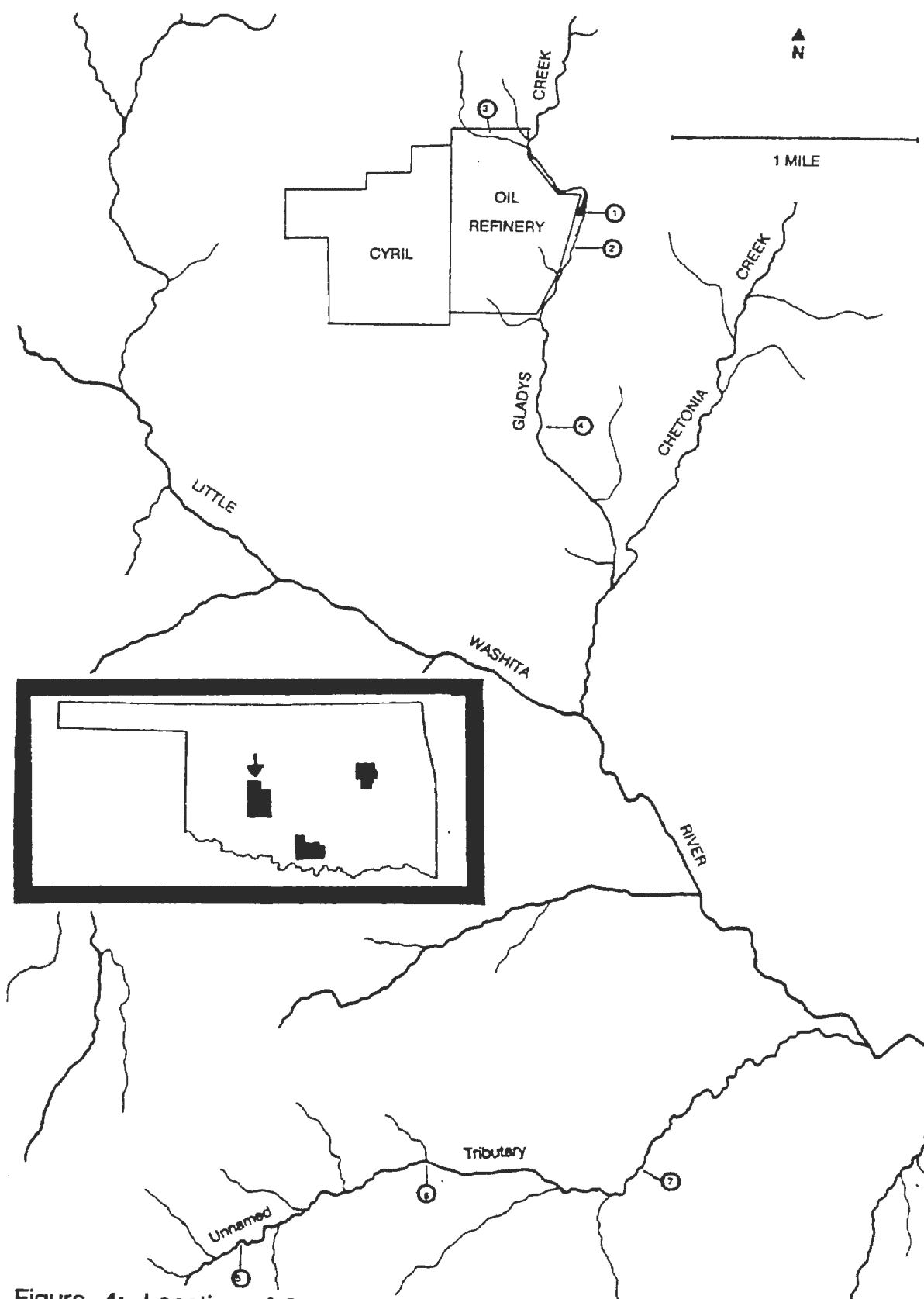


Figure 4: Location of Caddo County and sampling stations on Gladys Creek.

Station 2, Station 3, and Station 4. Station 1 was named Brown Pond and is formed by the damming of Gladys Creek and was located at NW $\frac{1}{4}$ NE $\frac{1}{4}$ S19 T5N R9W. Station 2 was 200 m below Brown Pond and had visible contaminated water leaching into the creek from the western banks. Station 3 was 800 m above Brown Pond at SW $\frac{1}{4}$ SW $\frac{1}{4}$ S18 T5N R9W and served as the reference station. Station 4 was 850 m below the southern oil refinery boundary at NE $\frac{1}{4}$ NW $\frac{1}{4}$ S30 T5N R9W and served as a downstream reference station.

The alternate reference stream for Gladys Creek was an unnamed tributary of the Little Washita River. It was approximately four miles south and three miles west of the refinery.

Okmulgee

Okmulgee is located in the east central part of the state in Okmulgee county (Figure 5). The Oklahoma Refining Company was operating an oil refinery here through the early 1980's. The refinery was capable of processing 24,000 barrels of crude oil per calendar day (Kinney, 1982). The creek of interest, Okmulgee Creek, flows from the north, along the eastern refinery boundary, through Okmulgee and on south to converge eventually with the Deep Fork of the Canadian River. Okmulgee Creek is known as Tar Creek by the people of Okmulgee because of the presence of oil in the creek apparently from spills. Sampling was performed at

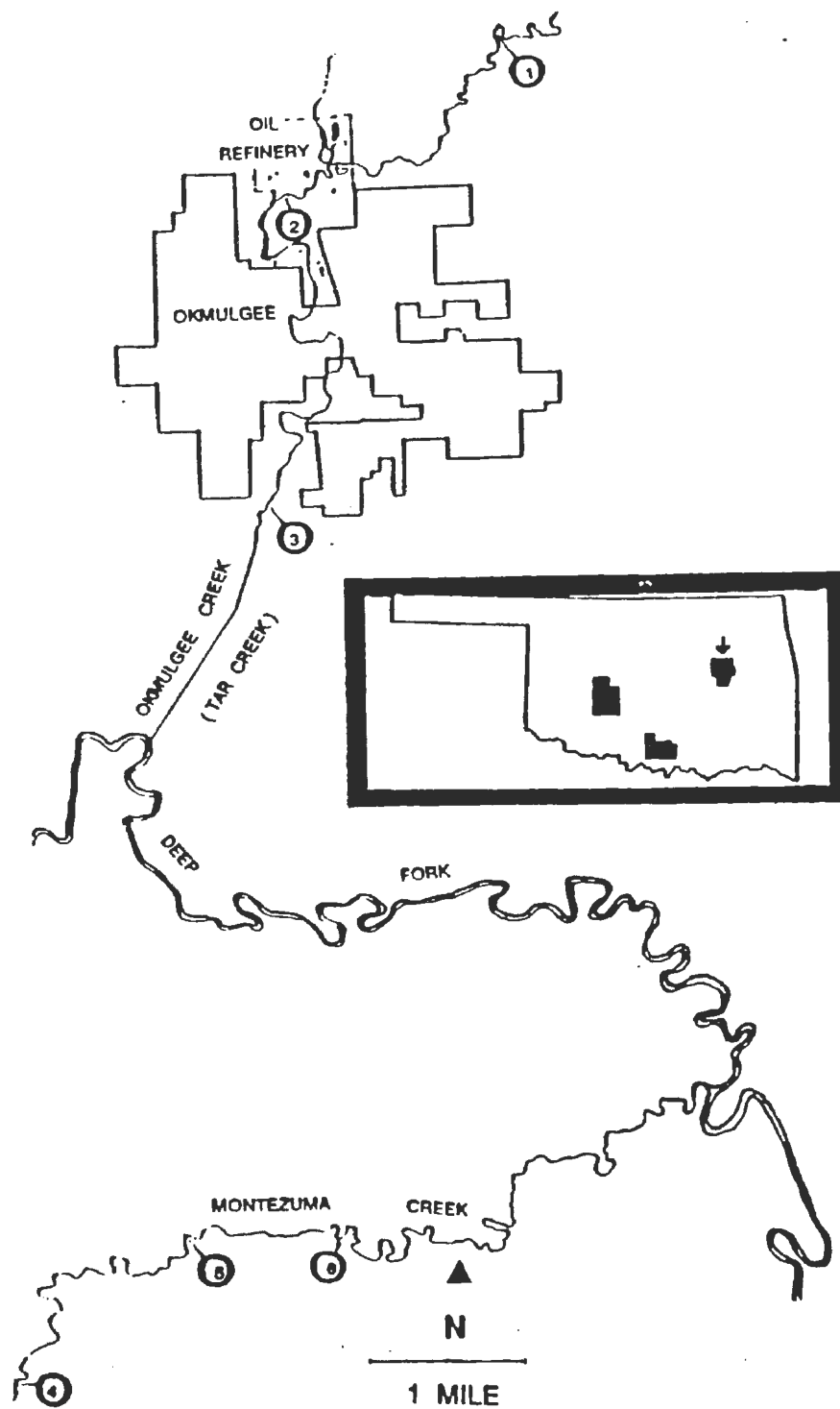


Figure 5: Location of Okmulgee County and sampling stations on Okmulgee Creek.

three stations (Figure 5): a reference station above the refinery site (Station 1) approximately one mile, NE $\frac{1}{4}$ NE $\frac{1}{4}$ S32 T14N R13E, at the state highway 52 bridge, on the refinery site at the point of discharge (Station 2) beneath the bridge crossing from the processing area to the tank farm, SE $\frac{1}{4}$ SE $\frac{1}{4}$ S31 T14N R13E, and below the site approximately one mile (Station 3), S $\frac{1}{2}$ SE $\frac{1}{4}$ S7 T13N R13E, below the 12th street bridge.

The alternate reference stream was Montezuma Creek. It was approximately four miles south and three miles west of the refinery (Figure 5).

Water and sediment sampling and analysis were consistent for all three sites and are described by the following methods. Fish sampling varied by site and is described by site following these methods. Samples were collected at the stations described above in the site descriptions.

Water Samples

Three samples of four liters each of water were taken. Water was collected in pre-cleaned, four-liter amber bottles. Samples were stored in the dark at 4°C until the time of extraction. Each four-liter sample was extracted on a Carbon-18 (C18) bonded solid phase extraction column (Bond Elut, Analytichem International, Product #607306). The columns were conditioned by passing two column volumes (12

ml) of methylene chloride through under a slight positive pressure, followed by two column volumes of reagent-grade water. The columns were not allowed to dry from this point on. The columns were then connected to a two-liter separatory funnel, and 4 liters of water were passed through under a slight vacuum. The columns were air dried under vacuum for 15 minutes to remove residual water prior to elution. Compounds were eluted with 40 ml of methylene chloride. This eluate was then passed through a 5 g column of sodium sulfate to remove excess water and rinsed twice with 2 ml of methylene chloride. The eluate was then concentrated by rotary evaporation in a 60°C water bath to 15 ml, transferred to a 15 ml concentrator tube, and finally concentrated to 1 ml in a 60°C water bath with nitrogen purge. The sample was then analyzed by gas chromatography/mass spectrometry. A VG Analytical TS-250 mass spectrometer connected to a Hewlett-Packard 5890A gas chromatograph was used for the analysis. A 30-meter, 0.32-mm inner diameter capillary column with an SE-54 bonded phase was used in the chromatograph. One to three microliters, depending on concentration, was injected and the sample was subjected to a programmed temperature gradient which raised the temperature 10°/minute from 50° to 280° C. The spectra of each peak was compared to reference spectra contained in the NBS on-line library (Milne and Heller, 1978; Heller and Milne, 1980).

Sediment Samples

Sediment samples were collected at the same locations described above. Three replicate samples of one liter each were taken in pre-cleaned amber bottles with teflon lid liners (Scientific Specialties Service, Product #B71132). The samples were stored at -40°C until extraction. In pilot studies, two separate methods of sediment extraction were employed. One was used by Malins and co-workers in studying Puget Sound sediments (1980) and the other by Fabacher and co-workers in studying Black River sediments (1988). These methods were compared for their ability to resolve compounds and the better of the two, that which provided the best separation of our compounds (Fabacher et al., 1988), by our lab, was used, with slight modifications described below, for the remainder of the study.

Dry Sediment Extraction with Fractionation on Neutral Alumina

The sediments were thawed and air dried under a hood for 24 hours prior to extraction. A 100 g aliquot of sediment was then powdered in a blender (Hamilton Beach, model #585-3). The sediment was extracted twice with 100 ml of a benzene:methanol (60:40) and twice with 100 ml of methylene chloride. At each step the slurry was shaken for two hours at 400 rpm on an orbital shaker. The sediment was allowed to settle out for 20 minutes, and the solvent was

then decanted off the top into a 500 ml solvent rinsed bottle. The extracts were concentrated with a rotary evaporator in a 60°C water bath to about 10 ml. This solution was transferred to a 15 ml concentrator tube with two, two ml rinses of methylene chloride. Finally, the sample was concentrated at 60°C under a continuous nitrogen stream to one ml. The sample was then fractionated on an alumina column.

An 11 x 250 mm glass chromatography column with a 200 ml solvent reservoir was fitted with a glass wool plug and filled with 9 g of neutral alumina activated at 200°C for 12 hours. Enough N-hexane was added to cover the alumina and the solution was shaken to remove any air bubbles. A small layer of sand was placed on top of the alumina to prevent disturbance when adding sample or solvent. Samples were applied and eluted with 400 ml of N-hexane. This first fraction contained mostly aliphatic hydrocarbons. The column was then eluted with 1000 ml of benzene. This fraction contained mostly aromatic hydrocarbons. Finally, the column was eluted with 1550 ml of chloroform and this final fraction contained mostly the nitrogen containing aromatic hydrocarbons. All three fractions were reduced by rotary evaporation to about 15 ml and resultant solutions were concentrated on a 60°C water bath under a nitrogen stream to one ml. The samples were then analyzed by gas chromatography/mass spectrometry. A VG Analytical TS-250

mass spectrometer was used with the same instrument conditions as described earlier for the water analysis. One to three microliters, depending on concentration, was injected and the sample was subjected to a programmed temperature gradient (10° /minute from 50° to 280° C). The spectra of each peak was compared to reference spectra, contained in the NBS on-line library (Milne and Heller, 1978; Heller and Milne, 1980) for compound identification.

Sampling of Fish populations, Ardmore

Each station was sampled for three consecutive transects of 75 meters each for a total of 225 meters. The areas of the creek that were over two meters wide were sampled with a 20' X 5' X 1/4" seine. Areas that were narrower and shallow were sampled with dip nets. The entire width of the creek was covered by passing the dip nets from side to side as the substrate was kicked to flush out the fish. Fish were immediately transferred to jars containing 10% neutral buffered formalin and later examined and identified in the lab. The communities were compared using the Shannon diversity index and the Percent Similarity and Morisita's indices that were described in the introduction.

Sampling of Fish Populations, Cyril

Fish were sampled in Gladys Creek at the stations described above. Station 1 was sampled first by a 50 foot

beach bag seine and secondly by four, two-hour sets of a 300' X 6' X 2" gill net. The gill nets were used to sample the bullhead population. The remaining stations were sampled with dip nets and seines as described in the Ardmore methods. Fish were counted and examined and any suspicious lesions were treated as described below. Bullhead from Station 1 were weighed and measured and the livers were removed, examined, and processed for histopathological examination by sectioning into five millimeter thicknesses and preserving overnight in 10% neutral buffered formalin followed by overnight rinsing in gently running tapwater. Sections were then embedded in paraffin and stained with hematoxylin and eosin and one fullface section of 5 μ M was microscopically examined. The Gulf Coast Research Lab in Ocean Springs, Mississippi, under the direction of Dr. William Hawkins, performed evaluation of liver samples. The communities were compared using the Shannon diversity index and the Percent Similarity and Morisita's indices.

Sampling of Fish Populations, Okmulgee

Fish were sampled by electroshocking and seining at the stations described earlier. A stream section of 225 meters was sampled at each station. A Smith-Root pulsed D.C. shocking unit was used. The unit was set-up for stream-side shocking and was placed in a small, four foot by three foot, boat and was floated behind the sampling crew. Fish were

identified, counted, and examined. Bullheads were weighed and measured. Livers from bullheads were removed and processed as described in the Cyril section above. The communities were compared using the Shannon diversity index and the Percent Similarity and Morisita's indices.

Results

Water and Sediment Sampling Results, Ardmore

A total of 13 compounds were identified in the water and sediment samples (Table VI). The Station 1 water sample had one compound: benzyl butly phthalate. The Station 1 sediment sample had seven compounds.

Decamethylcyclopentasiloxane is suspected to be an artifact arising from silicone stopcock grease used on the glassware. The Station 2 water sample was free of compounds. The sediment sample contained three compounds: 1,3-dimethylbenzene, 2,3,4-trimethyl-1,4-pentadiene, and 2,3,3-1,4-pentadiene.

The Station 3 water sample contained three compounds: benzyl butyl phthalate, 2,1,1-(1,1-dimethylethyl)propanoic acid, and 2,2-dimethyl-1,2-diphenylethanone. The sediment sample contained four compounds: 1,3-dimethylbenzene, (1-methylethyl)benzene, 2,3,4-trimethyl-1,4-pentadiene, and decamethylpentasiloxane. The decamethylcyclopentasiloxane is suspected to be an artifact from the silicone stopcock grease used on the glassware.

Appendix H contains a list of all compound spectra and their ratings of comparison to the library matches. For our study only those compounds with excellent or good library matches were considered positively identified and included in the following table.

TABLE VI
ARDMORE WATER(W) AND SEDIMENT(S) ANALYSIS

COMPOUND	STATION		
	1(D)	2(U)	3(I)
Benzyl butyl phthalate	W		W
2,6,10,14-Tetramethylhexadecane	S		
1,3-Dimethylbenzene	S	S	S
Propylbenzene	S		
(1-Methylethyl)benzene	S		S
1-Ethyl-3-methylbenzene	S		
1,4-Diethylbenzene	S		
2-Ethyl-1,4-dimethylbenzene	S		
2,3,4-Trimethyl-1,4-pentadiene		S	S
2,3,3-Trimethyl-1,4-pentadiene		S	
2-1,1-(1,1-Dimethylethyl)propanoic acid			W
2,2-Dimethyl-1,2-diphenylethanone			W
Decamethylpentasiloxane			S

(U = upstream, I = impacted, D = downstream)

Fish Sampling Results, Ardmore

A total of 365 fish were collected comprising six taxa (Table VII). The species diversity (Shannon Index) was highest for Station 3 (Table VIII), the impacted station, with an H' of 1.525, an H_{max}' of 2.584, and an evenness (H'/H_{max}') of 0.59. The highest density (number of fish per unit area) was at Station 1, the downstream station, with 211 individuals. Based on community similarity indices, Stations 1 and 2 and Stations 1 and 3 were the most similar with Percent of Similarities of 83.962% and 81.531% respectively and Morisita's Indices of 0.979 and 0.966 respectively. Similarity indices also indicated that Stations 2 and 3 were less similar with a Percent Similarity of 65.493% and a Morisita's Index of 0.894.

The diversities and similarities were not comparable to the alternate stream. On the alternate stream, the upstream station had the highest diversity ($H'=0.917$). The impacted station on Sand Creek was most similar to the downstream station on the alternate stream. Frequencies were not large enough to show any significant differences.

TABLE VII

FISH SPECIES AND NUMBERS

Ardmore

Species	Station					
	1(D)	2(U)	3(I)	4(R)	5(R)	6(R)
Gambusia (<u>Gambusia affinis</u>)	178	9	93	0	0	0
Bluegill (<u>Lepomis macrochirus</u>)	15	0	24	1	0	6
Red Shiner (<u>Notropis lutrensis</u>)	17	0	14	0	0	8
Green Sunfish (<u>Lepomis cyanellus</u>)	0	0	3	1	0	0
Largemouth Bass (<u>Micropterus salmoides</u>)	0	0	2	0	0	0
Central Stoneroller (<u>Campostoma anomalum</u>)	2	0	6	0	0	2
Longear Sunfish (<u>Lepomis megalotus</u>)	0	0	0	0	0	1

(U = upstream, I = impacted, D = downstream, R = Reference)

TABLE VIII

DIVERSITY INDICES FOR ARDMORE

	1(D)	Station 2(U)	3(I)
Total number of taxa	4	1	6
Total number of individuals	212	29	142
Shannon Diversity (H')	0.821	0	1.525
H_{max}'	2.000	0	2.584
Evenness	0.4105	0	0.590

(D = Downstream, U = Upstream, I = Impacted)

COMMUNITY SIMILARITY INDICES FOR ARDMORE

	1 & 2	Station 2 & 3	1 & 3
Number of taxa present at both stations	1	6	6
Percent Similarity	83.962	65.493	81.531
Morisita's Index	0.979	0.894	0.966

Water and Sediment Sampling Results, Cyril

Water and sediment samples contained 24 compounds (Table IX). Water from Station 1 revealed no compounds. Station 1 sediment contained five compounds: 5,6,7,7a-tetrahydro-4,7,7a-trimethyl-(S)-2(4H)-benzofurenone, 1,3-dimethylbenzene, 4-hydroxybenzenesulfonic acid, methylbenzene, and ethylbenzene.

Station 2 water contained six compounds as was expected due to visible contamination leaching in to the water from the creek banks. Station 2 sediment contained 12 compounds.

Water from Station 3 contained no compounds above detection limits. Sediment contained two compounds: ethylbenzene, and 2,6,10-trimethyldodecane.

Water from Station 4 contained no compounds above detection limits. Station 4 sediment contained one compound: α,β -dimethylbenzeneethanol.

Water samples taken from all stations for BTEX analysis revealed that none of these compounds were present above detection limits ($<0.005\mu\text{g/l}$) at any station.

TABLE IX

CYRIL WATER(W) AND SEDIMENT(S) ANALYSIS

COMPOUND	STATION			
	1(P)	2(I)	3(U)	4(D)
5,6,7,7a-Tetrahydro-4,7,7a-trimethyl-(S)-2(4H)-benzofuranone	S			
1,3-Dimethylbenzene	S			
4-Hydroxybenzenesulfonic acid	S			
Methylbenzene	S			
Ethylbenzene	S		S	
2,4-Dimethyl-2,3-heptadien-5-yne		W		
1-Methyl-2-(1-methylethyl)benzene		W		
1,2,3,4-Tetramethylbenzene		W,S		
1,2,3,5-Tetramethylbenzene		W		
1-Methylnaphthalene		W		
1,5-Dimethylnaphthalene		W,S		
2-Ethyl-1,4-dimethylbenzene		S		
1-Ethyl-3,5-dimethylbenzene		S		
Diethylmethylbenzene		S		
Ethyl-1,2,4-trimethylbenzene		S		
2,4-Dimethyl-1-(1-methylpropyl)-benzene		S		
Pentamethylbenzene		S		
1-Methylnaphthalene				

TABLE IX Continued

COMPOUND	STATION			
	1(P)	2(I)	3(U)	4(D)
1,2,3,4-Tetrahydro-1,5,8-trimethylnaphthalene		S		
1-Ethylnaphthalene		S		
1,6-Dimethylnaphthalene		S		
1-Bromo-4-(2-phenylethyl)benzene		S		
2,6,10-Trimethyldodecane			S	
α,β -Dimethylbenzeneethanol				S

(P = Pond, I = impacted, U = Upstream, D = downstream)

Fish Sampling Results, Cyril

A total of 134 fish were sampled comprising six taxa (Table X). Several species of fish were observed at Station 1, Brown Pond. Due to the extremely soft bottom, brush, and extremely high conductivity, a single seining was done to evaluate the general population and gill nets were set to sample the bullhead population.

Thirteen bullhead were taken from Station 1 and histopathology results are shown in Appendix C. Histologic analysis revealed that 23% of the bullheads had pigment deposits in the livers. This is comparable to percentages exhibited by fish from other contaminated sites. Also, 31% had parasitic cysts. This is higher than percentages seen at some reference sites. One bullhead had a small vacuolated focus resembling a clear-cell focus that is considered a pre-neoplastic lesion in rats. Since the bullhead taken at Station 2 were juveniles, the livers were not taken for histopathology.

Station 3, the upstream station, had the highest diversity with an H' of 1.585 and an H_{max}' of 1.585 (Table XI). Station 4 also had a high diversity with an H' of 1.362 and an H_{max}' of 1.585. The highest density was seen at Station 4, the downstream station. Community similarity indices indicated that Stations 2 and 4 and Stations 3 and 4 had fairly equal similarities with Percent Similarities of 46.341 and 43.902 respectively and Morisita's Indices of

0.703 and 0.512 respectively. Stations 2 and 3 had no similarity.

Sampling on the alternate stream provided very little information for community comparisons. The upstream station was the only station in which fish were caught on the alternate stream. It is unclear as to why almost no fish were caught in the alternate stream. The habitats and water flow were very similar.

TABLE X

FISH SPECIES AND NUMBERS

Cyril

Species	Station						
	1(P)	2(I)	3(U)	4(D)	5(R)	6(R)	7(R)
Gambusia (<u>Gambusia affinis</u>)	25	10	0	19	0	0	0
Bluegill (<u>Lepomis macrochirus</u>)	15	0	6	18	0	0	0
Bullhead (<u>Ameiurus melas</u>)	17	2	0	0	0	0	0
Green Sunfish (<u>Lepomis cyanellus</u>)	3	0	6	0	2	0	0
Largemouth Bass (<u>Micropterus salmoides</u>)	5	0	0	4	0	0	0
White Crappie (<u>Pomoxis annularis</u>)	4	0	0	0	0	0	0

(P = Pond, I = impacted, U = Upstream, D = downstream,
R = Reference)

TABLE XI

DIVERSITY INDICES FOR CYRIL

	Station		
	2(I)	3(U)	4(D)
Total number of taxa	2	2	3
Total number of individuals	12	12	41
Shannon Diversity (H')	1.0271	1.585	1.362
Hmax'	1.585	1.585	1.585
Evenness	0.6480	1.0	0.8593

(I = Impacted, U = Upstream, D = Downstream)

COMMUNITY SIMILARITY INDICES FOR CYRIL

	Station		
	2 & 3	3 & 4	2 & 4
Number of taxa present at both stations	0	1	1
Percent Similarity	0.00	43.902	46.431
Morisita's Index	0.00	0.512	0.703

Water and Sediment Sampling Results, Okmulgee

A total of 17 compounds were positively identified in the water and sediment samples (Table XII). Water analysis at Station 1 revealed no compounds above detection limits. Sediment samples from Station 1 contained 12 compounds.

No compounds were seen above detection limits in the water at Station 2. Station 2 sediment contained two compounds: 2,3,5-trimethylphenanthrene, and 1,1-dichloro-2,2-difluoroethane.

Station 3 water contained one compound: 3,5-dimethylcyclohexanol. Station 3 sediment contained three compounds: diethyl phthalate, pheanthrene, and 2,3,4-trimethyl-1,4-pentadiene.

TABLE XII

OKMULGEE WATER(W) AND SEDIMENT(S) ANALYSIS

COMPOUND	STATION		
	1(U)	2(I)	3(D)
<u>1,2,3,4-Tetramethylbenzene</u>	S		
<u>Naphthalene</u>	S		
<u>1-Ethylidene-1H-indene</u>	S		
<u>Diethyl phthalate</u>	S		S
<u>Benzyl butyl phthalate</u>	S		
<u>1,3-Dimethylbenzene</u>	S		
<u>Propylbenzene</u>	S		
<u>(1-Methylethyl)benzene</u>	S		
<u>1-Ethenyl-2-methylbenzene</u>	S		
<u>1,2-Diethylbenzene</u>	S		
<u>1-Ethyl-2,3-dimethylbenzene</u>	S		
<u>(1-Methylpropyl)benzene</u>	S		
<u>2,3,5-Trimethylphenanthrene</u>		S	
<u>1,1-Dichloro-2,2-difluoroethane</u>		S	
<u>3,5-Dimethylcyclohexanol</u>			W
<u>Phenanthrene</u>			S
<u>2,3,4-Trimethyl-1,4-pentadiene</u>			S

(U = Upstream, I = Impacted, D = Downstream)

Fish Sampling Results, Okmulgee

A total of 183 fish were captured in Okmulgee Creek comprising 13 taxa (Table XIII). Histological examination revealed that 75% of the bullheads at Station 1 had normal livers compared to 40% at Station 2. Also, 20% from Station 2 had parasitic cysts and none from Station 1. Station 2 also had a high percentage, 60%, of the bullheads that had reactive/degenerative foci compared to none at Station 1. Bullhead from Station 3 were juveniles and livers were not taken. Station 1 had the highest diversity with an H' of 3.3058 and an H_{max}' of 3.584 (Table XIV). Station 3 had the highest density. Similarity indices indicated that Stations 2 and 3 are the most similar with a Percent Similarity of 40.302 and a Morisita's Index of 0.444.

Results from sampling at Montezuma Creek showed that the creeks were not similar. Station 5, the midstream station on Montezuma Creek, had the highest diversity. On Okmulgee Creek, the highest diversity was seen at Station 1, the upstream station. Frequencies were too low to show any significant differences between the numbers of fish among the stations.

TABLE XIII

FISH SPECIES AND NUMBERS

Species	Station					
	1(U)	2(I)	3(D)	4(R)	5(R)	6(R)
Green Sunfish (<u>Lepomis cyanellus</u>)	0	16	2	0	0	0
Channel Catfish (<u>Ictalurus punctatus</u>)	0	0	1	1	0	0
Slough Darter (<u>Etheostoma gracile</u>)	0	0	2	0	0	0
Gambusia (<u>Gambusia affinis</u>)	0	0	1	5	0	0
Bullhead (<u>Ameiurus melas</u>)	8	5	3	0	0	0
Largemouth Bass (<u>Micropterus salmoides</u>)	6	2	0	0	0	0
Carp (<u>Cyprinus carpio</u>)	3	1	1	0	0	0
Bluegill (<u>Lepomis macrochirus</u>)	3	11	17	1	5	1
White Crappie (<u>Pomoxis annularis</u>)	2	1	1	0	0	0
Warmouth (<u>Chaenobryttus gulosus</u>)	0	23	7	0	0	0
Red Shiner (<u>Notropis lutrensis</u>)	0	3	21	1	1	0
Ghost Shiner (<u>Notropis buechanani</u>)	0	0	2	0	0	0

TABLE XIII Continued

<u>Species</u>	Station					
	1(U)	2(I)	3(D)	4(R)	5(R)	6(R)
Bullhead Minnow (<u>Pimephales vigilax</u>)	0	0	21	0	0	0
Gizzard Shad (<u>Dorosoma cepedianum</u>)	0	0	0	0	1	5

(U = Upstream, I = Impacted, D = Downstream, R = Reference)

TABLE XIV

DIVERSITY INDICES FOR OKMULGEE

	Station		
	1(U)	2(I)	3(D)
Total number of taxa	5	8	12
Total number of individuals	22	62	79
Shannon Diversity (H')	3.306	2.681	2.704
H_{max}'	3.584	3.584	3.584

(U = upstream, I = impacted, D = downstream)

COMMUNITY SIMILARITY

	Station		
	1 & 2	1 & 3	2 & 3
Number of taxa present at both stations	5	4	7
Percent Similarity	28.152	19.965	40.302
Morisita's Index	0.293	0.227	0.444

Discussion

Ardmore

There were differences in the number and classes of compounds found in the water and sediments at the impacted, upstream, and downstream stations. There were about three times as many anthropogenic compounds present at the impacted and downstream stations compared to the upstream station. In addition, non-point sources are probably contributing to the contaminant load in Sand Creek. Immediately upstream from Station 2 is the old city dump. There are possibly leachates from run-off entering the creek at that point.

There were many more fish caught at the impacted and downstream stations compared to the upstream station. Similarly, these two stations had higher species diversities than the upstream station. There was also more water at these locations. The refinery (Station 3) and the wastewater treatment plant (WWTP)(Station 1) are probably contributing fairly significant amounts of water to the stream. A report by the Oklahoma Water Resources Board (OWRB) (1989) and one by Stanley Engineering (1985) both showed that water flows increase below areas where effluents are being discharged. This could be providing a more varied environment for the fish and thus densities and diversities would rise. However, the diversity did go down between the

impacted station and the downstream station. The increased organic load from the WWTP could possibly be affecting the fish population at that point. Further sampling farther downstream would be needed to make a determination. Similar results have been reported near other wastewater treatment plants. The OWRB (1985) reported that the WWTP at Cushing, Oklahoma, was causing an impact on the fish population of Cottonwood Creek which receives the effluent. The species diversity was lowered below the WWTP and was also lower than the reference creek.

There was a severe infestation of parasites on the *Gambusia* caught at Station 3. The parasites were myxozoans and were found in high numbers on most of the *Gambusia* caught at Station 3. They were not found at the other two stations. The increased pollution at Station 3 was probably a contributing factor for the parasite infestation. Kuperman (1992) showed that parasites can be indicators of pollution.

Sampling on an alternate creek provided very little information. Very few fish were caught and statistical analysis could not be performed. It did show again, however, that the additional water being introduced by the refinery and WWTP was probably increasing the habitat availability to the fish.

Further sampling of the fish, water, and sediments is needed to make any definitive statements about the effects of the refinery on the fish population.

Cyril

There were five time as many compounds at the impacted station compared to the two upstream stations and 13 times as many as the downstream station. Station 2 (impacted) was the only station with compounds in the water.

Although fish numbers were low, the upstream and downstream stations had higher diversities than the impacted station. Only 46% of the bullhead had no visible lesions in the liver. This was lower than the 60% observed in the channel catfish population from Tulsa. Thirty-one percent had parasitic cysts. This was lower than the impacted stations at Tulsa but higher than the reference station. Since no bullheads were collected at the upstream or downstream stations, no statistical analysis was performed. One bullhead did have a lesion consistent with pre-neoplastic lesions in rats (Farber and Cameron, 1980).

Sampling of the alternate creek provided no information except that Gladys Creek contained better habitat for fish. It is possible that Gladys Creek is supplied by a spring and thus maintains its flow year round where the alternate creek may not.

Based on our results, there is an impact on the fish population. Further sampling of the bullhead population in Brown Pond would probably produce more lesions indicative of pre-neoplastic conditions.

Okmulgee

There were differences in the number and classes of compounds found in the water and sediments at the impacted, upstream, and downstream stations. There were six times as many compounds present at the upstream station compared to the other two stations.

Compounds such as the phthalate esters, and pyrenes are common to aquatic environments associated with this type of contamination sources. The phthalate esters are plasticizers in many plastics and are released into the environment as the plastics breakdown. The cyclic hydrocarbons are by-products formed in the refining of crude petroleum and during the incomplete combustion of fossil fuels. The fact that more compounds were identified upstream indicates that other sources beside the refinery are contributing to the pollution in the creek. Station 2, the station at the refinery, was far from clean. The sediment was tar-like and smelled like petroleum. The fact that only two compounds were positively identified there was due to the difficulty of separating complex chemical mixtures enough to obtain positive identification (see

chromatograms OKS-2-1 and OKS-2-2). These chromatograms represent a typical "hydrocarbon hump" typical of the complex mixtures associated with oil refineries. Appendix H contains a listing of all compounds tentatively identified. They were not positively identified, but it is reasonable to suspect that they are correctly identified and present. If all compounds listed had been positively identified, there would be as many compounds at Station 2 as at Station 1. Common compounds such as pyrenes, anthracenes, and chrysenes were tentatively identified at Station 2.

Diversity indices and histological examination show that Station 1, the upstream station, was in better shape biologically than Station 2. The diversity was highest and there was a higher percentage of fish with normal livers at Station 1. Station 2 had a lower diversity than both of the other stations. Also, 60% of the fish at Station 2 had livers with reactive/degenerative focus.

Based on our results, there are anthropogenic contaminants in Okmulgee Creek. There are compounds present that are in the classes of compounds that have been shown to cause morphological deformations and neoplastic lesions in fish. We also can say that there are differences in the community parameters of the fish. These may be caused by the contamination from the oil refinery or by other non-point sources along the creek.

Further sampling of the fish, water, and sediments is needed to make any definitive statements about the effects of the refinery on the fish population.

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APPENDICES

APPENDIX E

CHROMATOGRAMS AND MASS SPECTRA

Ardmore, Oklahoma

The following are chromatograms and mass spectra for water and sediment samples from Ardmore. The accession labels are as follows:

Accession Label	Page
ARDW-1 = Water from Station 1	163-164
ARDW-2 = Water from Station 2	165
ARDW-3 = Water from Station 3	166-168
ARDS-1-1 = Sediment from Station 1, fraction #1	169-170
ARDS-1-2 = Sediment From Station 1, fraction #2	171-175
ARDS-1-3 = Sediment from Station 1, fraction #3	176
ARDS-2-1 = Sediment from Station 2, fraction #1	177
ARDS-2-2 = Sediment from Station 2, fraction #2	178-179
ARDS-2-3 = Sediment from Station 2, fraction #3	180
ARDS-3-1 = Sediment from Station 3, fraction #1	181-182
ARDS-3-2 = Sediment from Station 3, fraction #2	183-186
ARDS-3-3 = Sediment from Station 3, fraction #3	187

The mass spectra are presented as two spectra. The top spectra is that of the sample with the peak number in the upper right corner. The bottom spectra is that of the library match corresponding to the sample. The name and molecular formula of the compound are given at the top. Also, the CAS registry number is listed, if available, at the top right following the label "RFN". In the interest of being concise, spectra were only included once per occurrence.

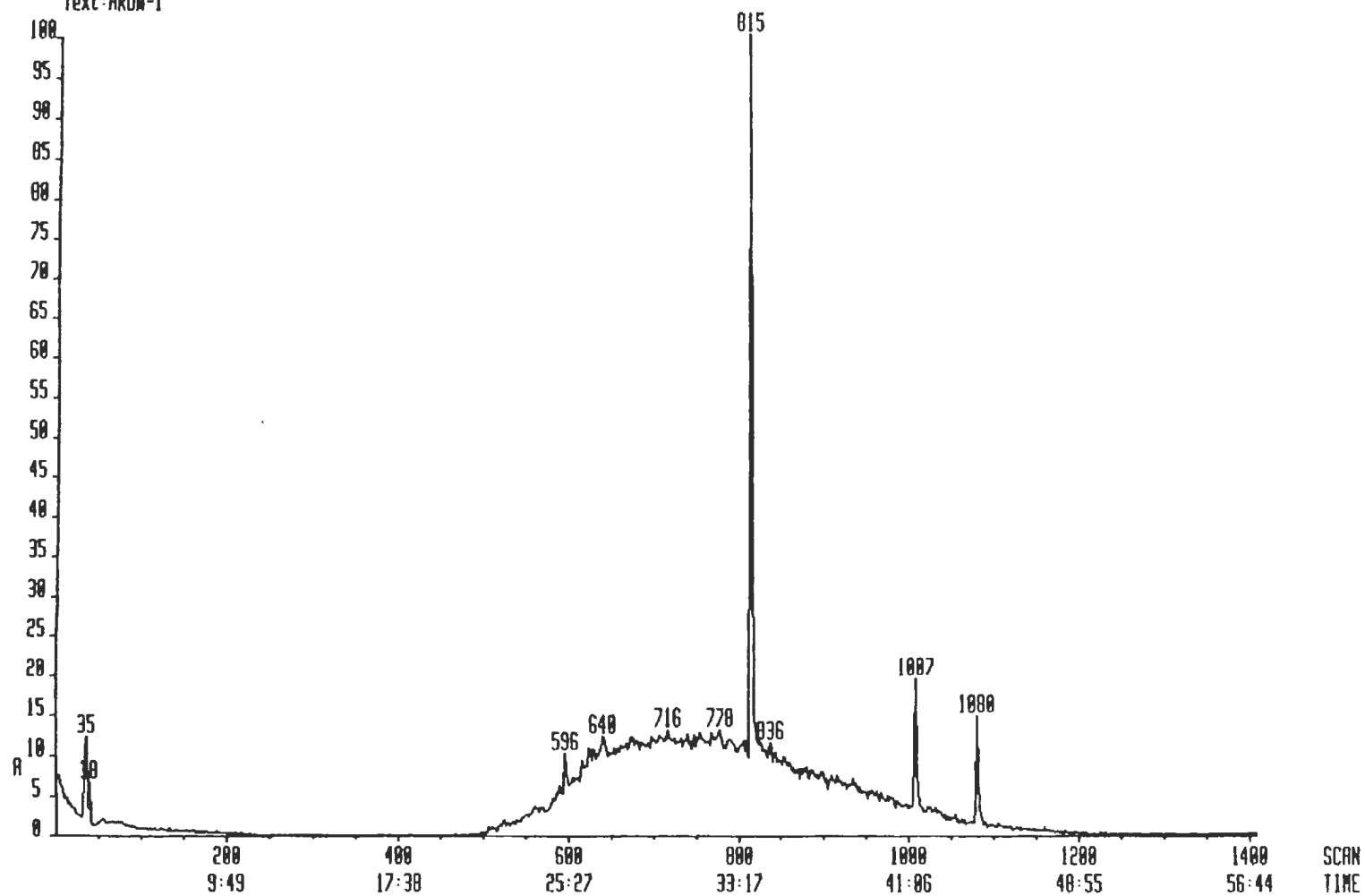
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Text:AROW-I

(E1+)

Sys:DEFAULTS

IHP

A: 2434



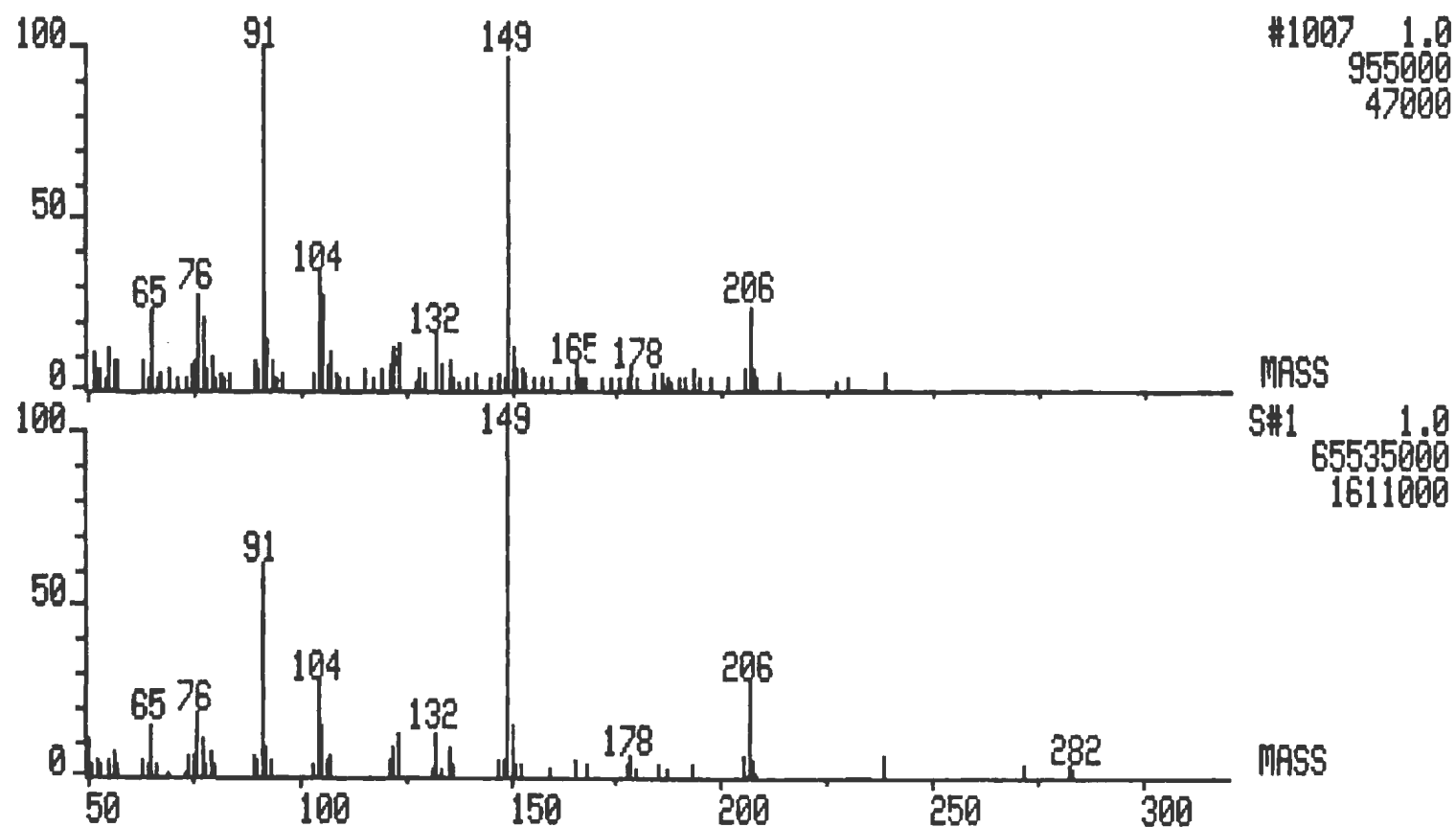
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C19.H20.O4.

+0:00:00

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Lib:NBS

30320 Bpk: 149 Mwt: 312



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(E1+)

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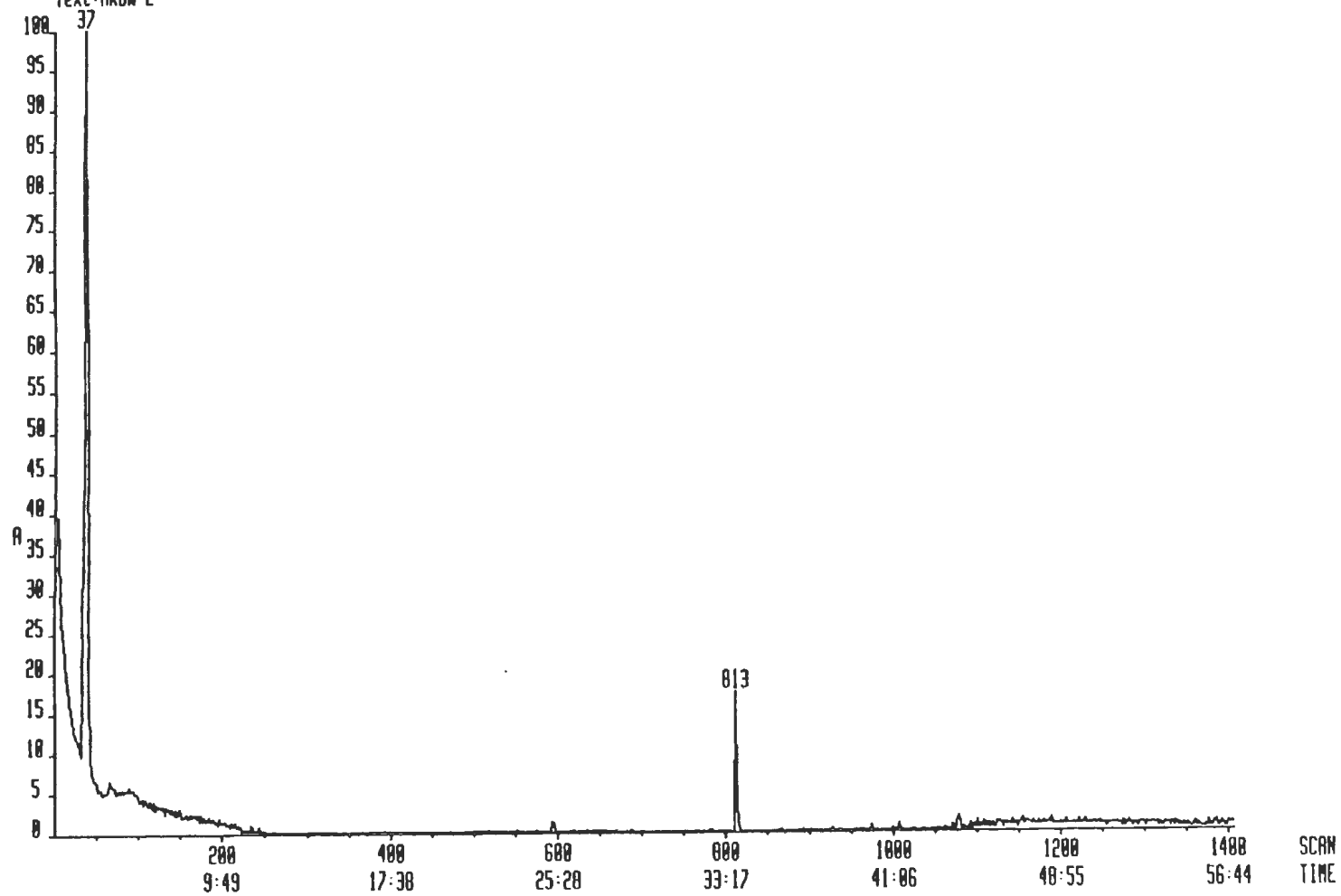
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Chromatogram Identifiers : A:ATIC

A:

401

Text:ARDW-2



IHP
R: 9085

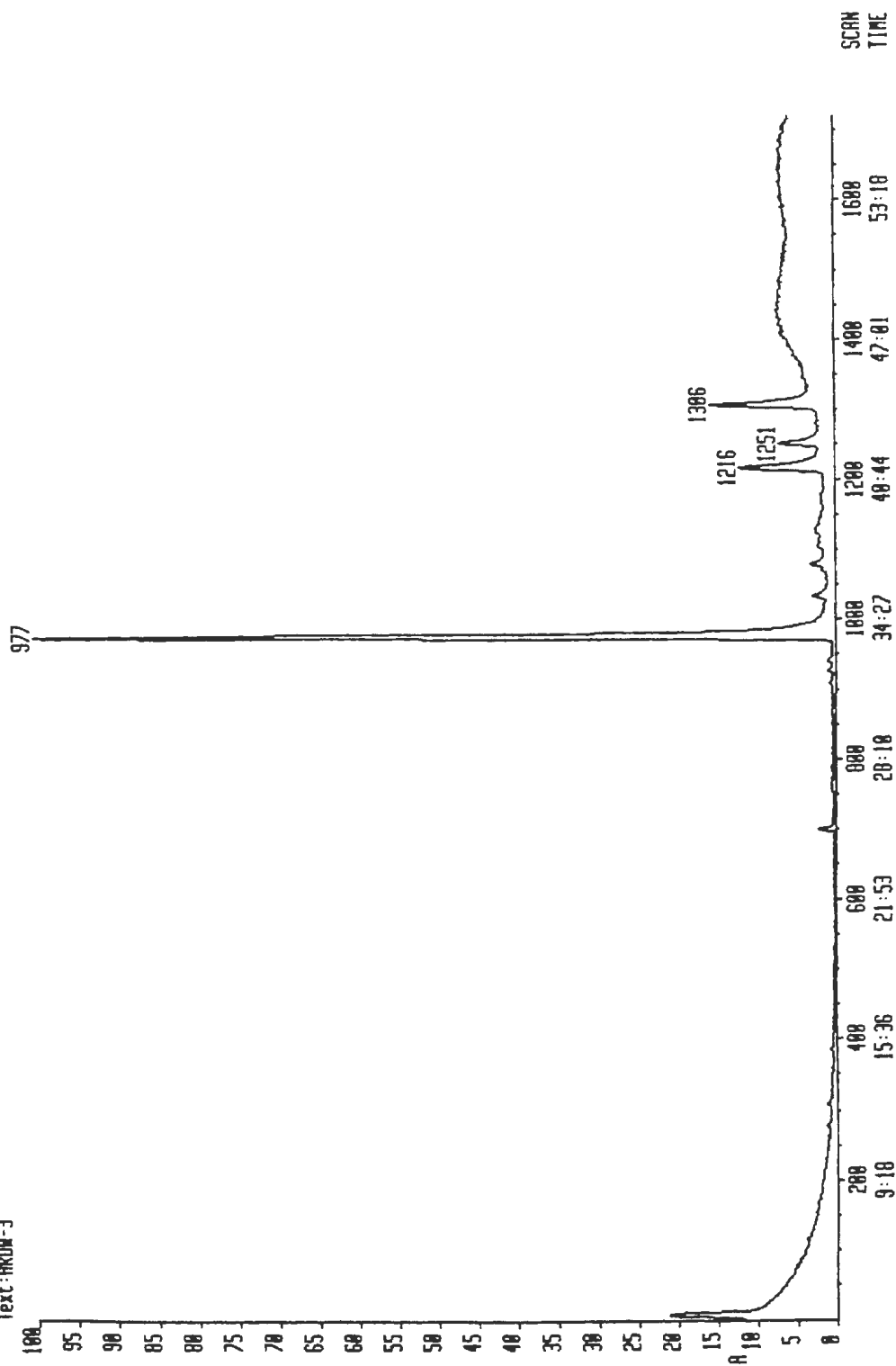
Sys: DEFAULTS

(E1+)

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Chromatogram Identifiers : R : ATIC

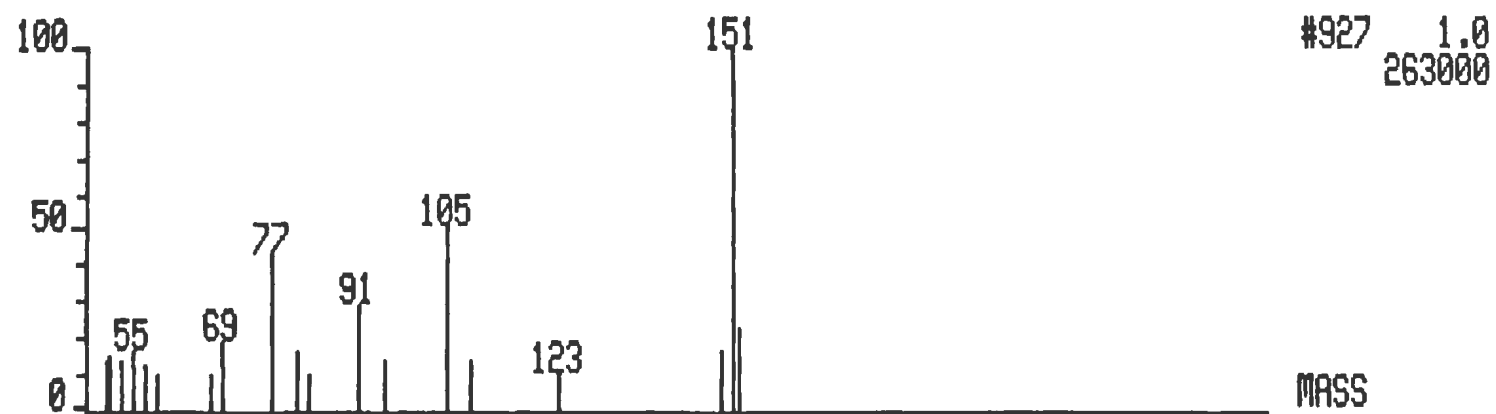
Text: AR004-3



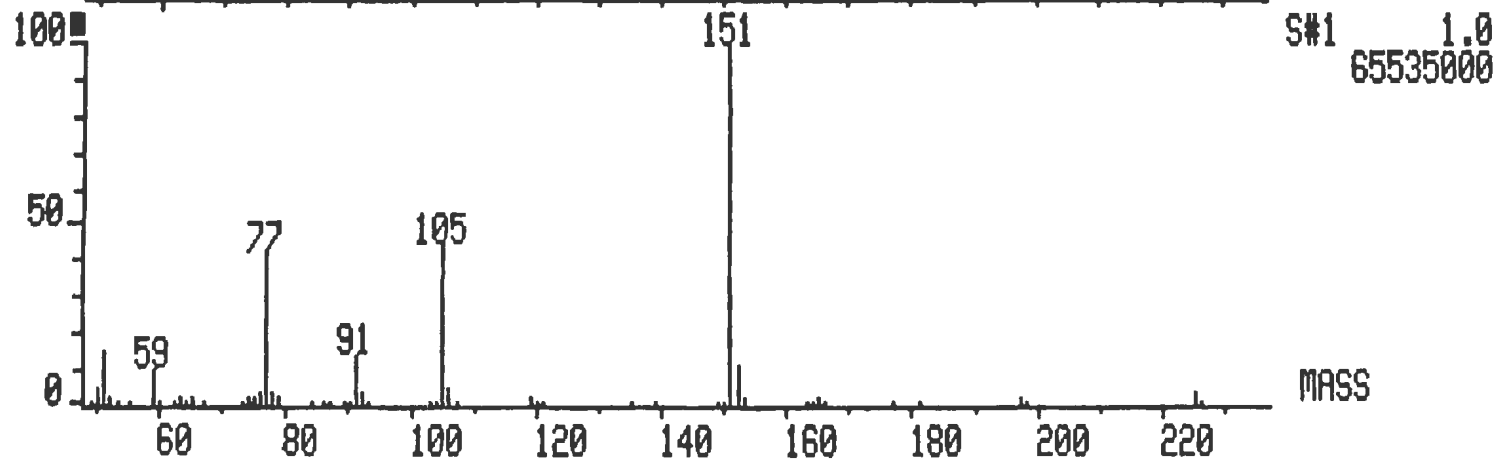
LIBFITS1#1* x1 Bgd=927 T0302305
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C16.H16.O3.

+0:00:00

p641 M777 r733 RFN:24650-42-8
Lib:NBS 24453 Bpk: 151 Mwt: 256



#927 1.0
263000



MASS
S#1 1.0
65535000

MASS

LIBFITS1#1* x1 Bgd=476 T0102302 +0:00:00

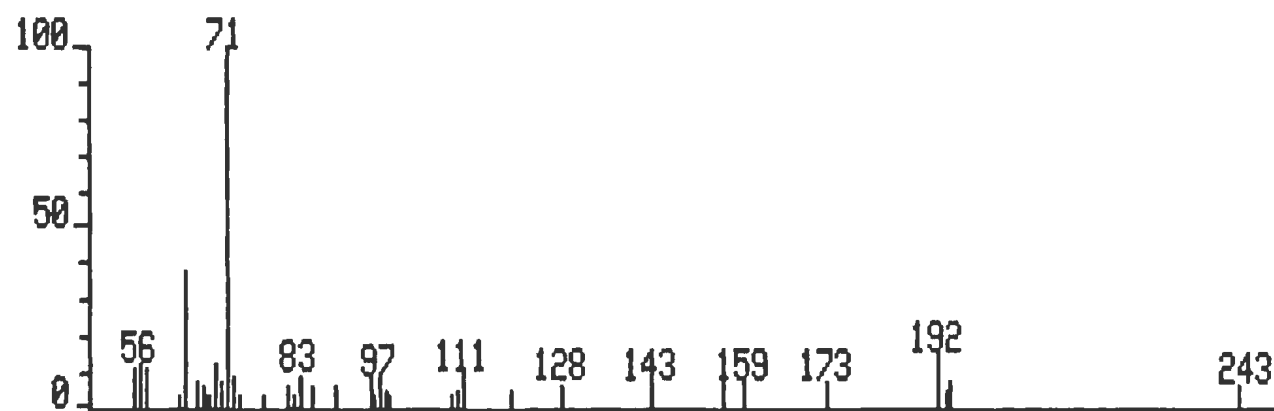
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p558 M842 r605 RFN:0-00-0

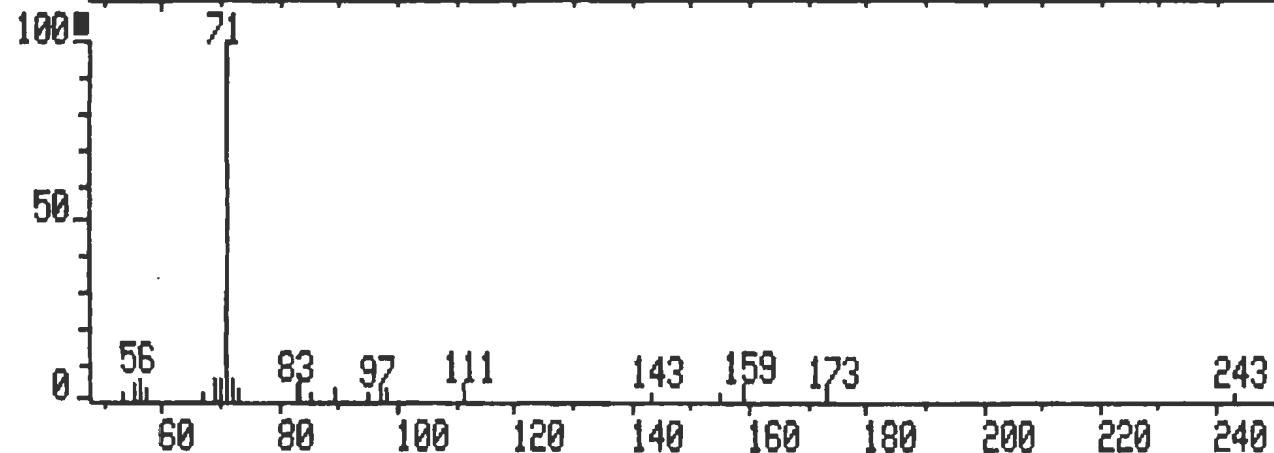
C16.H30.04.

Lib:NBS

27805 Bpk: 71 Mwt: 286



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862000
40000



MASS
S#1 1.0
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1060000

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(E1+)

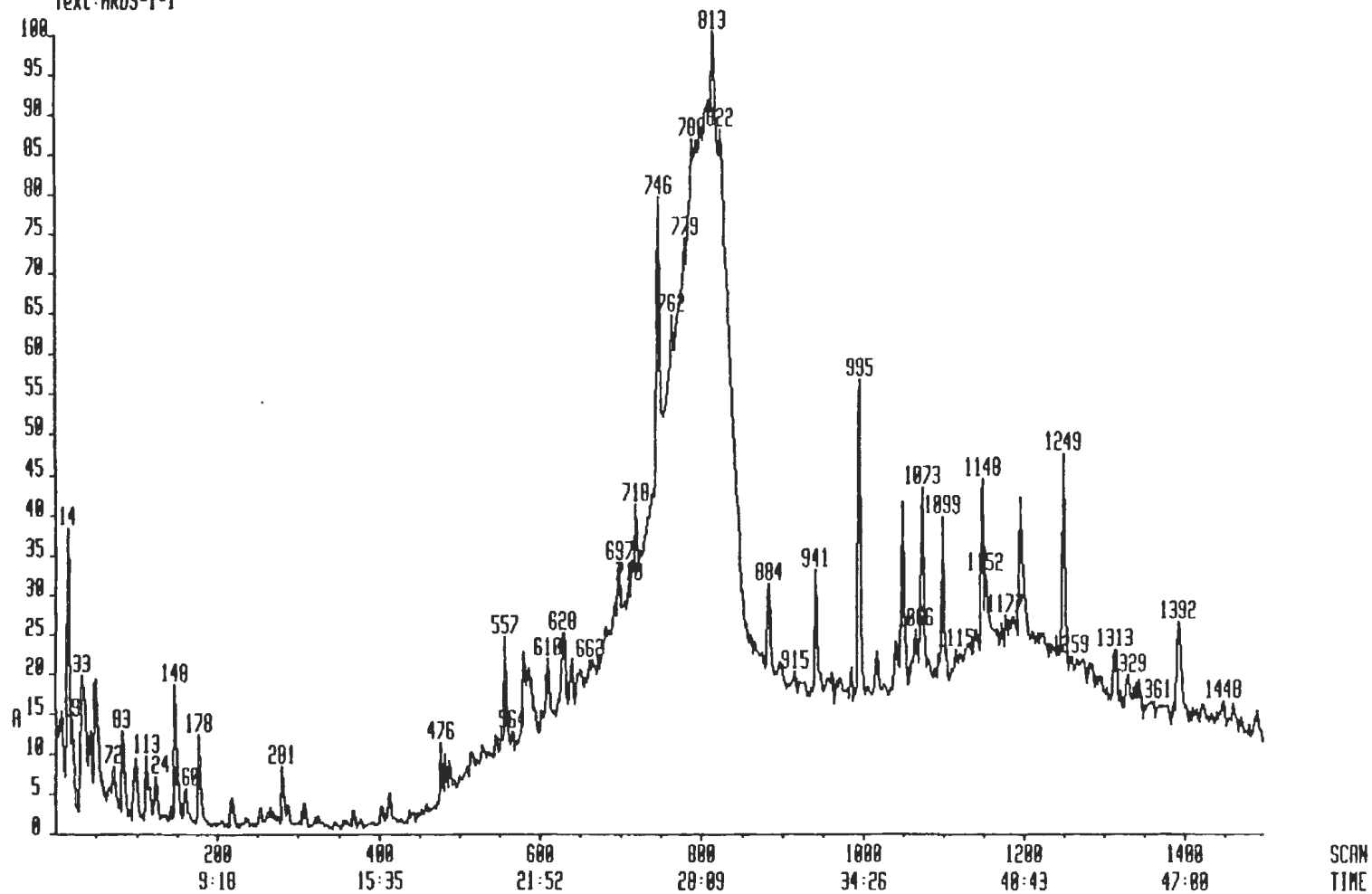
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IHP

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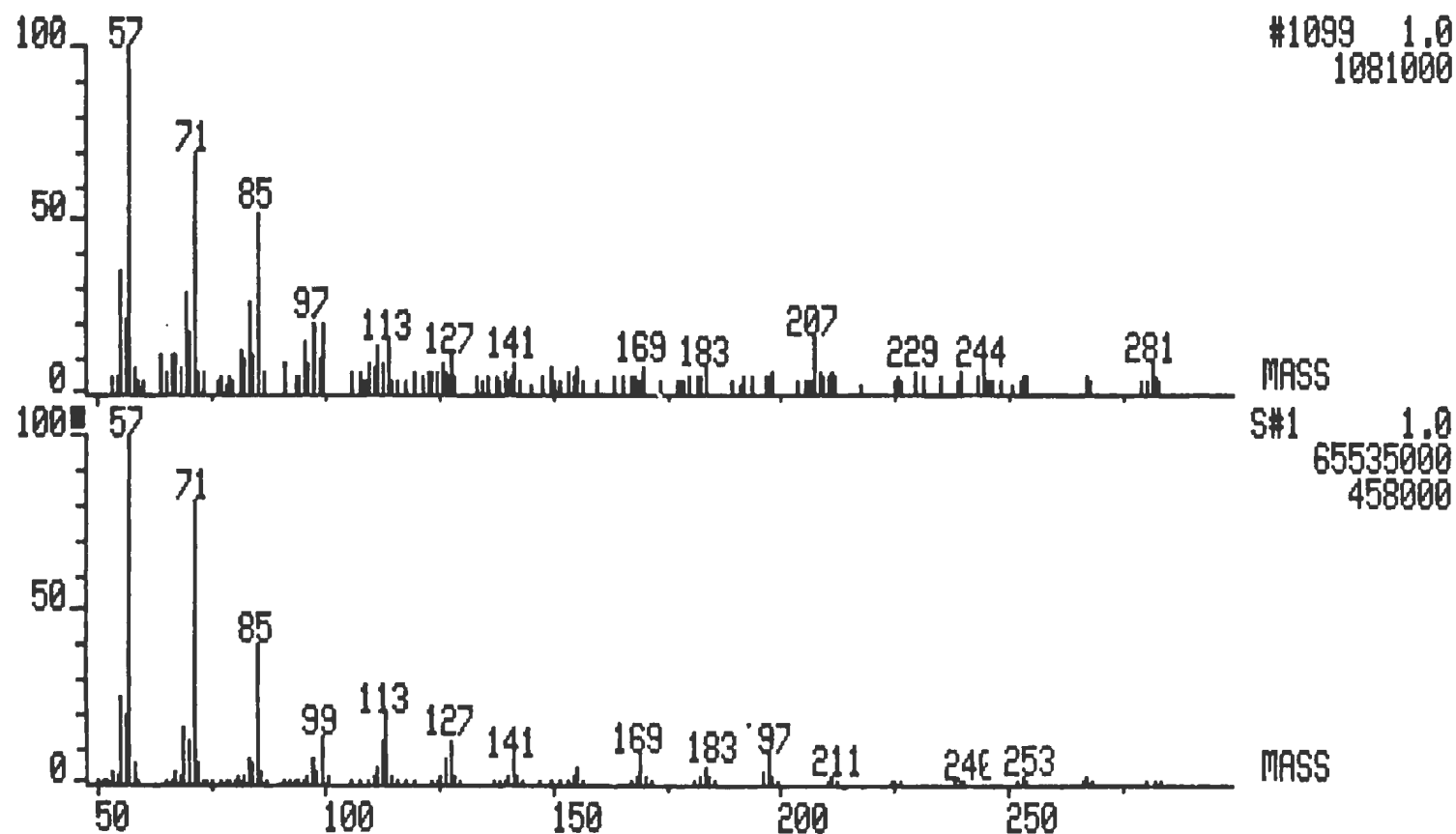
A: 1701

Text:ARDS-1-1



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 C20.H42.

+0:00:00
 p475 M650 r540 RFN:638-36-8
 Lib:NBS 27370 Bpk: 57 Mwt: 282



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(E1+)

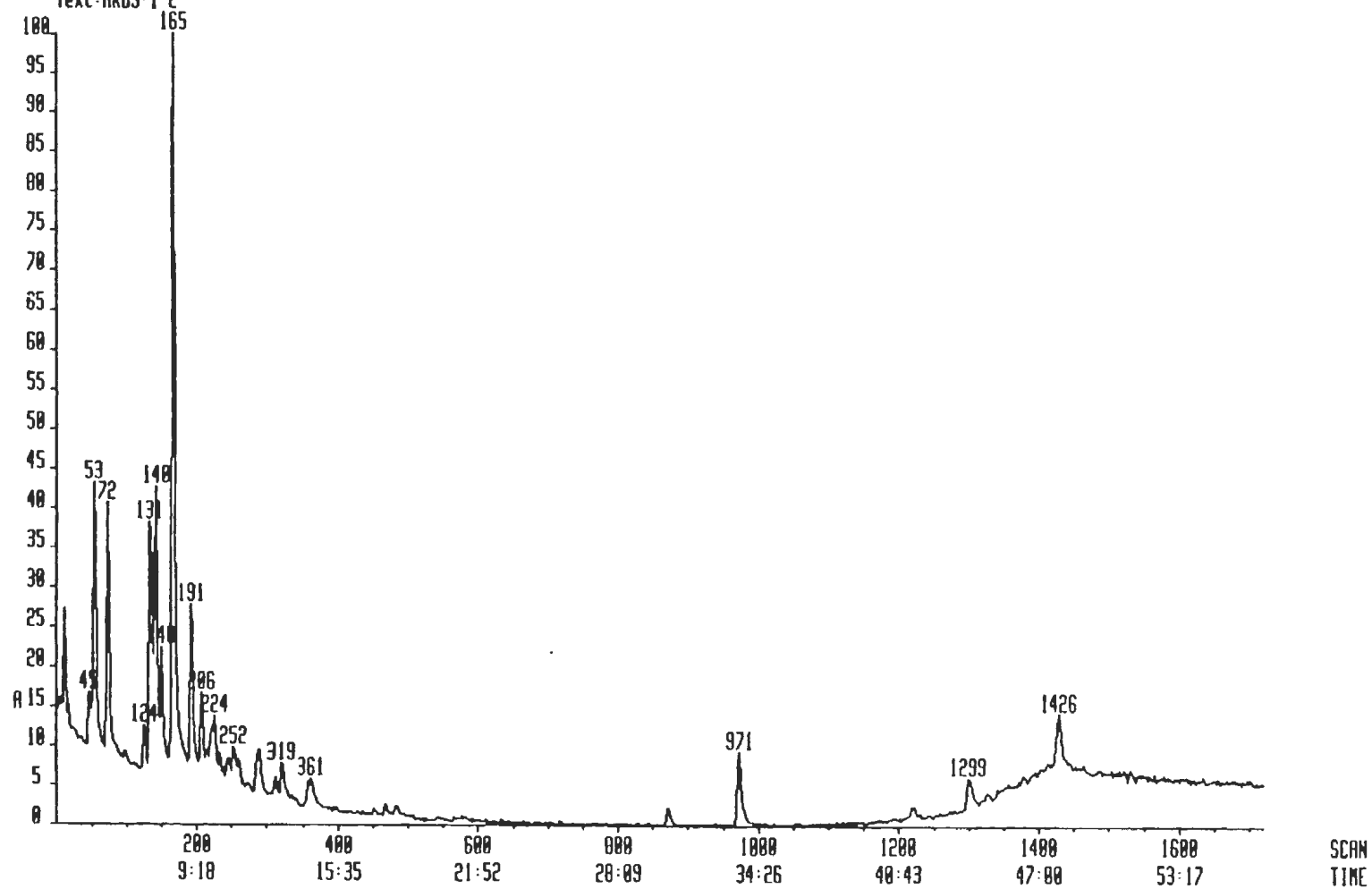
Sys:DEFAULTS

1HP

Chromatogram Identifiers : A :ATIC

A: 4095

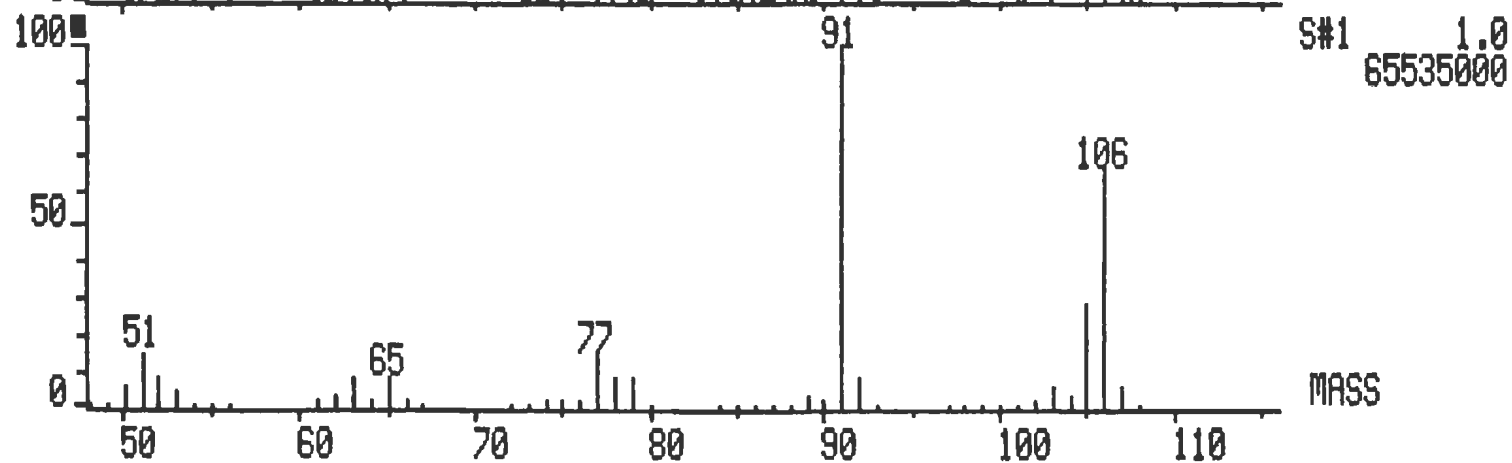
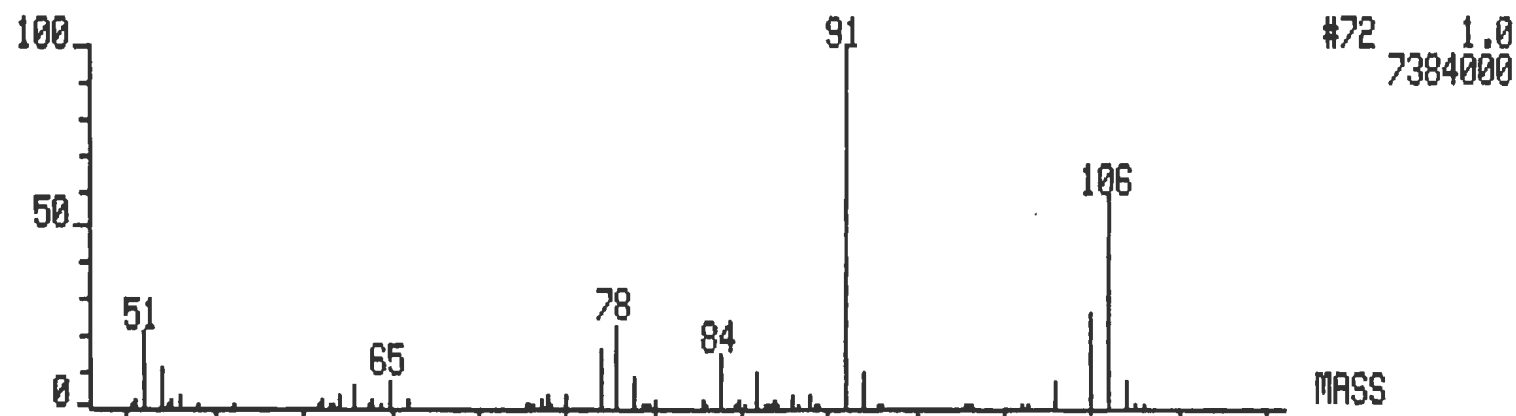
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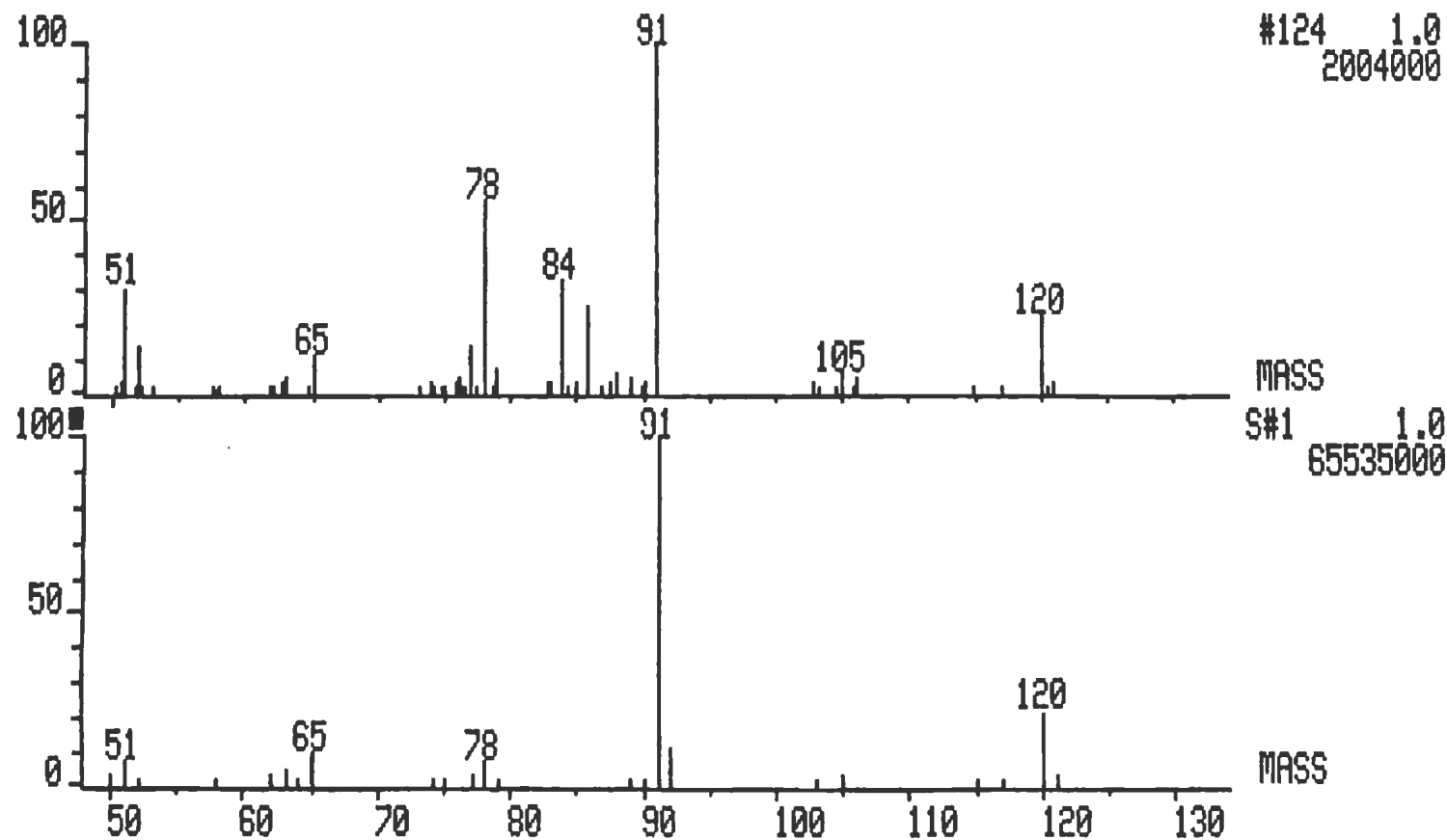
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Lib:NBS 1731 Bpk: 91 Mwt: 106



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C9.H12.

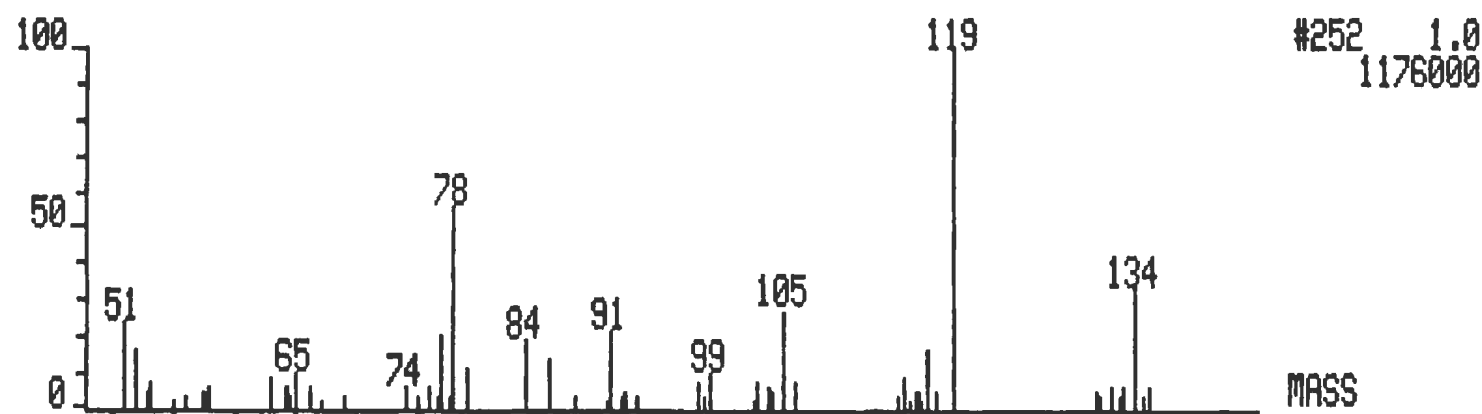
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Lib:NBS 3097 Bpk: 91 Mwt: 120

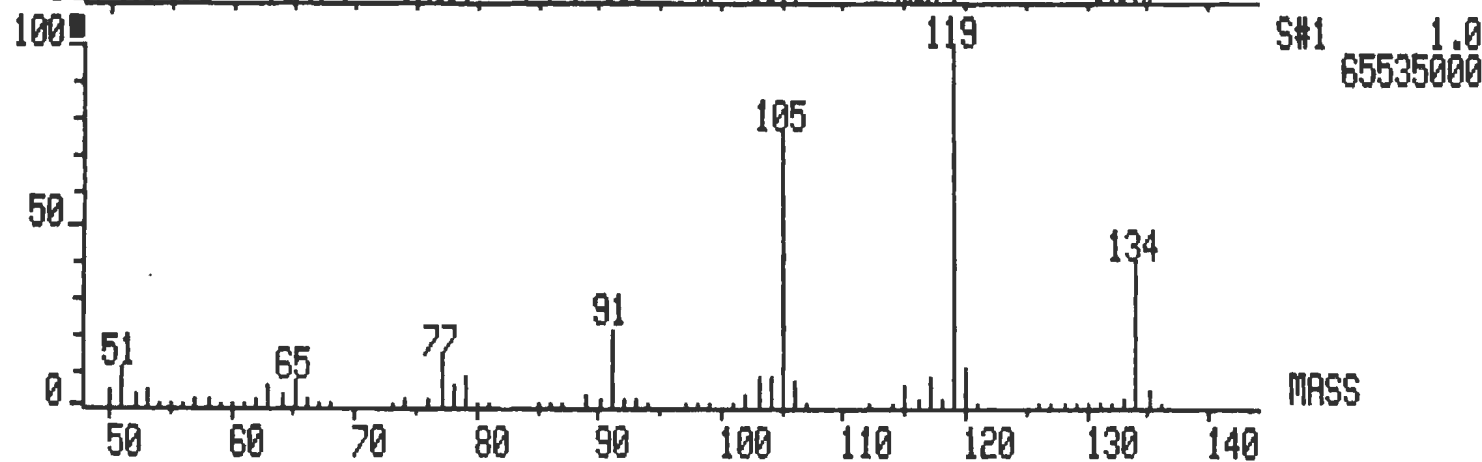


LIBFITS1#1* x1 Bgd=252 T0402301
BENZENE, 1,4-DIETHYL-
C10.H14.

+0:00:00
p703 M736 r771 RFN:105-05-5
Lib:NBS 4771 Bpk: 119 Mwt: 134



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MASS
S#1 1.0
65535000

MASS

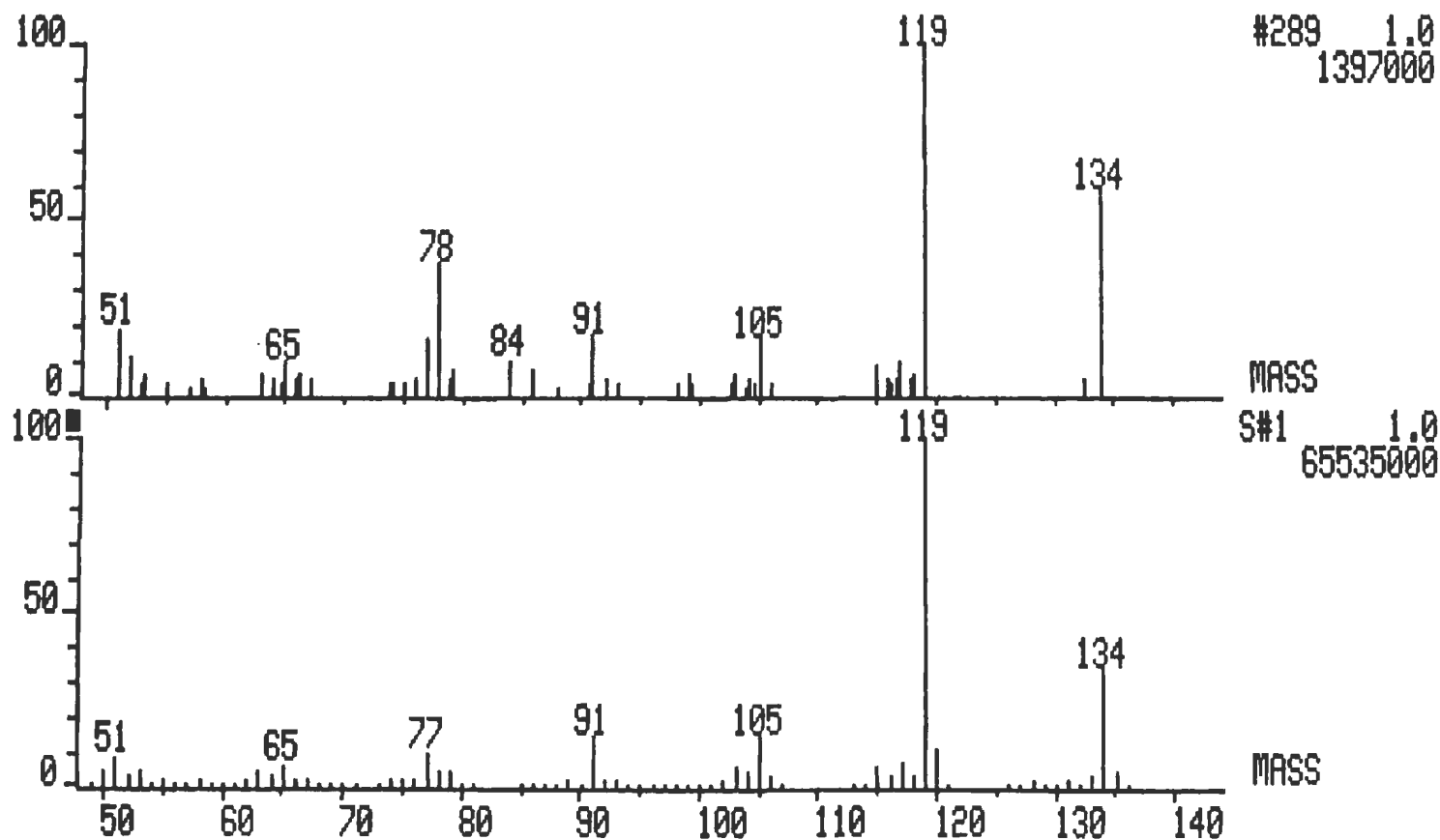
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C10.H14.

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Lib:NBS

4787 Bpk: 119 Mwt: 134



IHP
R: 4885

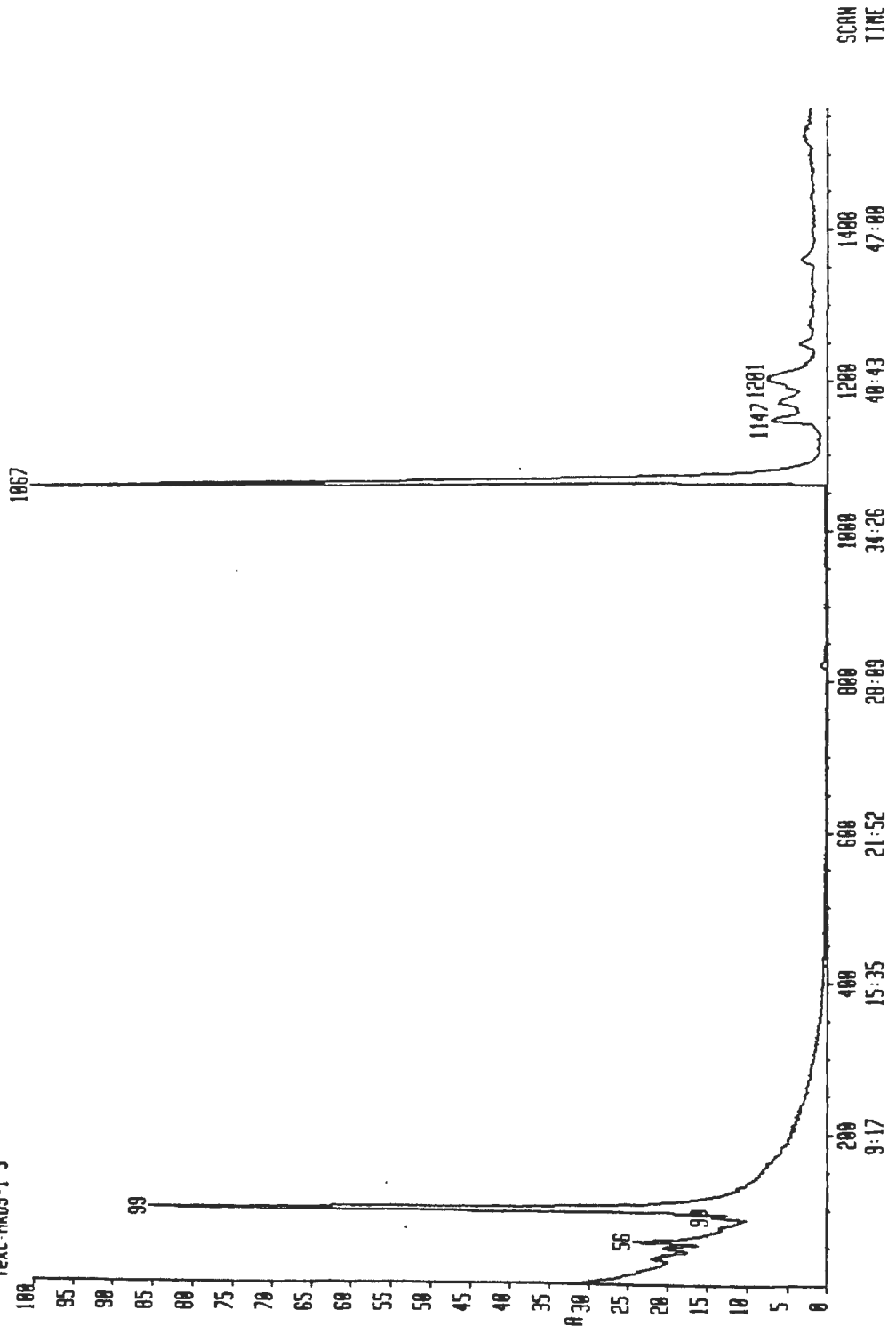
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Text:ARDS-1-3



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(E1+)

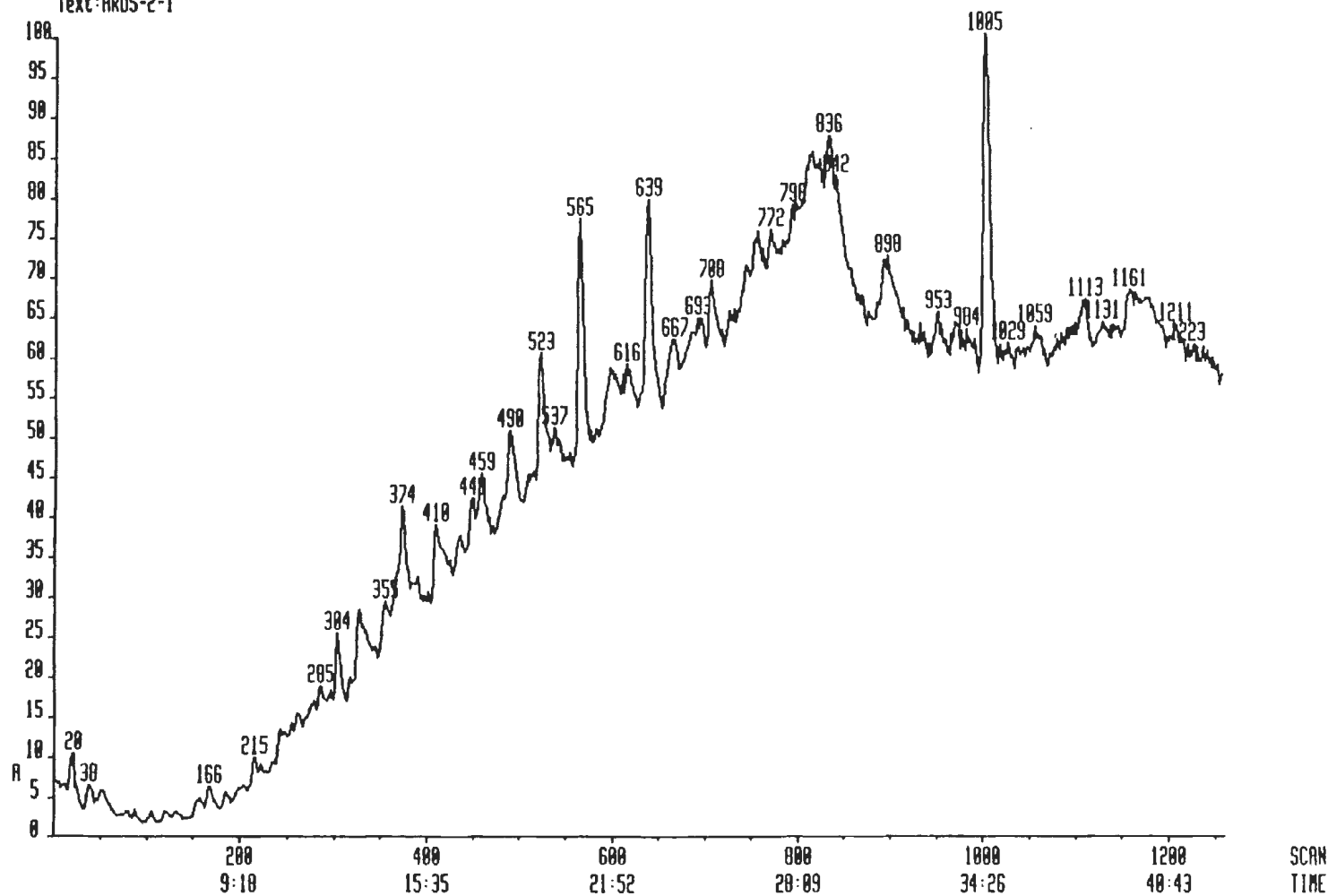
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IHP

Chromatogram Identifiers : A :ATIC

A: 4213

Text:ARDS-2-1



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A: 14436

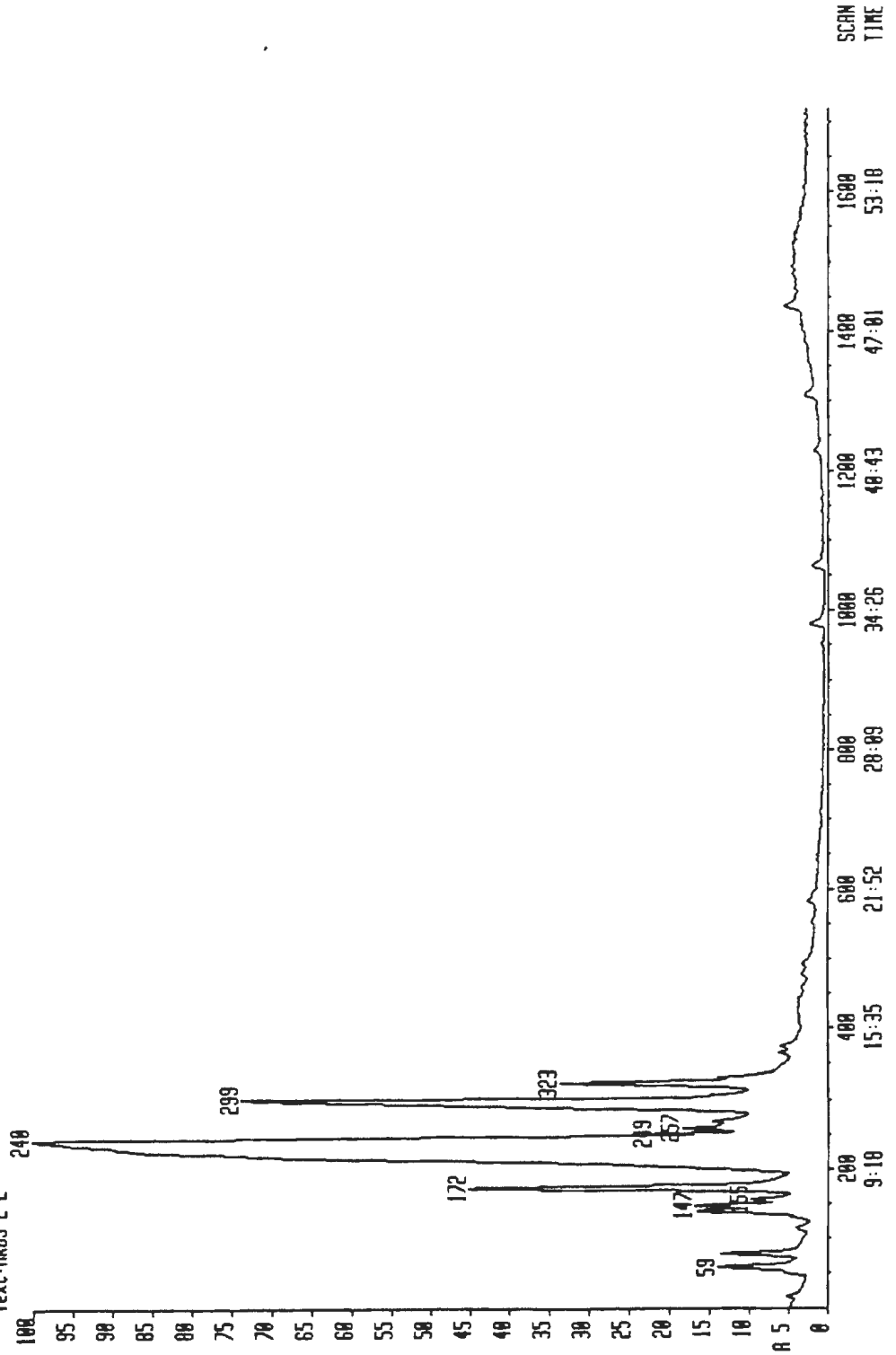
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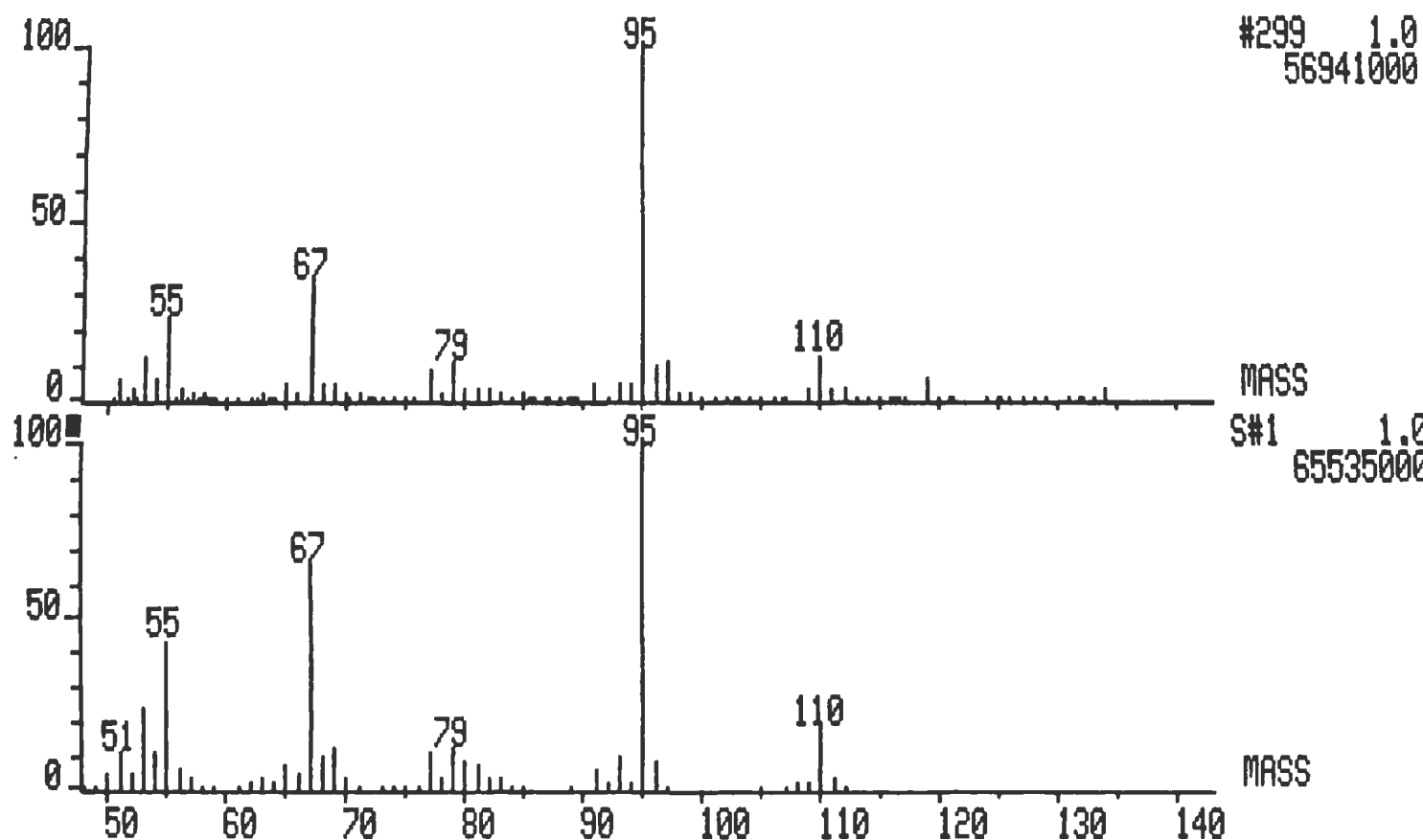
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Text: ARDS-2-2



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1,4-PENTADIENE, 2,3,3-TRIMETHYL-
C8.H14.

+0:00:00
p714 M768 r857 RFN:756-02-5
Lib:NBS 1973 Bpk: 95 Mwt: 110



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(E1+)

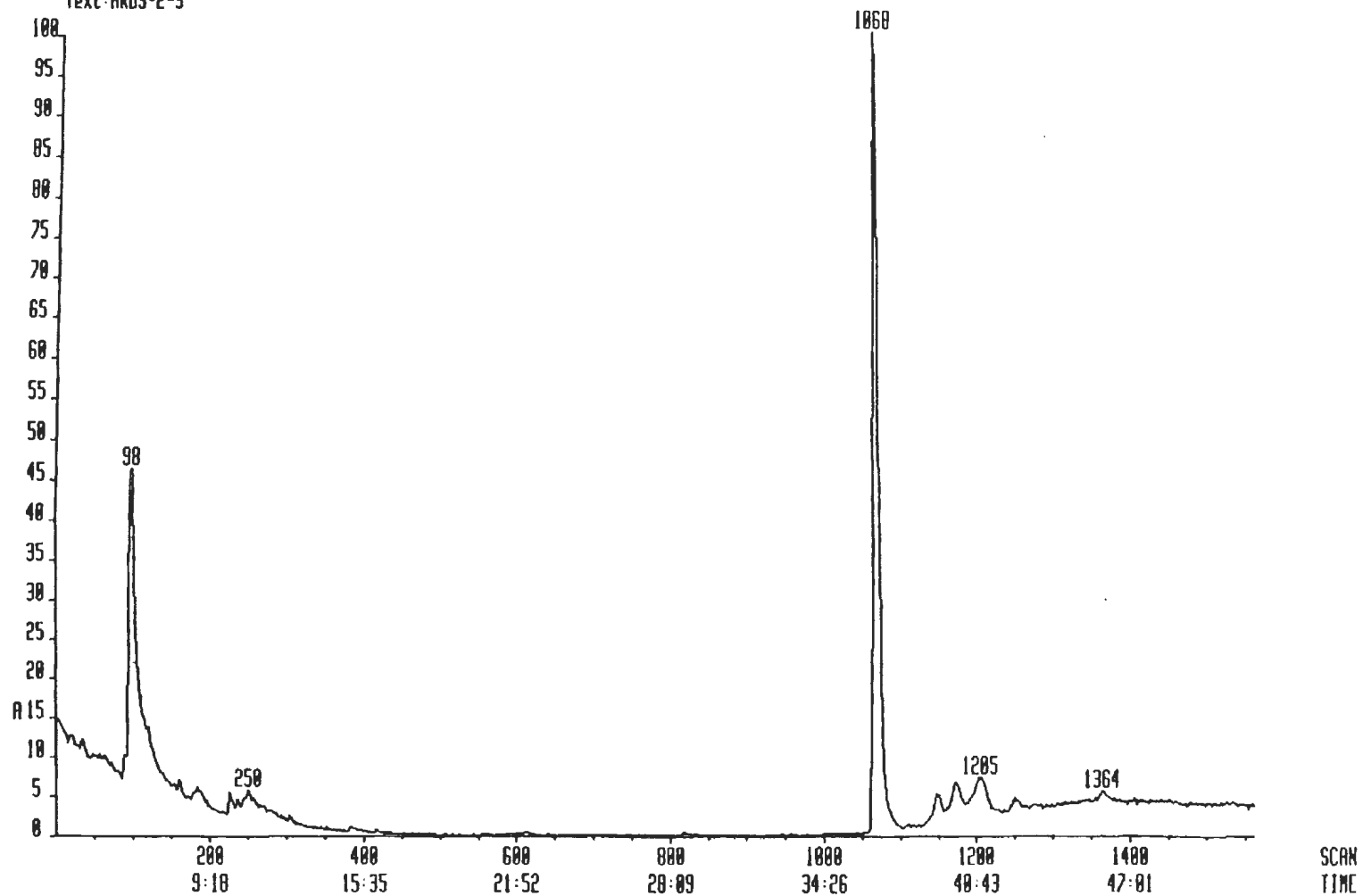
Sys:DEFAULTS

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Chromatogram Identifiers : A:AT1C

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Text:ARDS-2-3



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(E1+)

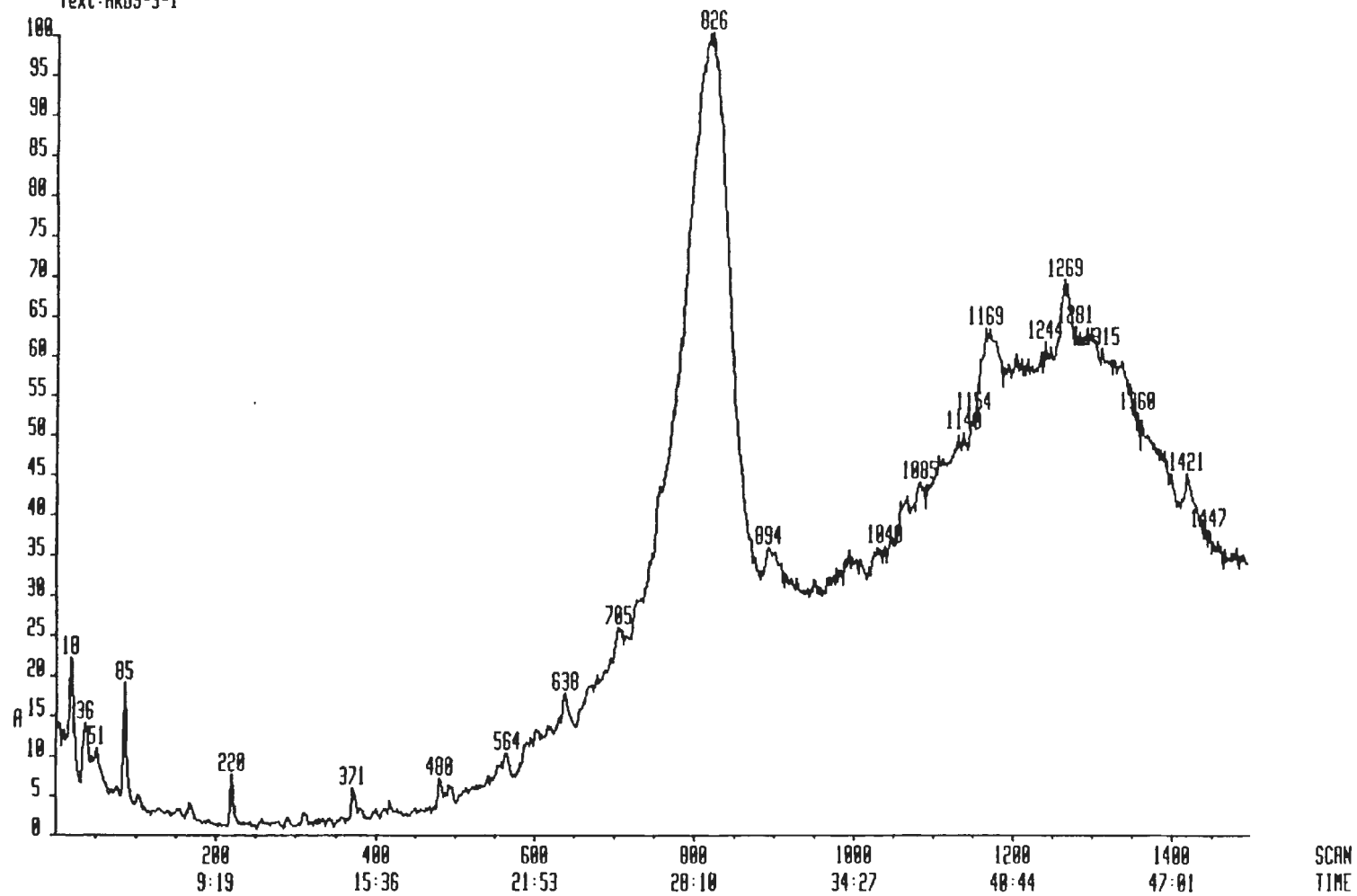
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IHP

Chromatogram Identifiers : A:ATIC

A: 1900

Text:ARDS-3-1



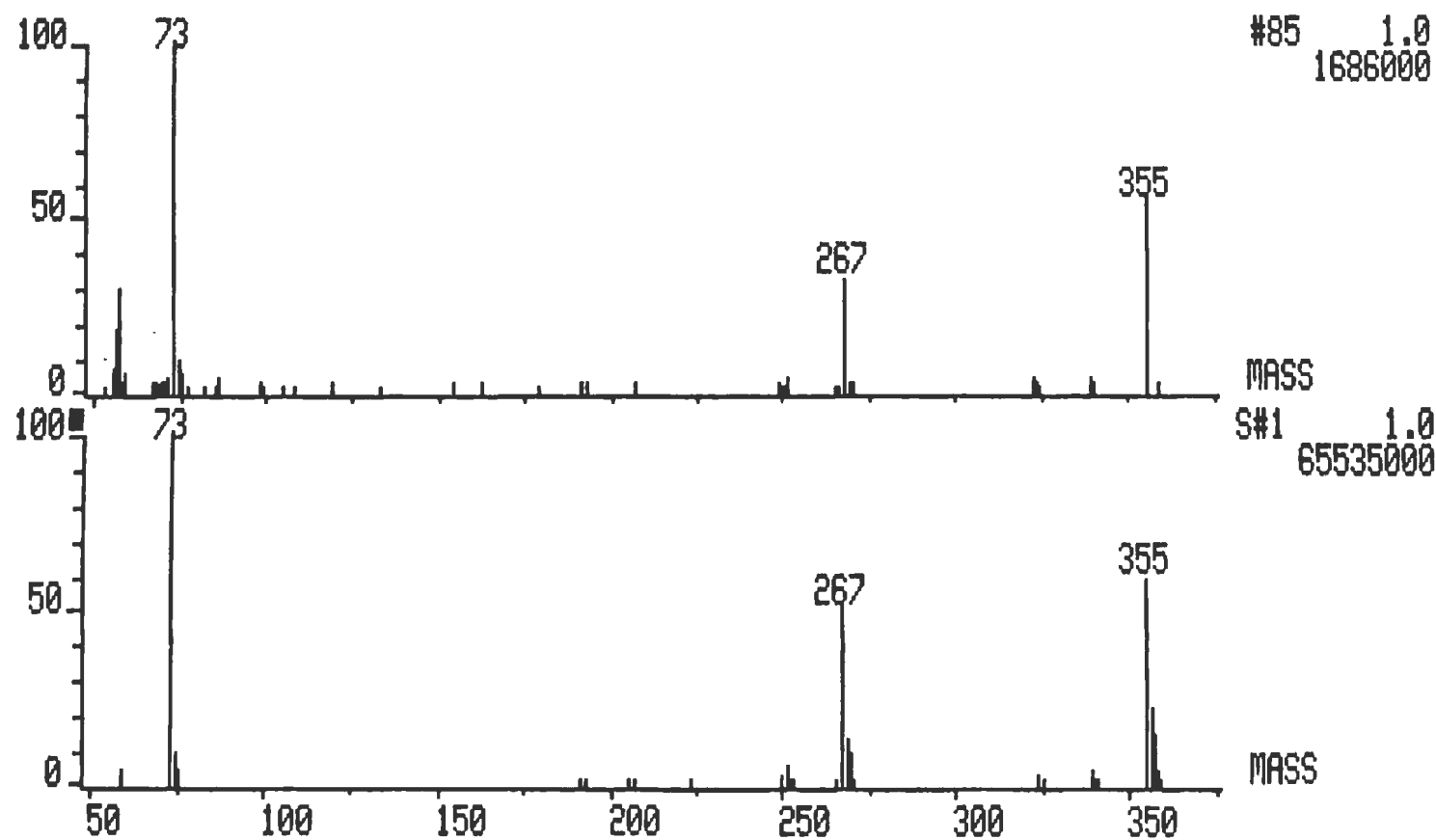
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p563 M684 r803 RFN:541-02-6

Lib:NBS

34673 Bpk: 73 Mwt: 370



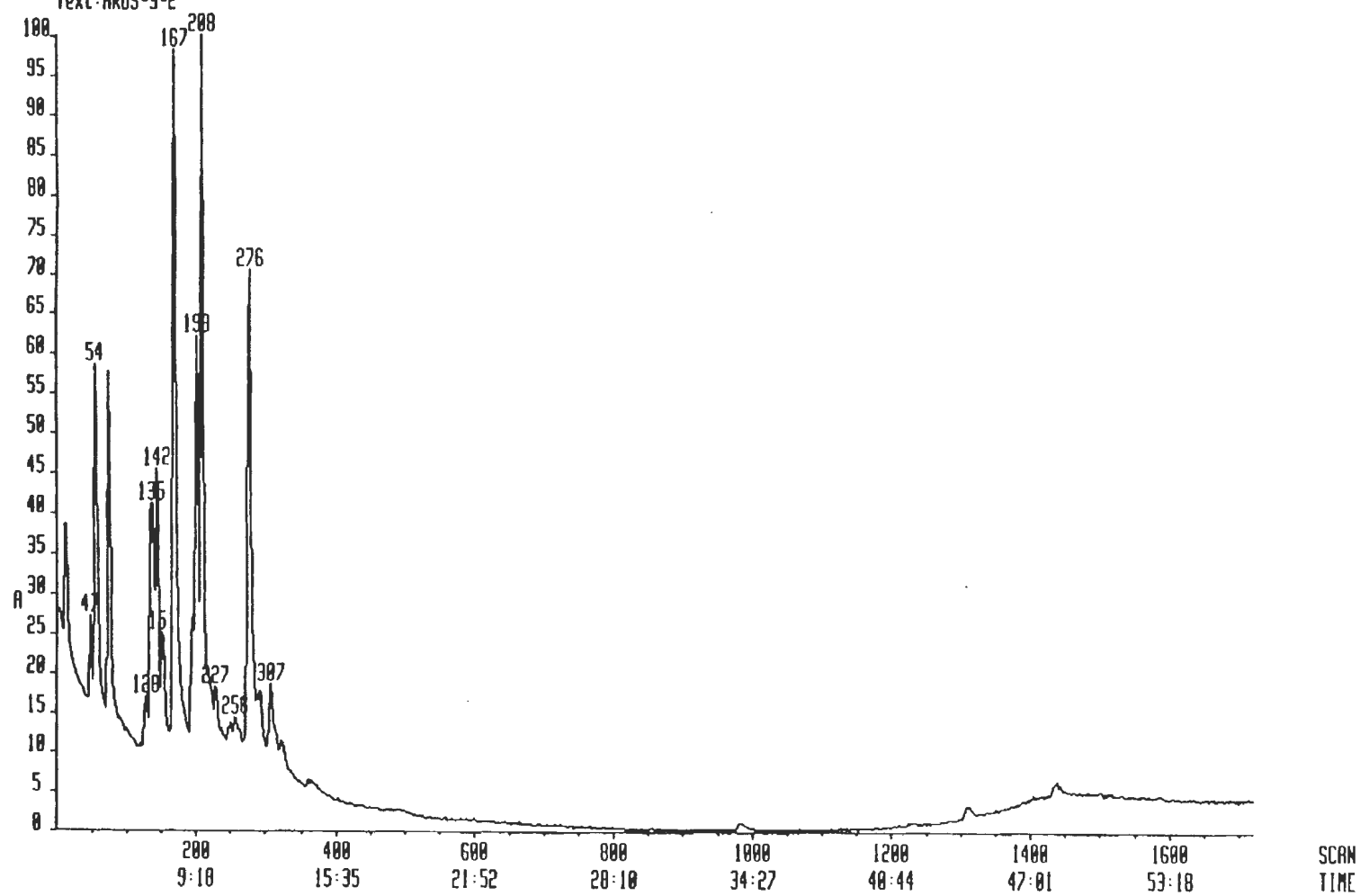
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(E1+)

Sys: DEFAULTS

IHP

A: 8505



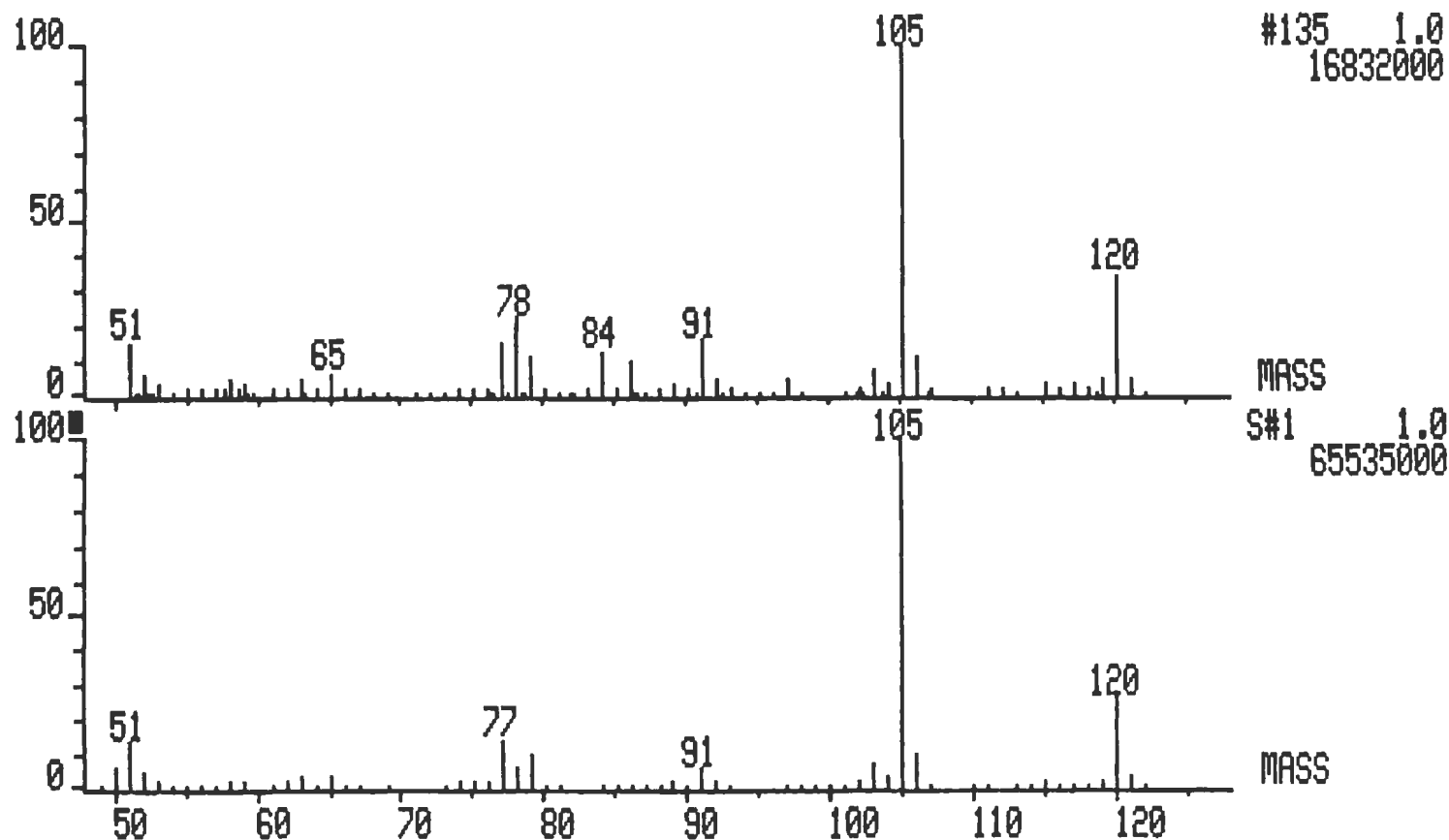
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C9.H12.

+0:00:00

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Lib:NBS

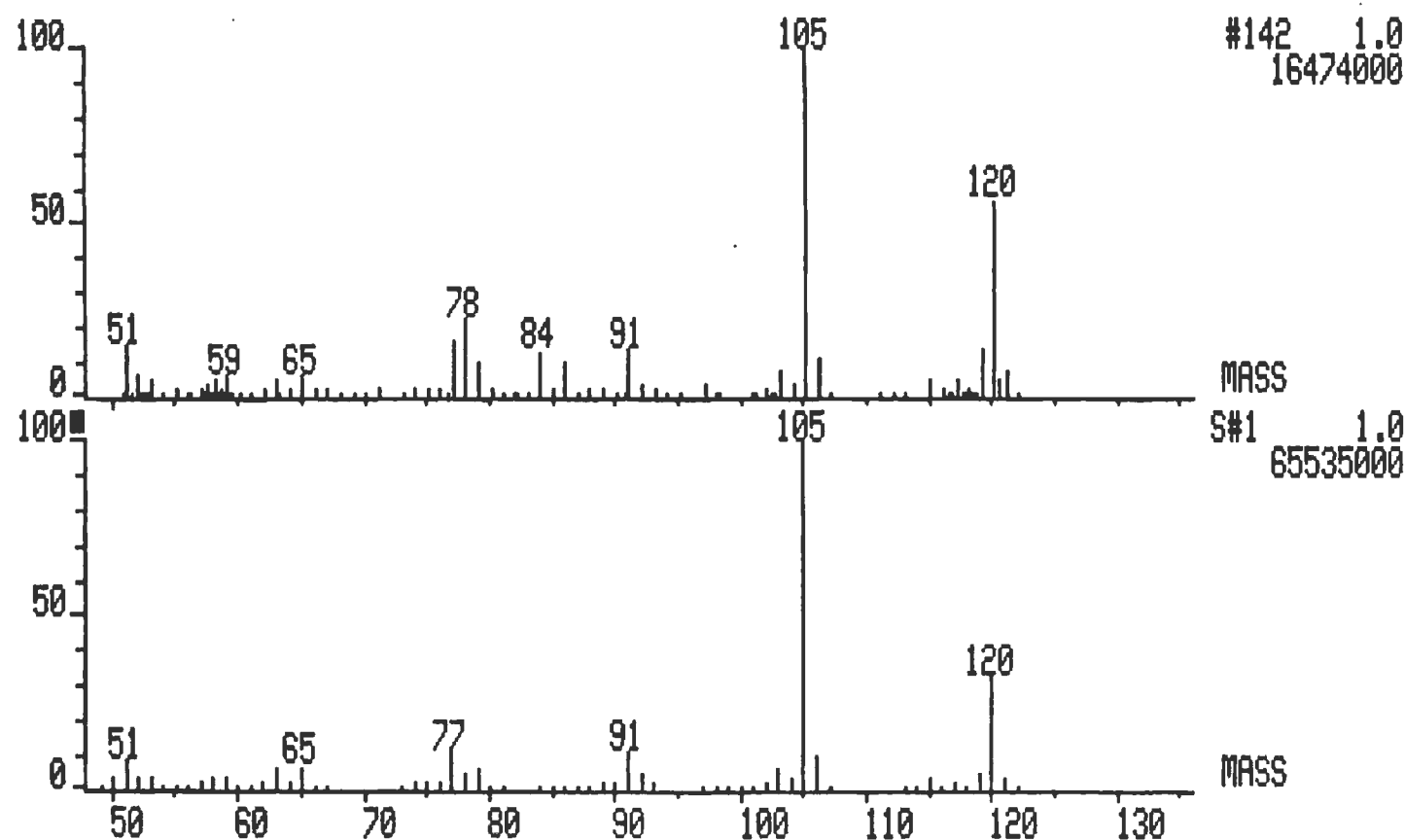
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C9.H12.

+0:00:00

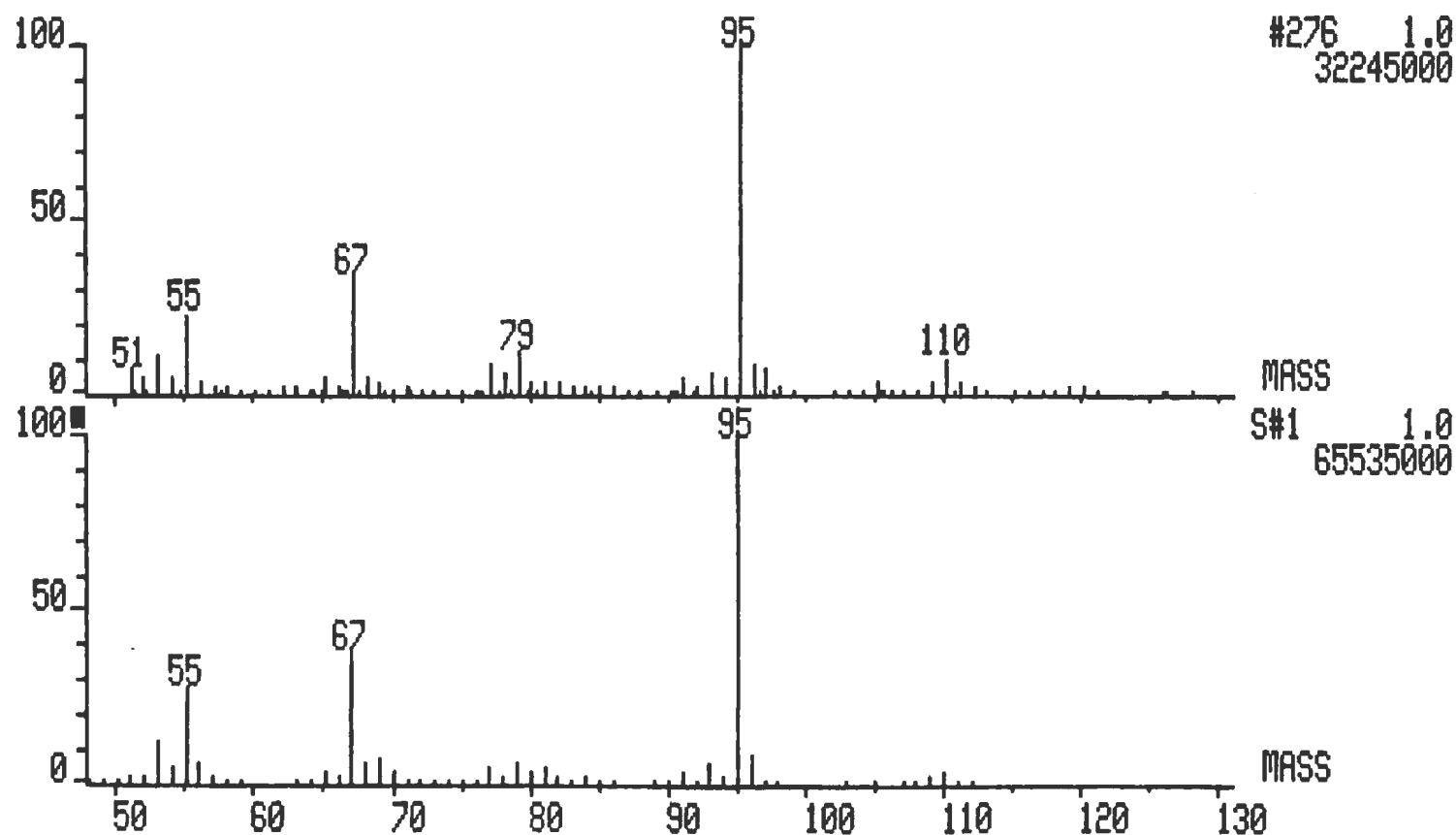
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Lib:NBS 3101 Bpk: 105 Mwt: 120



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1,4-PENTADIENE, 2,3,4-TRIMETHYL-
C8.H14.

+0:00:00

p779 M810 r881 RFN:72014-90-5
Lib:NBS 2024 Bpk: 95 Mwt: 110



T1202304 #1-1568 12-FEB-93 12:50 TS250
Chromatogram Identifiers : A:AT1C
Text:ARDS-3-3

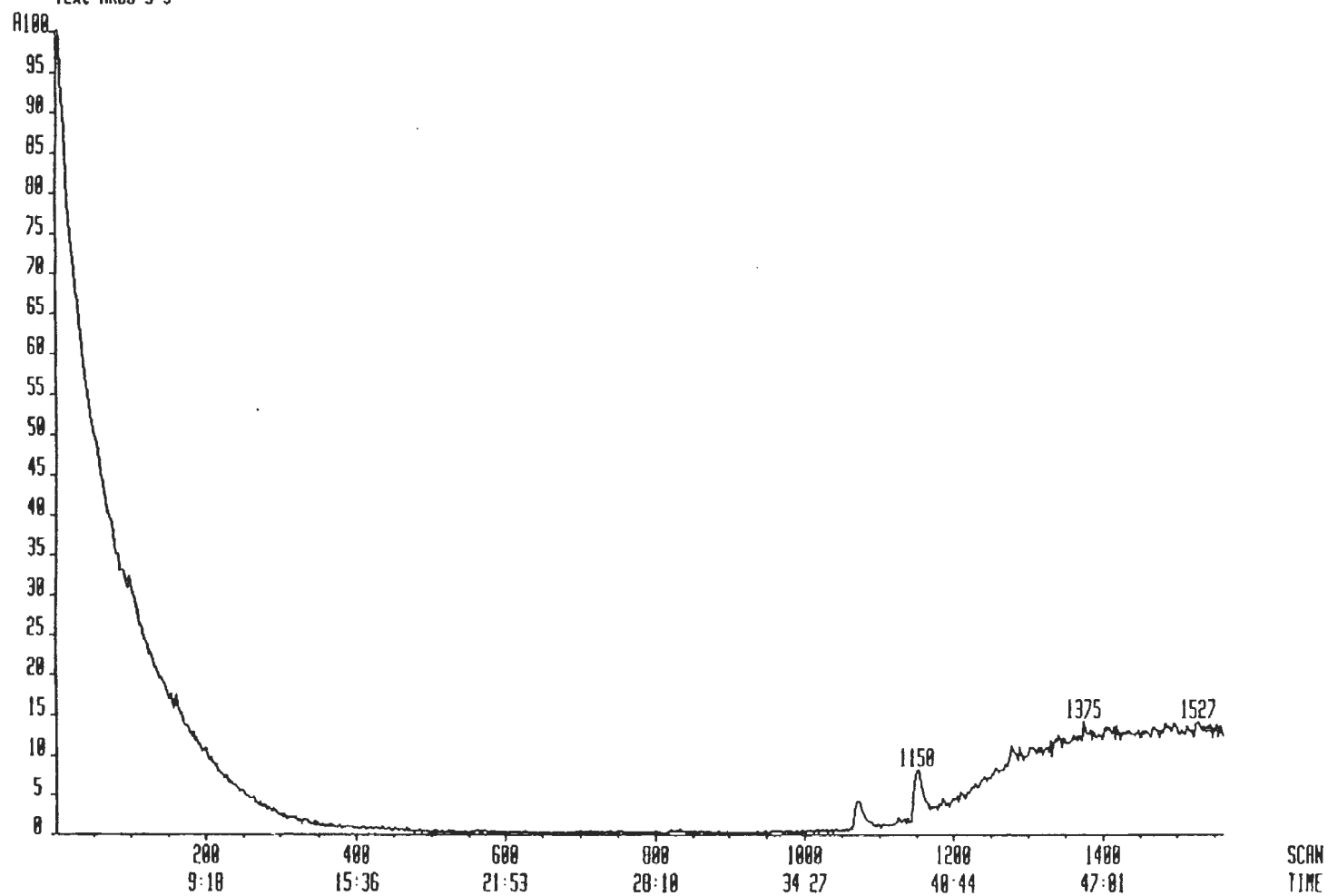
(E1*)

Sys:DEFAULTS

IHP

A:

2611



APPENDIX F

CHROMATOGRAMS AND MASS SPECTRA

Cyril, Oklahoma

The following are chromatograms and mass spectra for water and sediment samples from Cyril. The accession labels are as follows:

Accession Label	Page
CW-1 = Water from Station 1	191
CW-2 = Water from Station 2	192-199
CW-3 = Water from Station 3	200
CW-4 = Water from Station 4	201
CS-1-1 = Sediment from Station 1, fraction #1	202-203
CS-1-2 = Sediment From Station 1, fraction #2	204-206
CS-1-3 = Sediment from Station 1, fraction #3	207-209
CS-2-1 = Sediment from Station 2, fraction #1	210-217
CS-2-2 = Sediment from Station 2, fraction #2	218-219
CS-3-1 = Sediment from Station 3, fraction #1	220
CS-3-2 = Sediment from Station 3, fraction #2	221
CS-3-3 = Sediment from Station 3, fraction #3	222
CS-4-1 = Sediment from Station 4, fraction #1	223-224
CS-4-2 = Sediment from Station 4, fraction #2	225
CS-4-3 = Sediment from Station 4, fraction #3	226-229

The mass spectra are presented as two spectra. The top spectra is that of the sample with the peak number in the upper right corner. The bottom spectra is that of the library match corresponding to the sample. The name and molecular formula of the compound are given at the top. Also, the CAS registry number is listed, if available, at

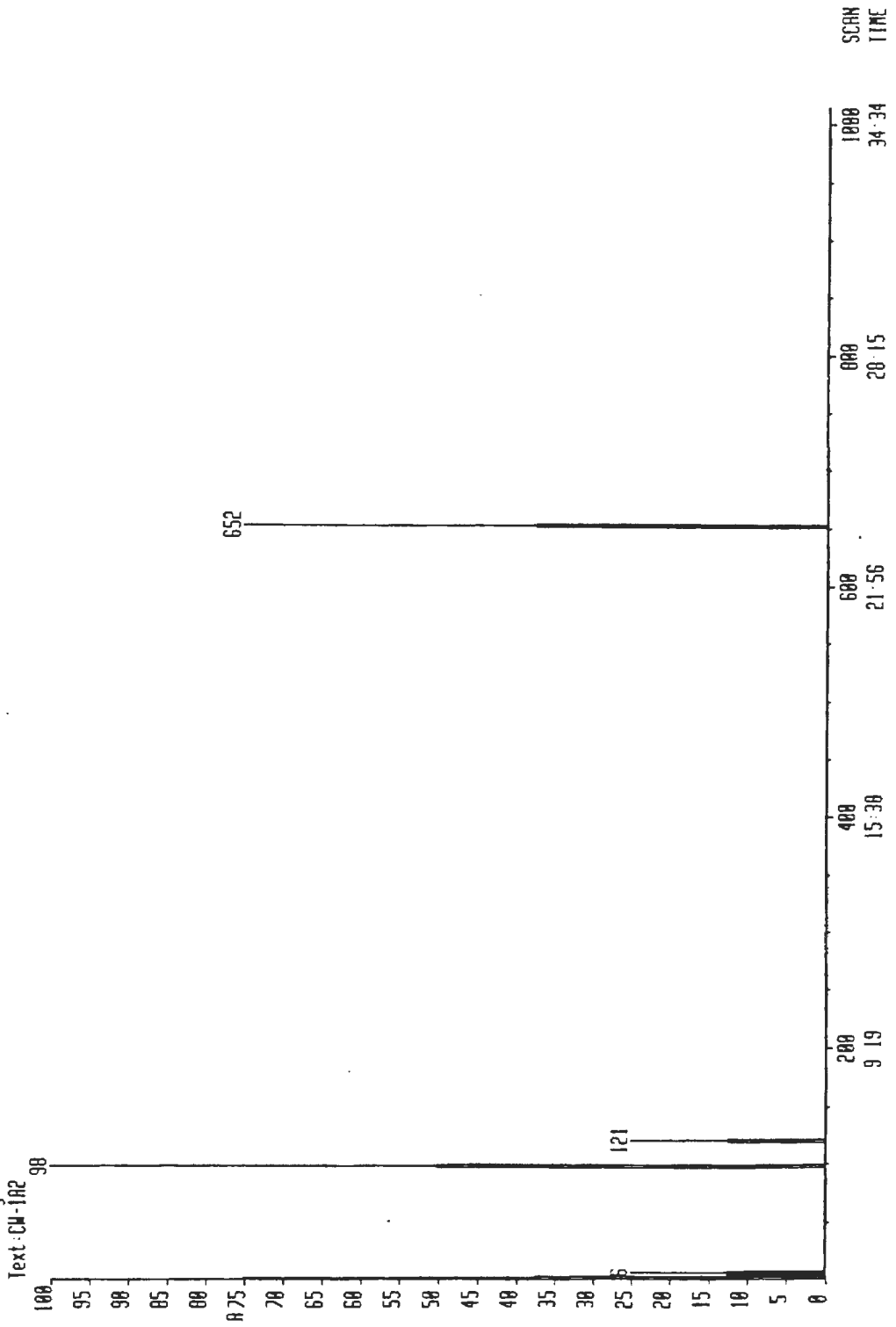
the top right following the label "RFN". In the interest of being concise, spectra were only included once per occurrence.

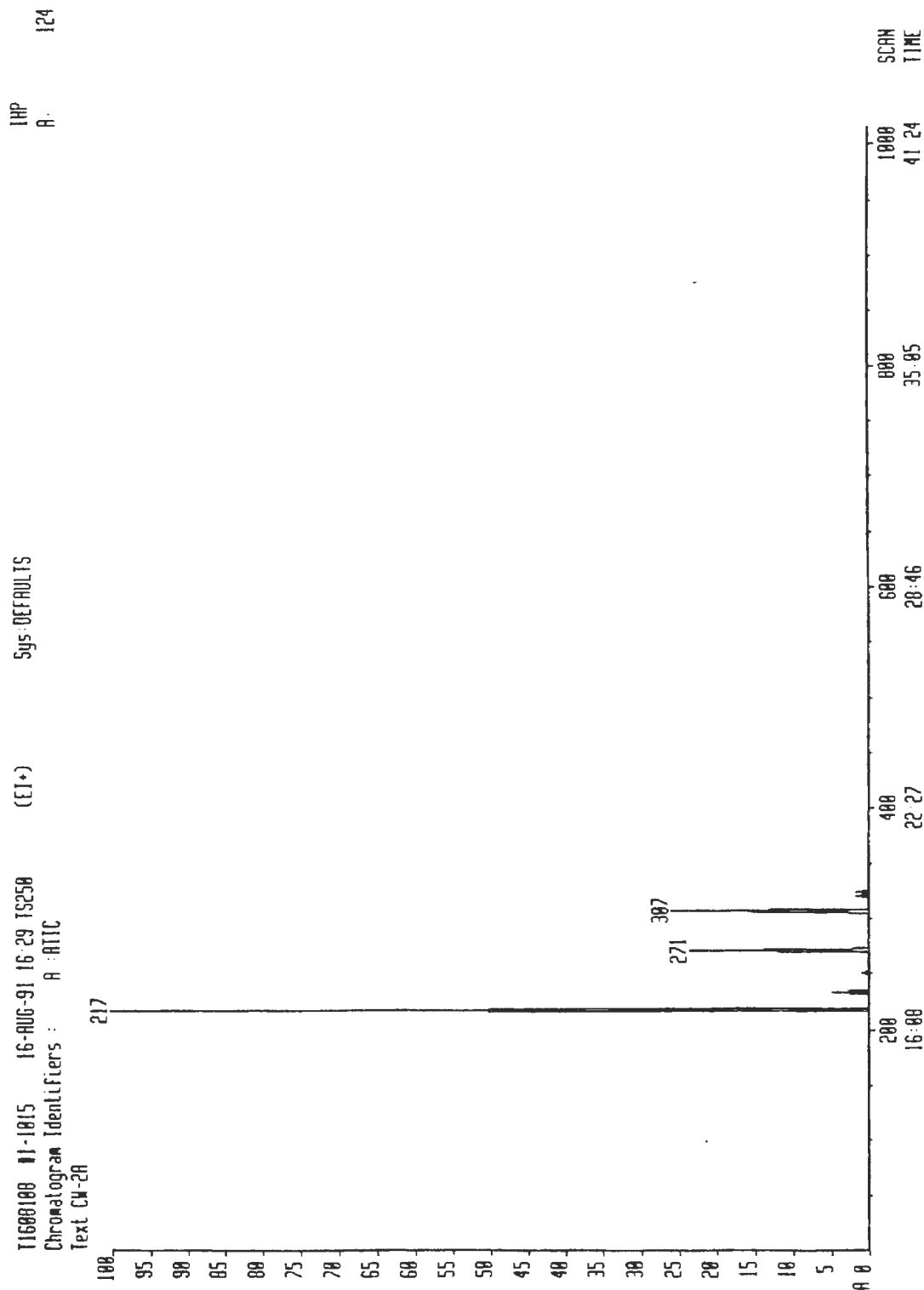
IHP
R: B

Sys-DEFAULTS

(EI+)

11600107 01-1015 16-AUG-91 15:45 TS250
Chromatogram Identifiers : A ATIC
Text:CU-IR2





LIBFITS1#1* x1 Bgd=217 T1608108
 2,3-HEPTADIEN-5-YNE, 2,4-DIMETHYL-
 C9.H12.

+0:00:00

p793 M793 r946 RFN:41898-89-9

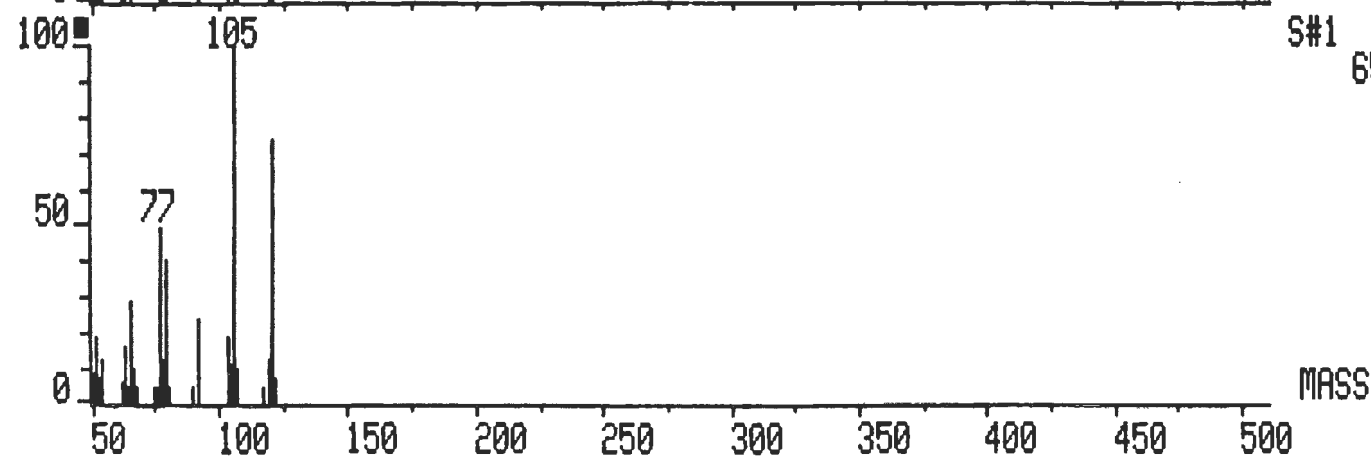
Lib:NBS

3115 Bpk: 105 Mwt: 120

#217 1.0
 465000



S#1 1.0
 65535000



T0509102 #1-1031

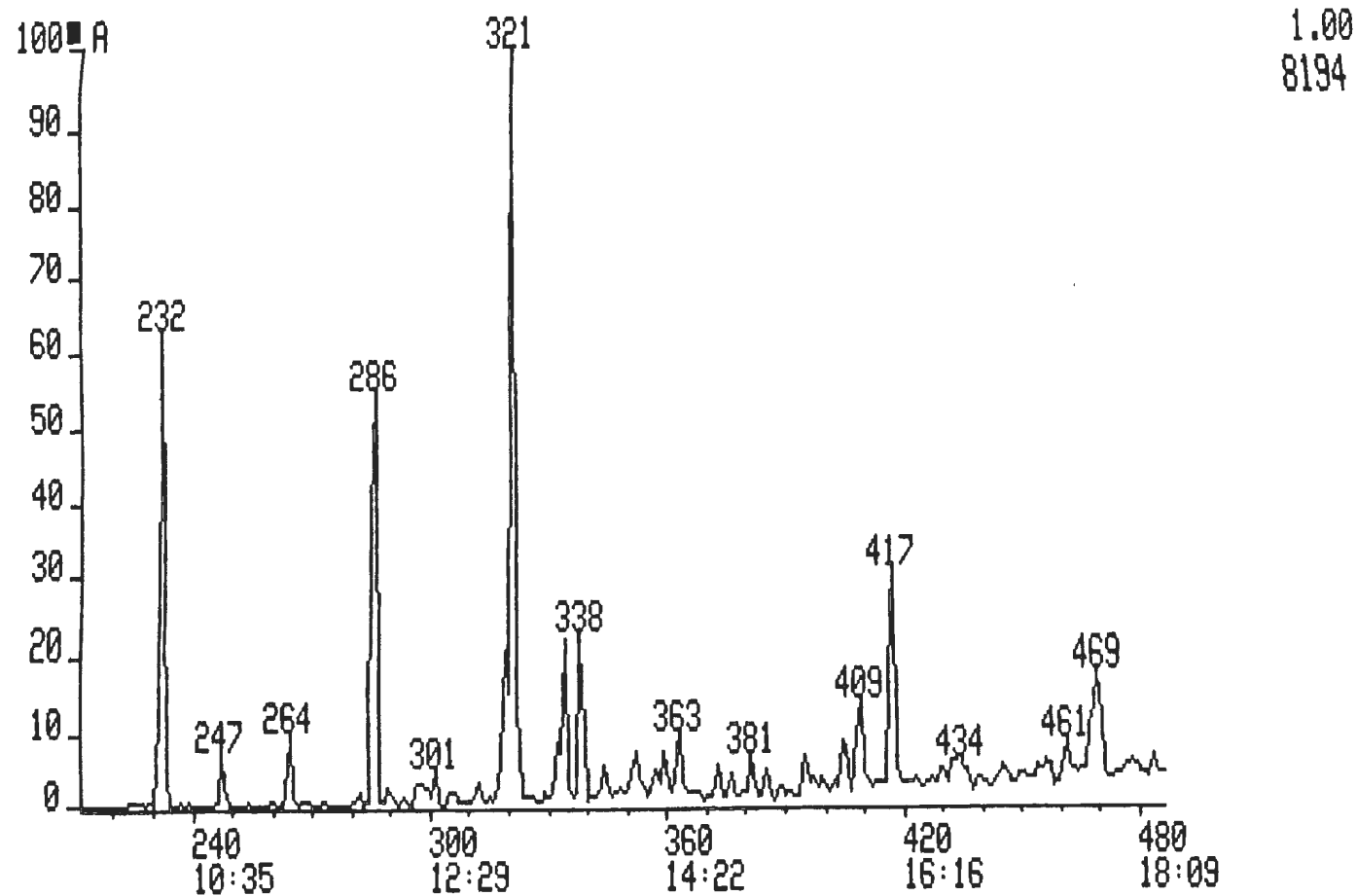
5-SEP-91 11:32 TS250

(EI+)

Sqs:DEFAULTS

A:ATIC

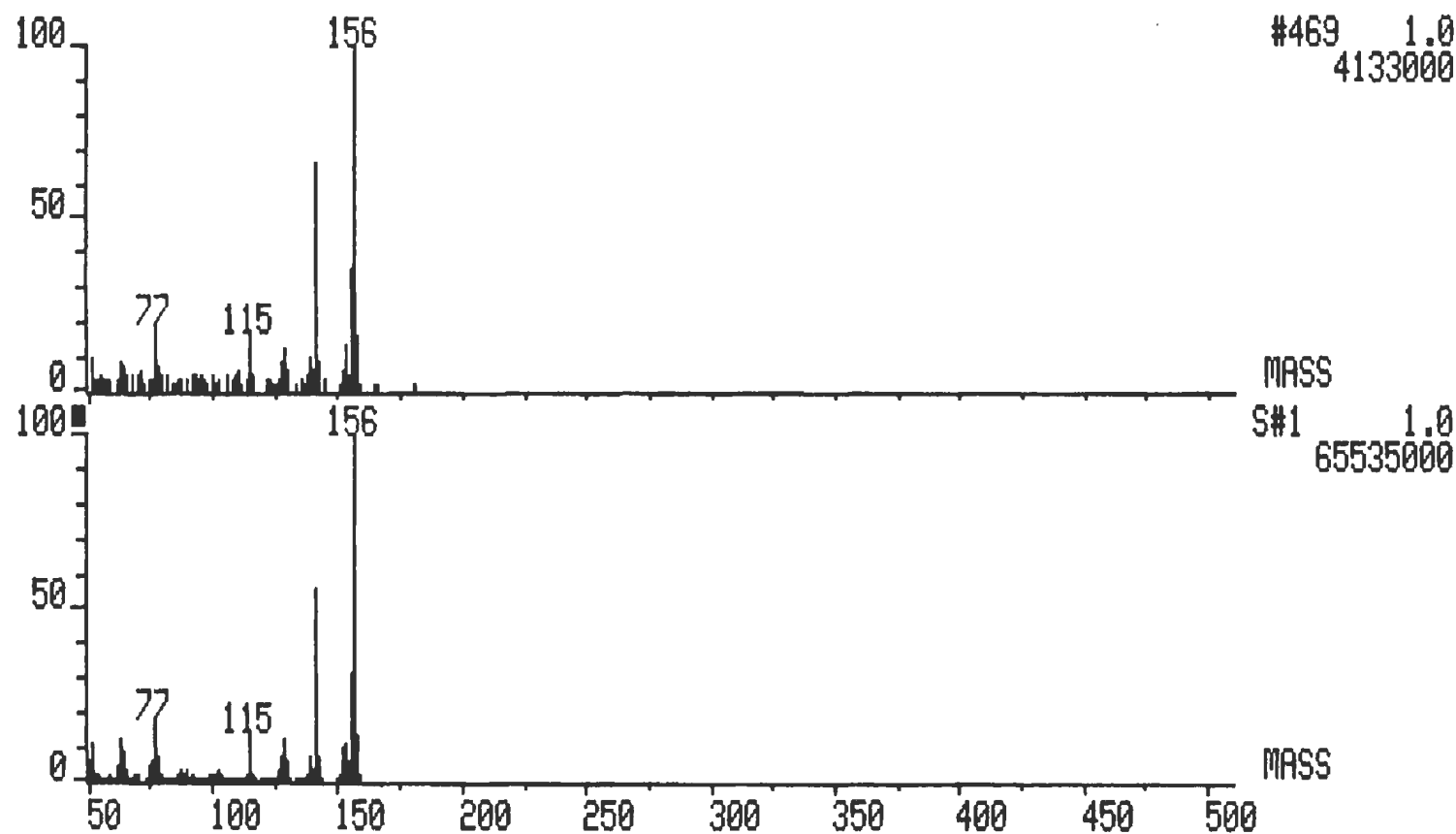
Text: CW 2A3



LIBFITS1#1* x1 Bgd=469 T0509102
NAPHTHALENE, 1,5-DIMETHYL-
C12.H12.

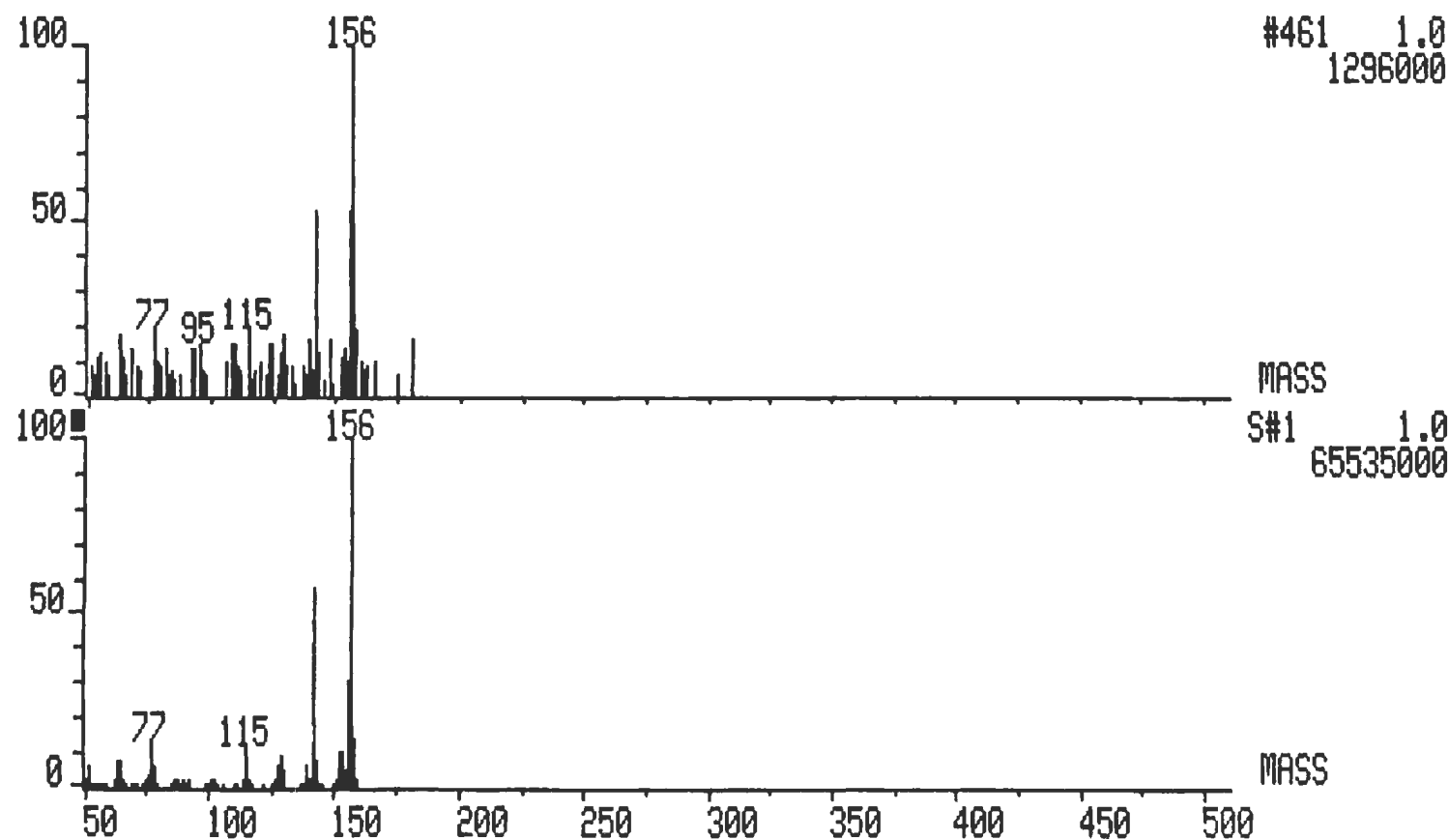
+0:00:00
p862 M887 r902 RFN:571-61-9
Lib:NBS 8745 Bpk: 156 Mwt: 156

#469 1.0
4133000



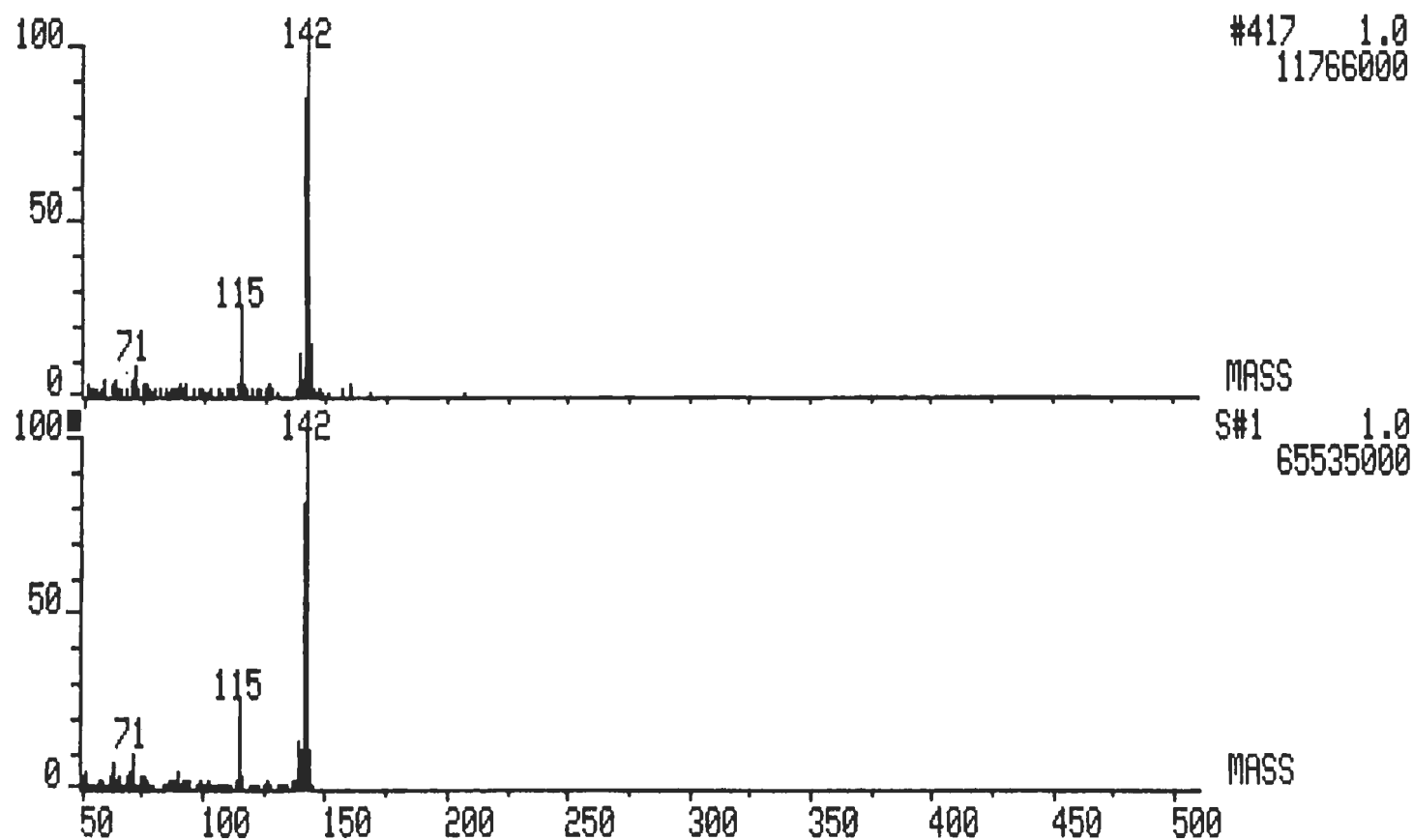
LIBFITS2#1* x1 Bgd=461 T0509102
NAPHTHALENE, 1,6-DIMETHYL-
C12.H12.

+0:00:00
p581 M823 r606 RFN:575-43-9
Lib:NBS 8749 Bpk: 156 Mwt: 156



LIBFITS1#1* x1 Bgd=417 T0509102
NAPHTHALENE, 1-METHYL-
C11.H10.

+0:00:00
p910 M944 r933 RFN:90-12-0
Lib:NBS 6111 Bpk: 142 Mwt: 142



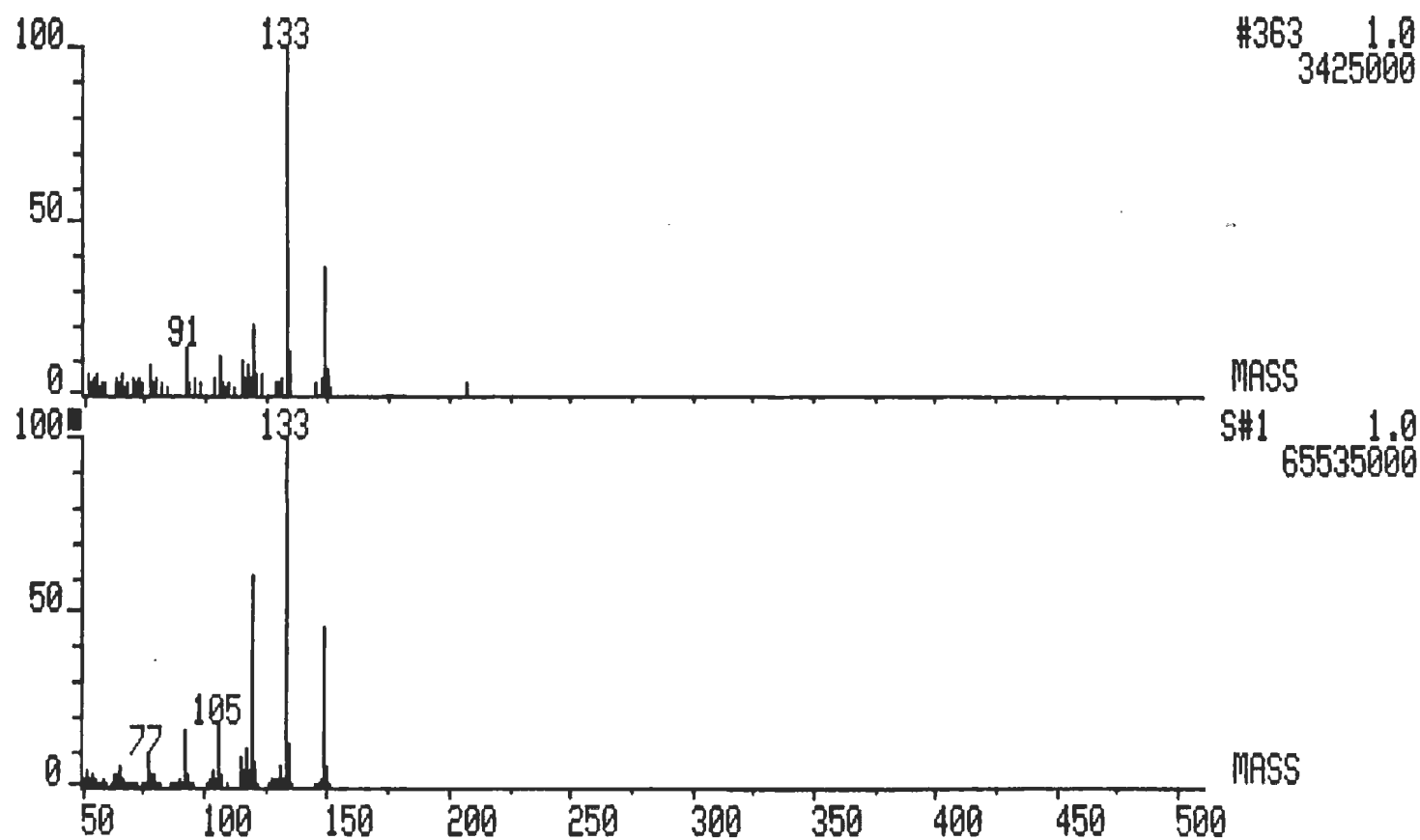
LIBFITS2#1* x1 Bgd=363 T0509102
 BENZENE, DIETHYLMETHYL-
 C11.H16.

+0:00:00

p864 M901 r900 RFN:25550-13-4

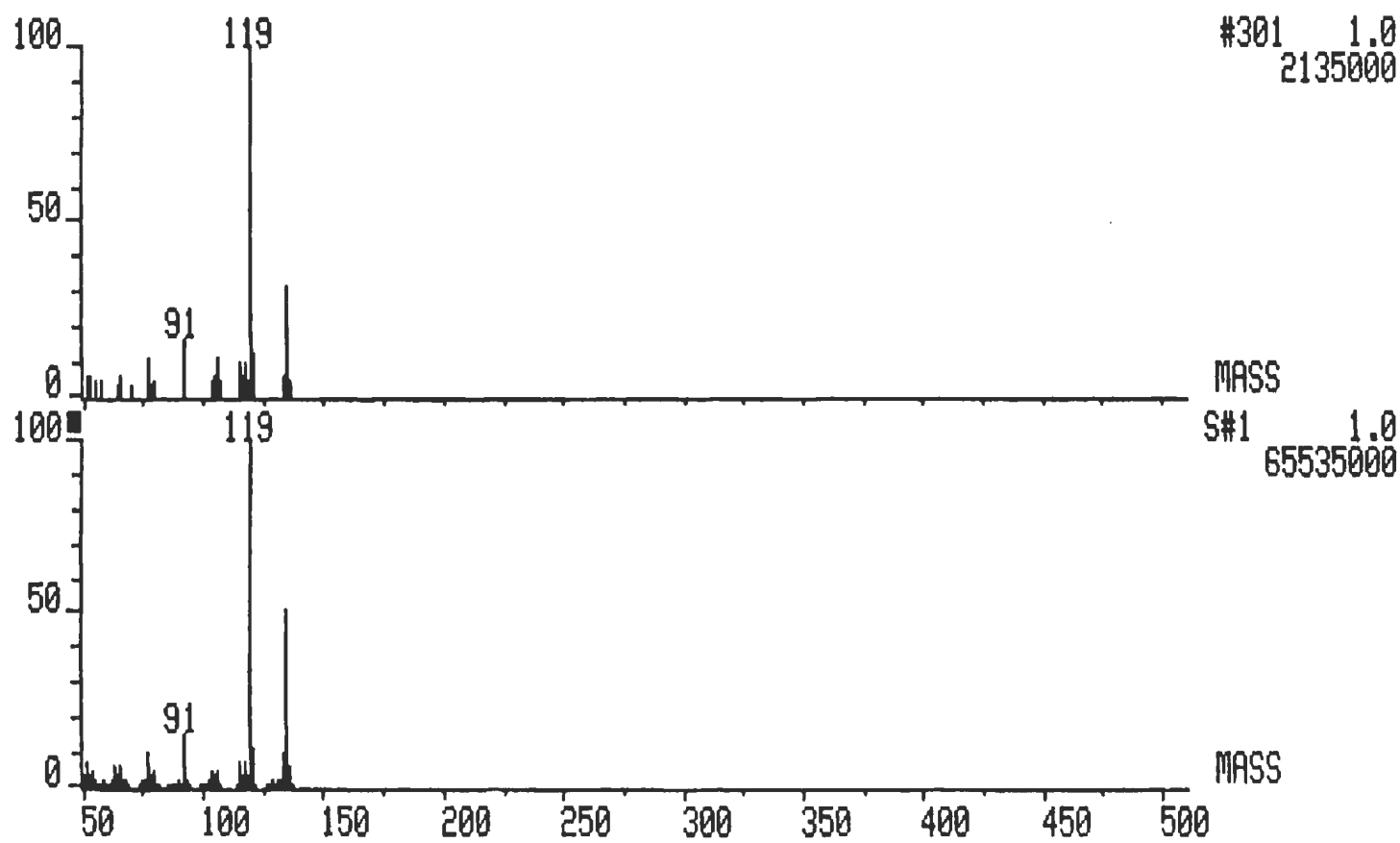
Lib:NBS

7187 Bpk: 133 Mwt: 148



LIBFITS1#1* x1 Bgd=301 T0509102
BENZENE, 1,2,3,4-TETRAMETHYL-
C10.H14.

+0:00:00
p862 M867 r962 RFN:488-23-3
Lib:NBS 4775 Bpk: 119 Mwt: 134



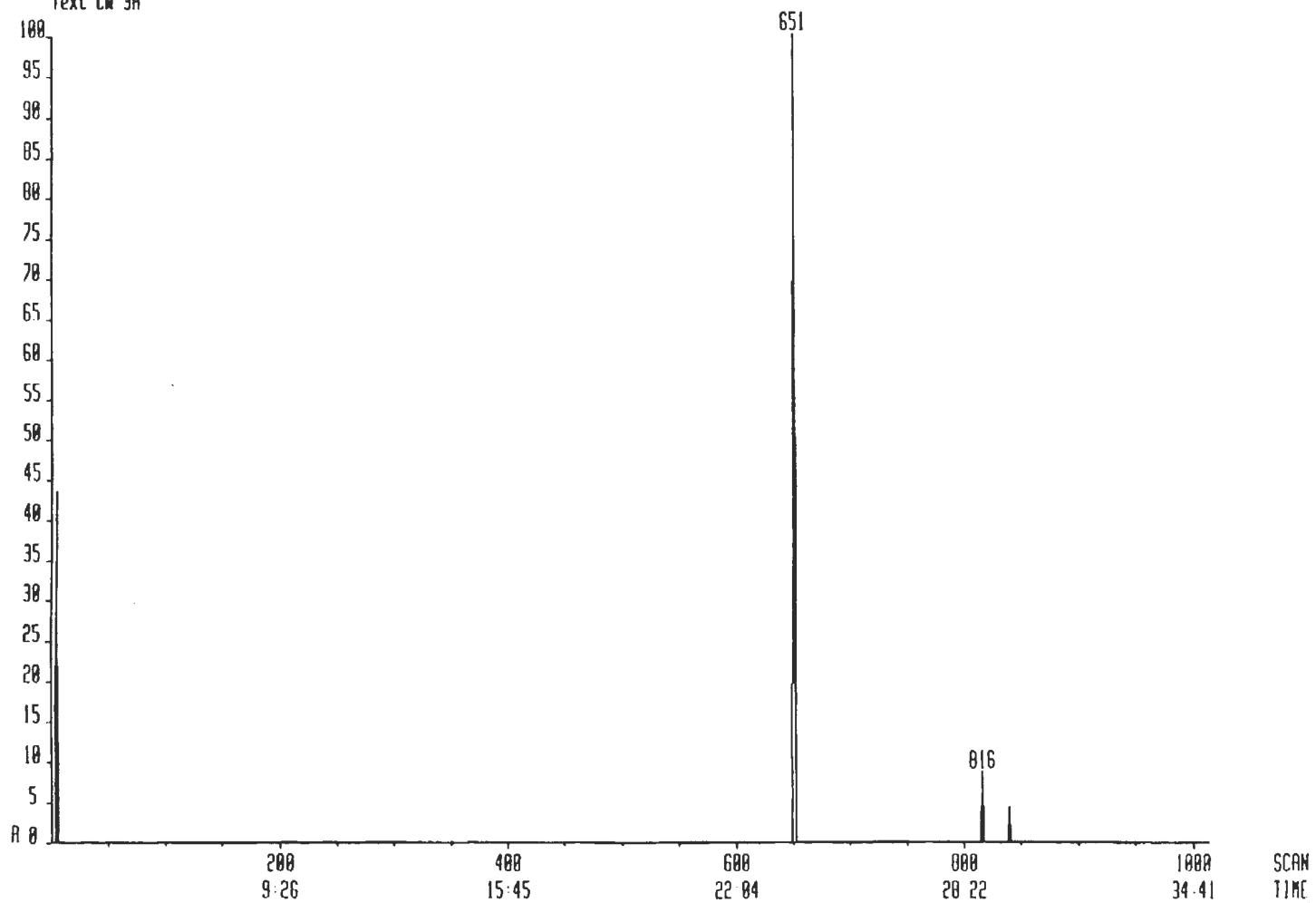
11900102 11-1014 19-AUG-91 10:01 TS250
Chromatogram Identifiers: A:ATIC
Text: CW 3A

(E1+)

Sys: DEFAULTS

IHP
A

23



12000101 11-1014 20-AUG-91 00:55 TS250

(EI+)

Sys DEFAULTS

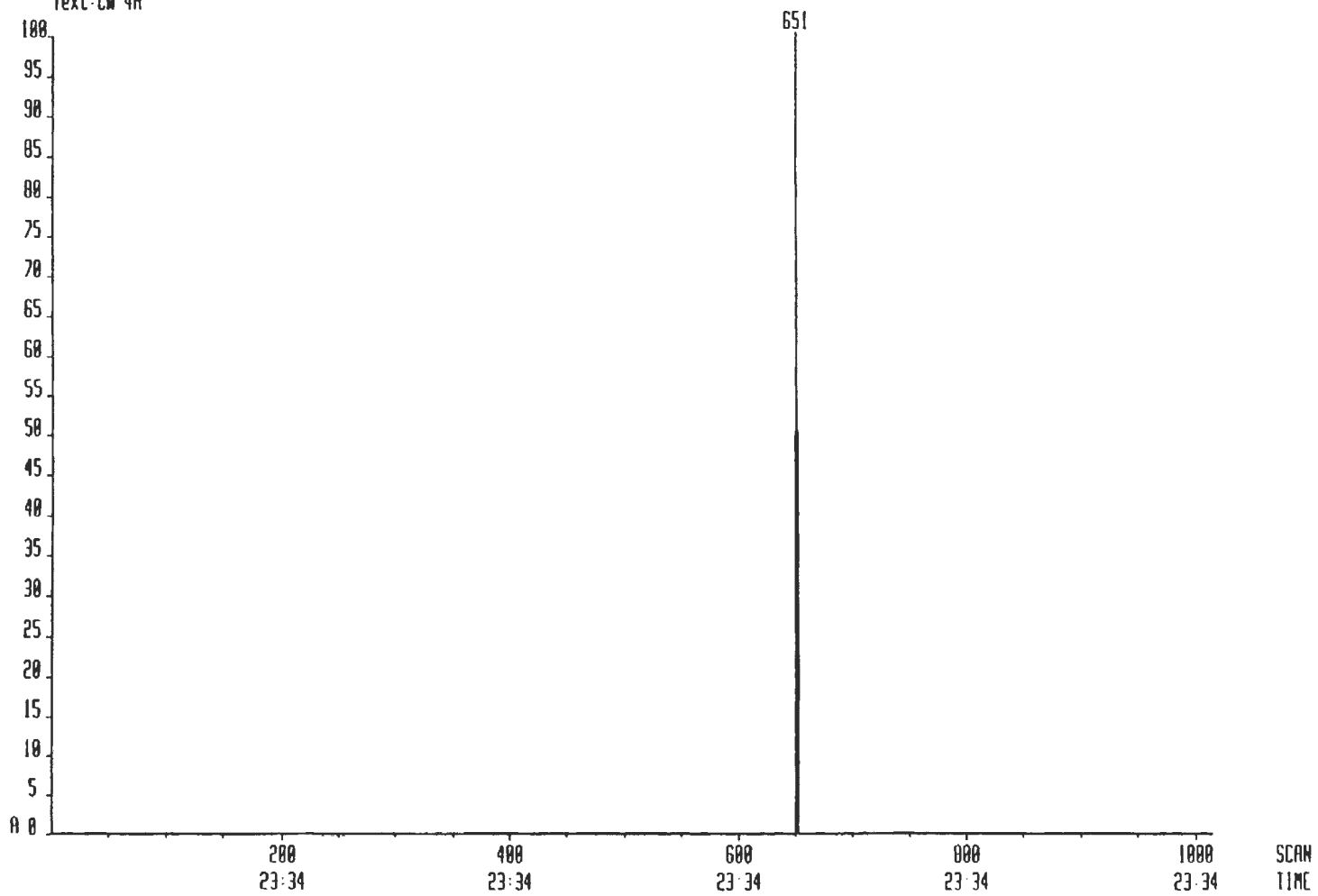
IHP

Chromatogram Identifiers: A: ATIC

R:

4

Text: CM 4A



T1307205 #1-1553

13-JUL-92 16:12 TS250

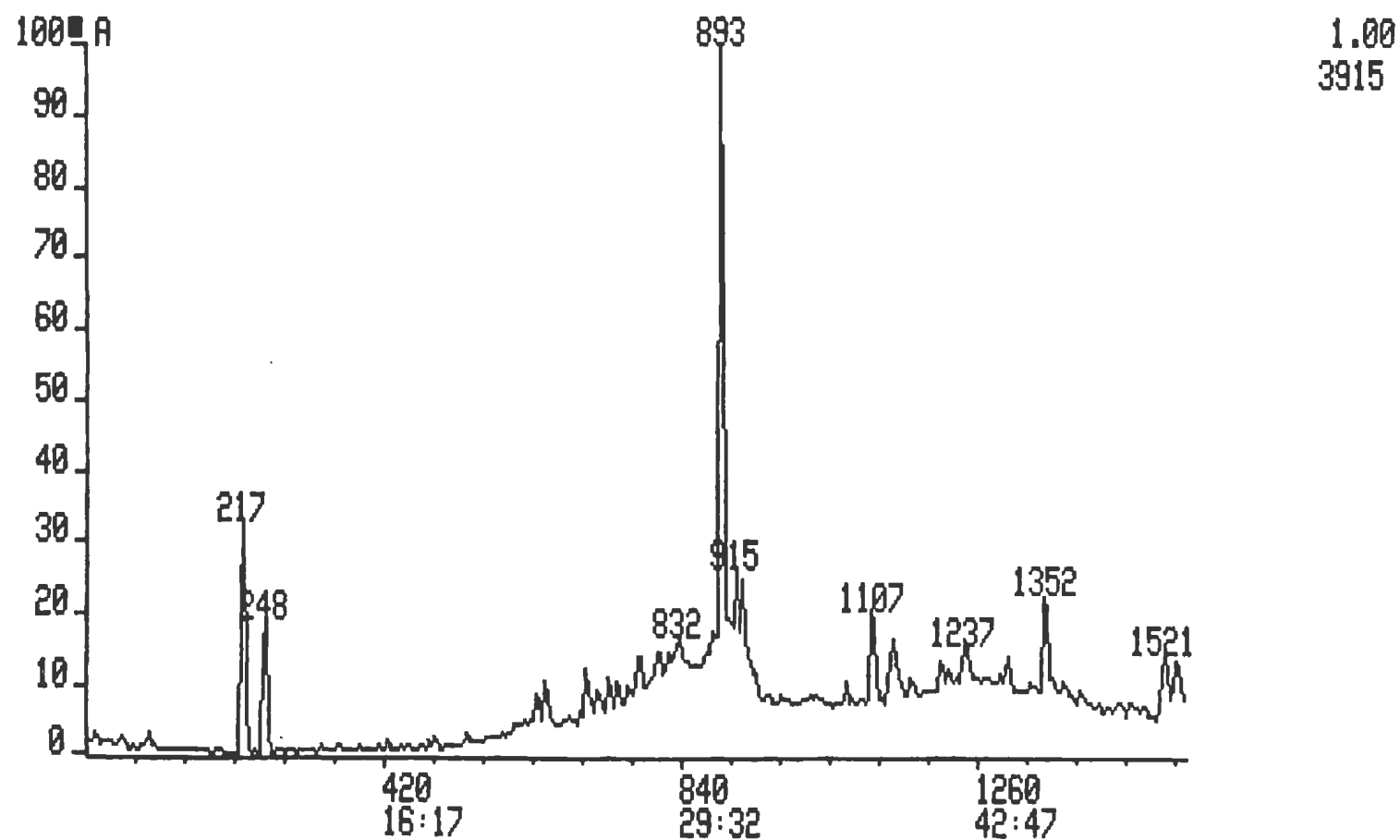
(EI+)

Sqs:DEFAULTS

A:ATIC

Text:C5-1A-1

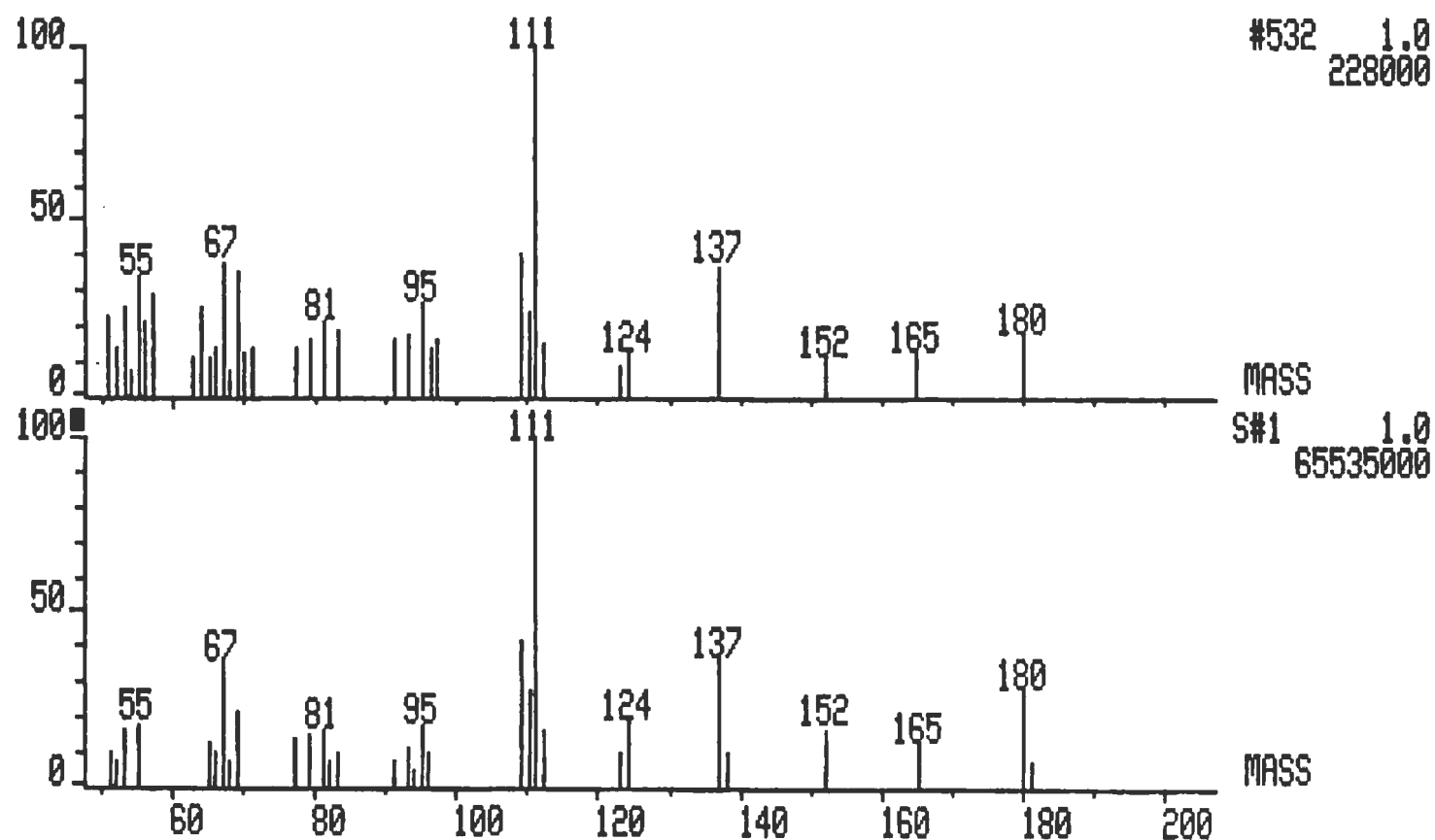
>



LIBFITS1#1* x1 Bgd=532 T1307205 +0:00:00

2(4H)-BENZOFURANONE, 5,6,7,7A-TETRAHYDRO-4,4,7A-T p735 M834 r848 RFN:17092-92-1

C11.H16.O2. Lib:NBS 13084 Bpk: 111 Mwt: 180



T1407203 #1-1775

14-JUL-92 11:47 TS250

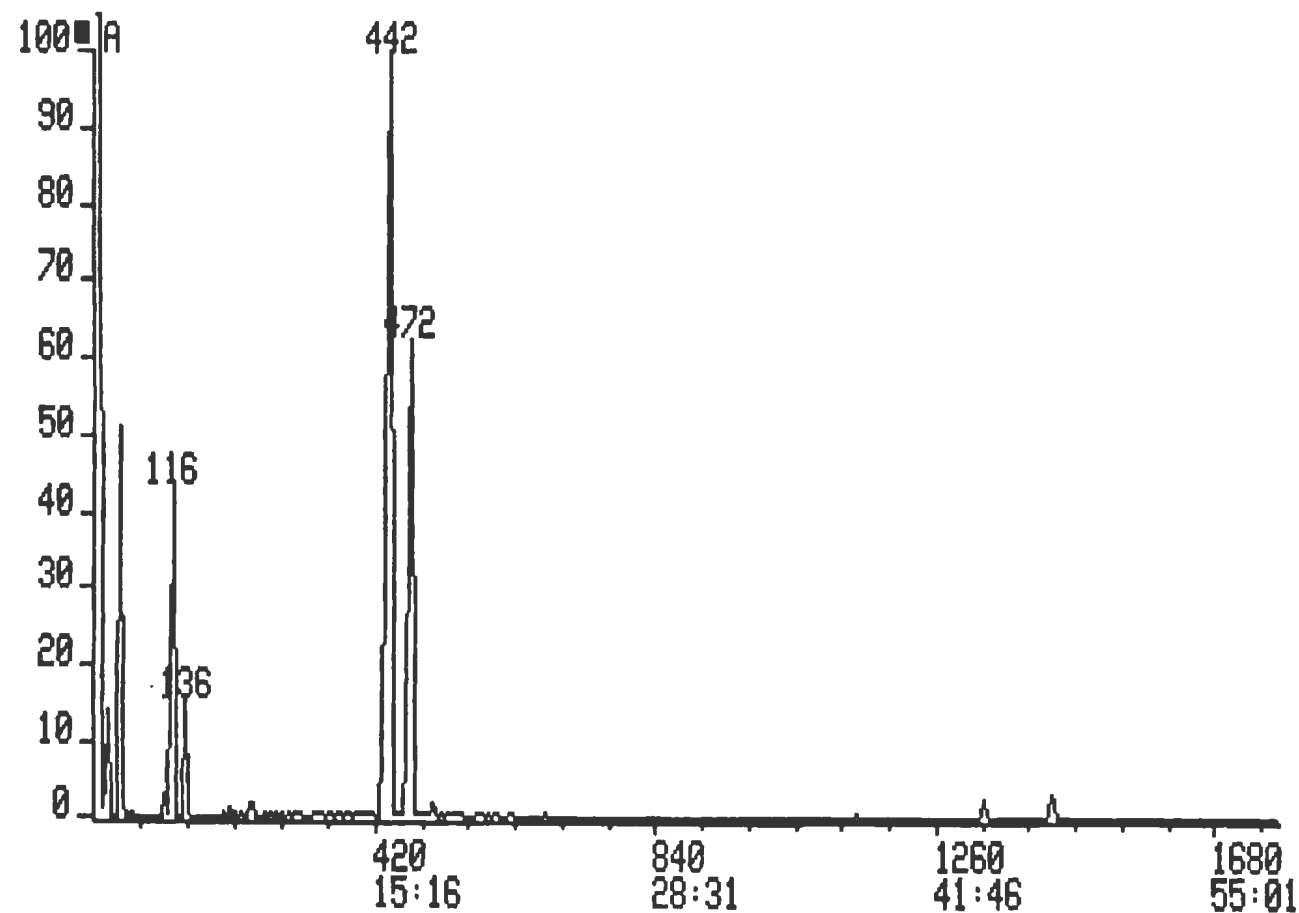
(EI+)

Sys:DEFAULTS

>

A:ATIC

Text:CS-1B-2

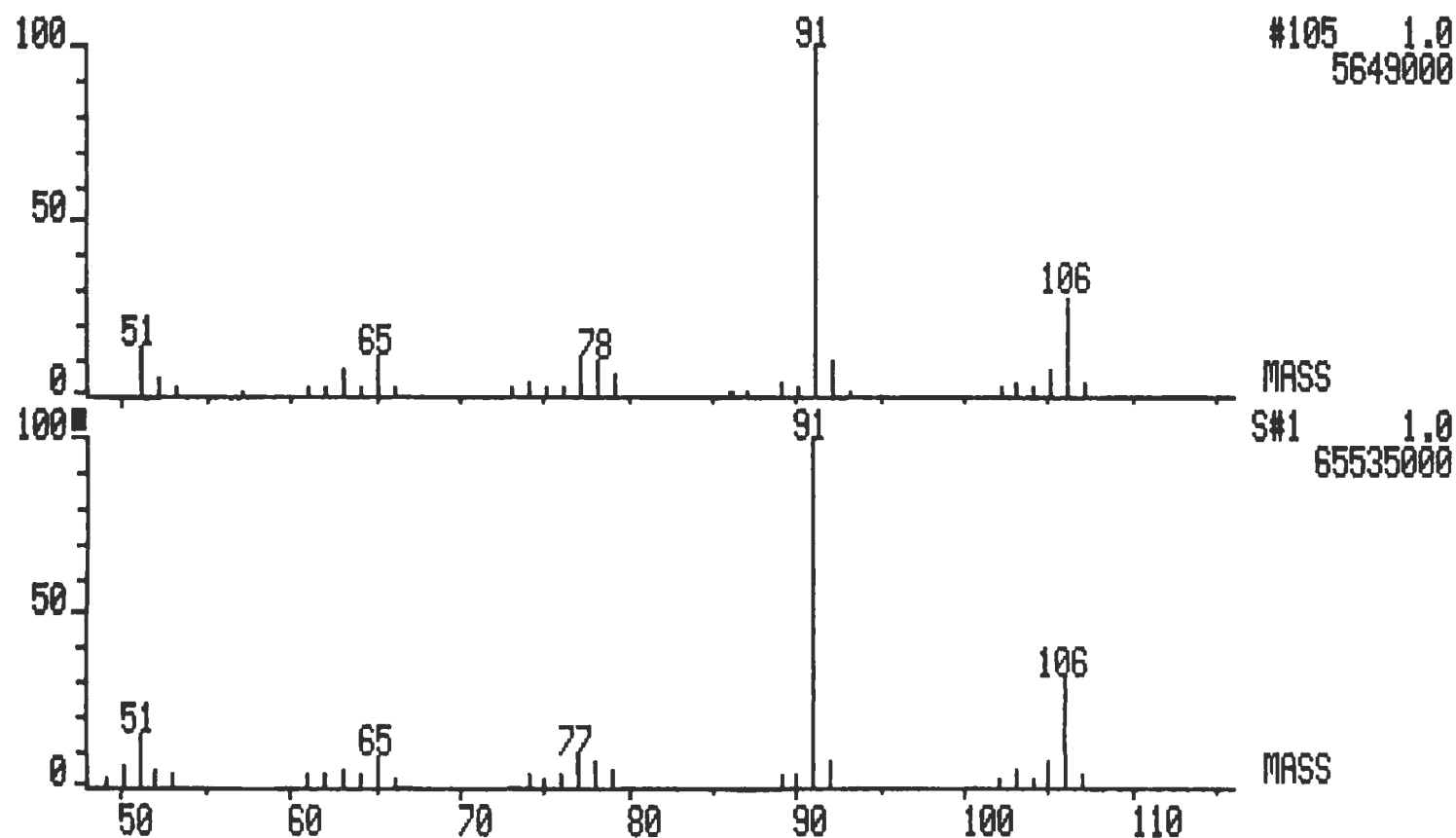


1.00
27943

LIBFITS1#1* x1 Bgd=105 T1407203
BENZENE, ETHYL-
C8.H10.

+0:00:00

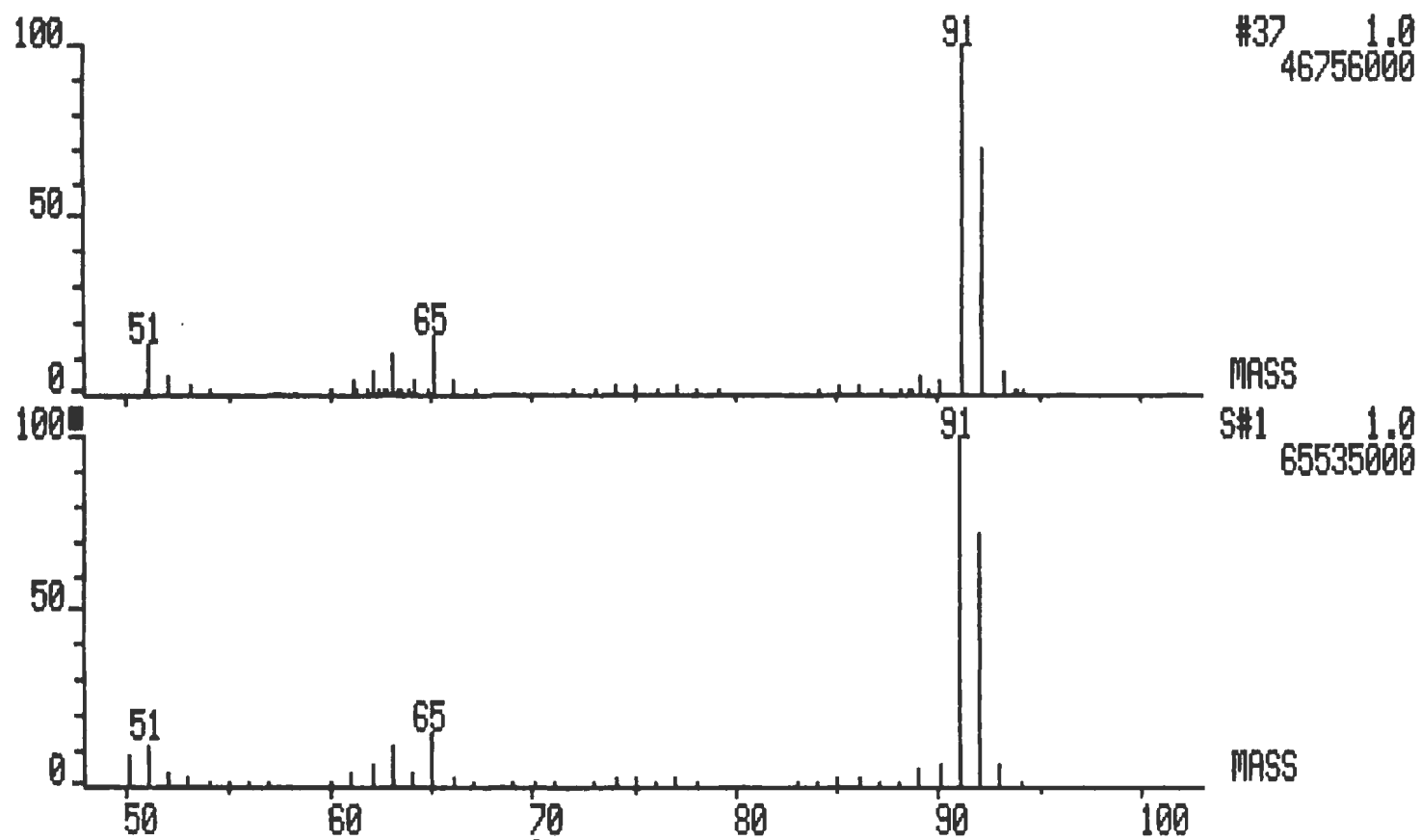
Lib:NBS p934 M946 r981 RFN:100-41-4
1729 Bpk: 91 Mwt: 106



LIBFITS2#1* x1 Bgd=37 T1407203
BENZENE, METHYL-
C7.H8.

+0:00:00

Lib:NBS p910 M911 r995 RFN:108-88-3
854 Bpk: 91 Mwt: 92



T1307204 #1-1616

13-JUL-92 15:13 TS250

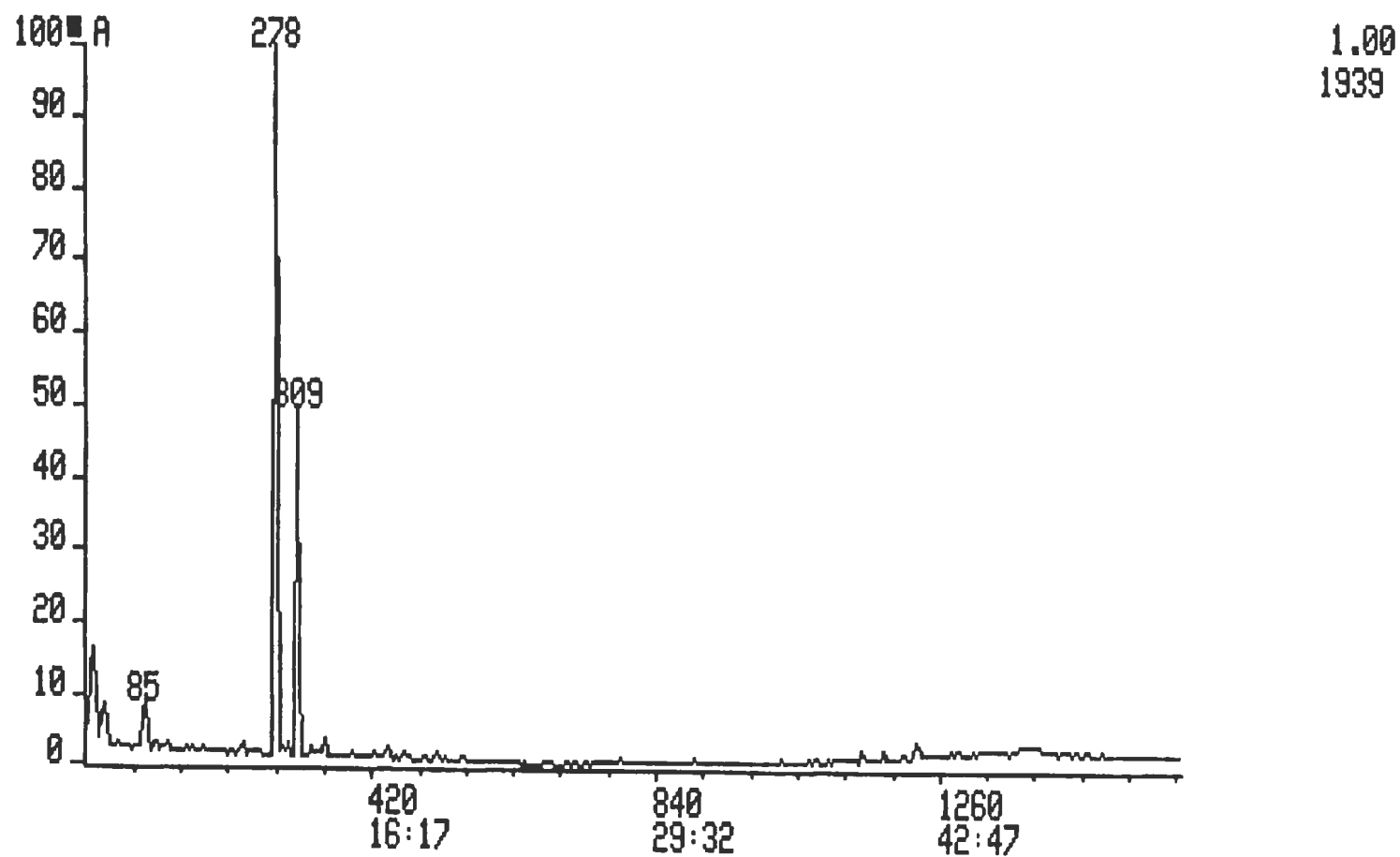
(EI+)

Sqs:DEFAULTS

A:ATIC

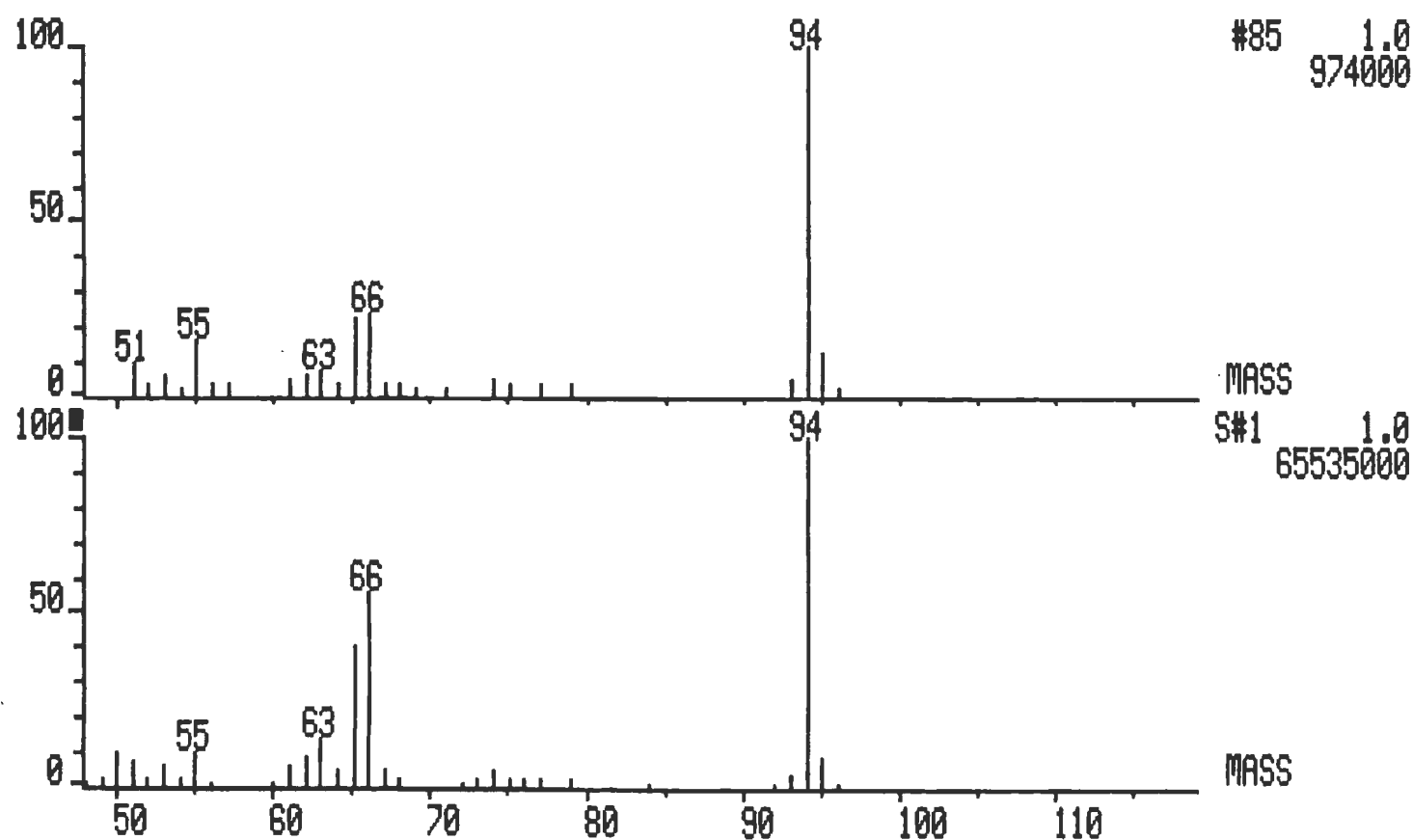
Text:C5-1A-3

>



LIBFITS1#1* x1 Bgd=85 T1307204
BENZENESULFONIC ACID, 4-HYDROXY-
C6.H6.O4.S.

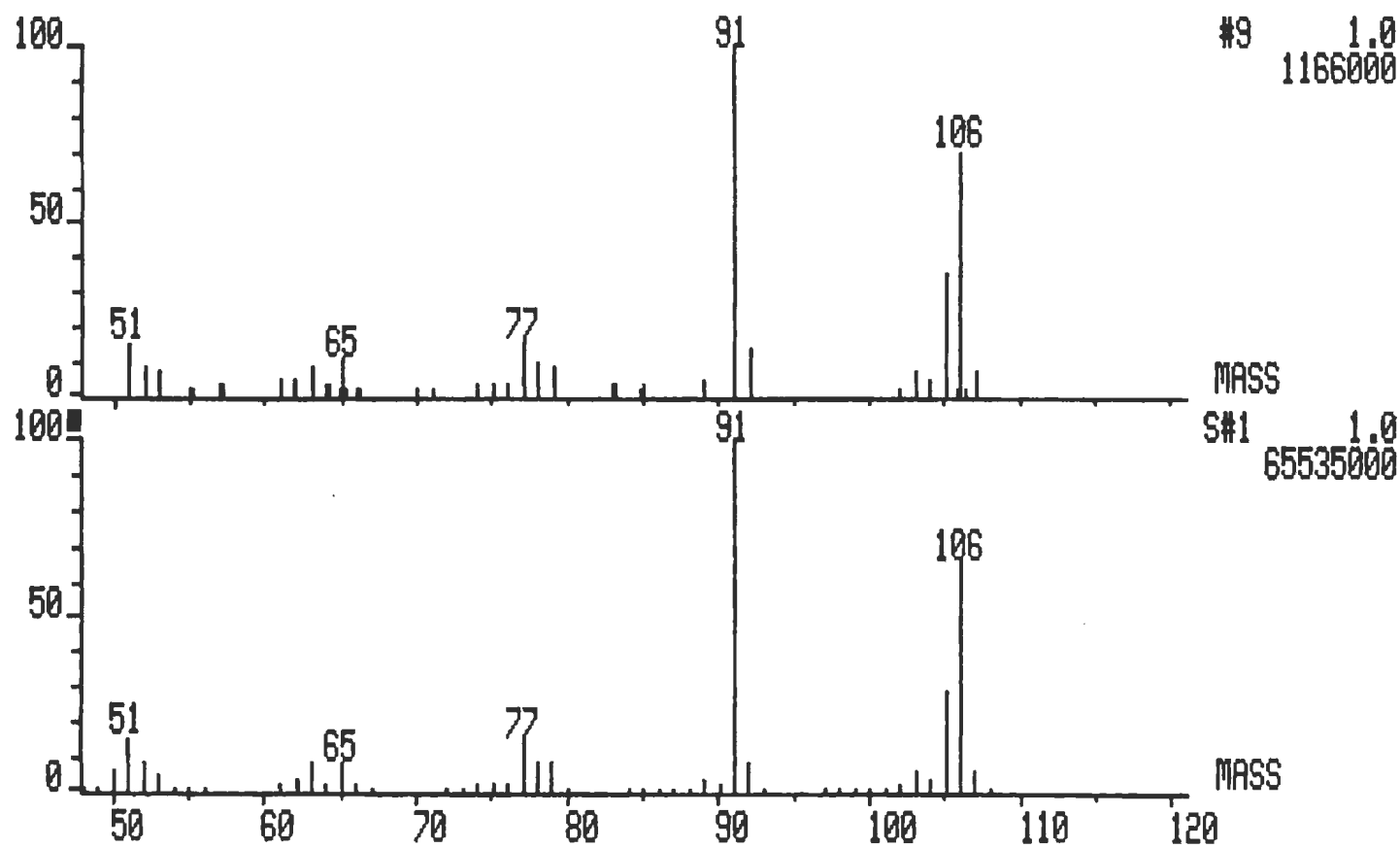
+0:00:00
p577 M831 r660 RFN:98-67-9
Lib:NBS 12146 Bpk: 94 Mwt: 174



LIBFITS1#1* x1 Bgd=9 T1307204
BENZENE, 1,3-DIMETHYL-
C8.H10.

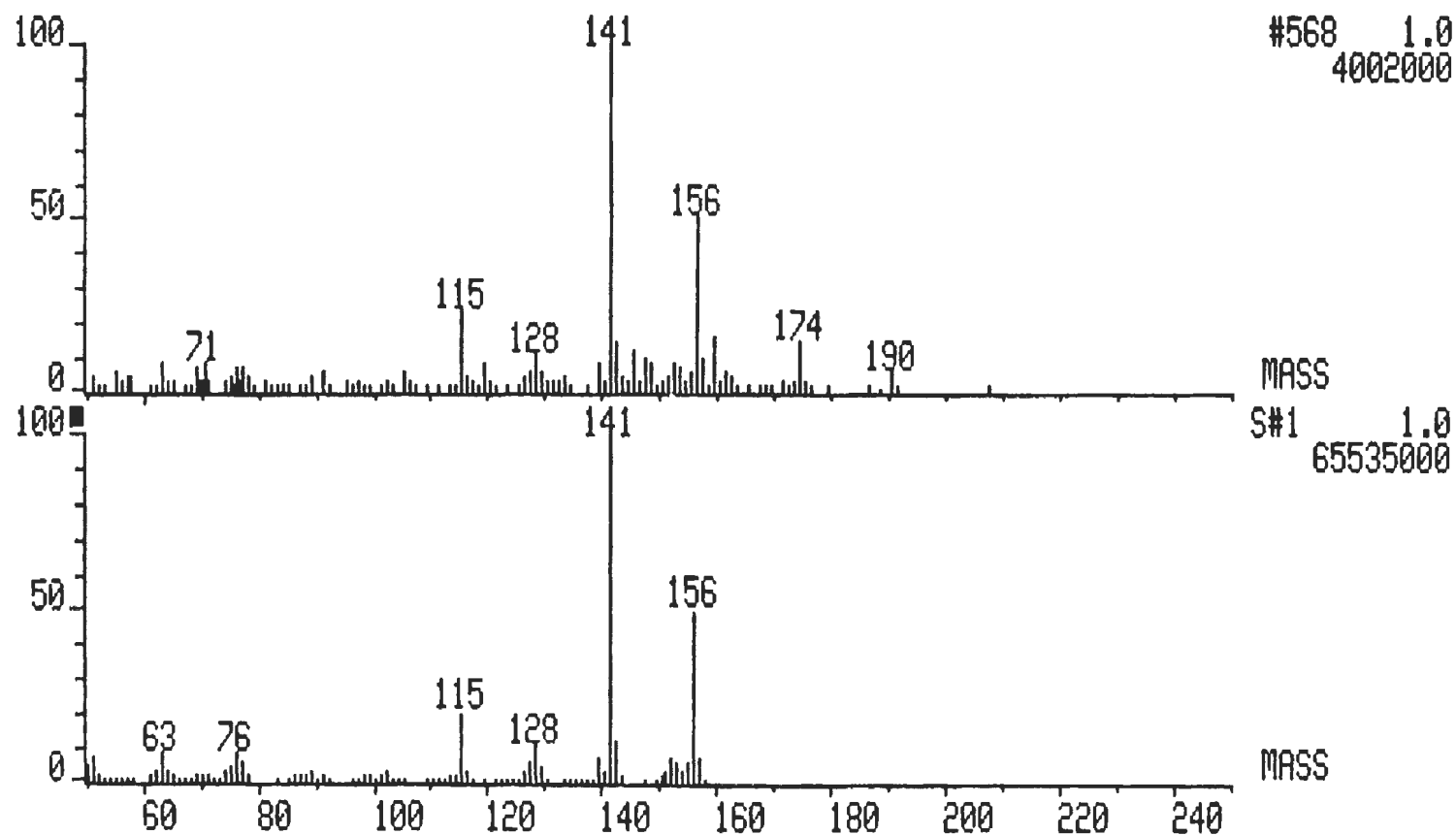
+0:00:00

p636 M919 r679 RFN:108-38-3
Lib:NBS 1731 Bpk: 91 Mwt: 106

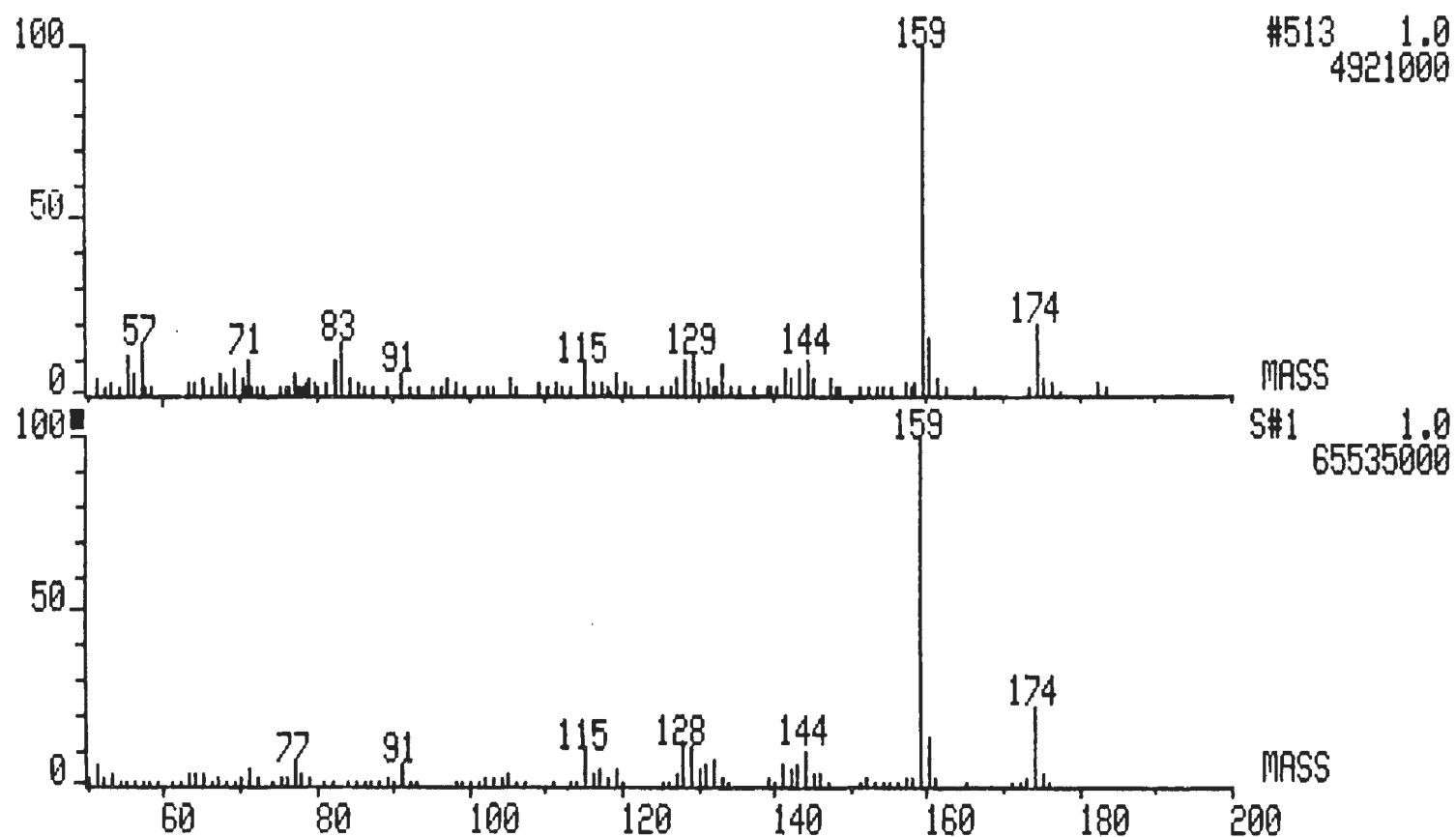


LIEFITS1#1* x1 Bgd=568 T1002201
NAPHTHALENE, 1-ETHYL-
C12.H12.

+0:00:00
p652 M875 r668 RFN:00-00-0
Lib:NBS 8754 Bpk: 141 Mwt: 156



LIBFITS2#1* x1 Bgd=513 T1002201 +0:00:00
 NAPHTHALENE, 1,2,3,4-TETRAHYDRO-1,5,8-TRIMETHYL- p785 M862 r810 RFN:21693-51-6
 C13.H18. Lib:NBS 12114 Bpk: 159 Mwt: 174



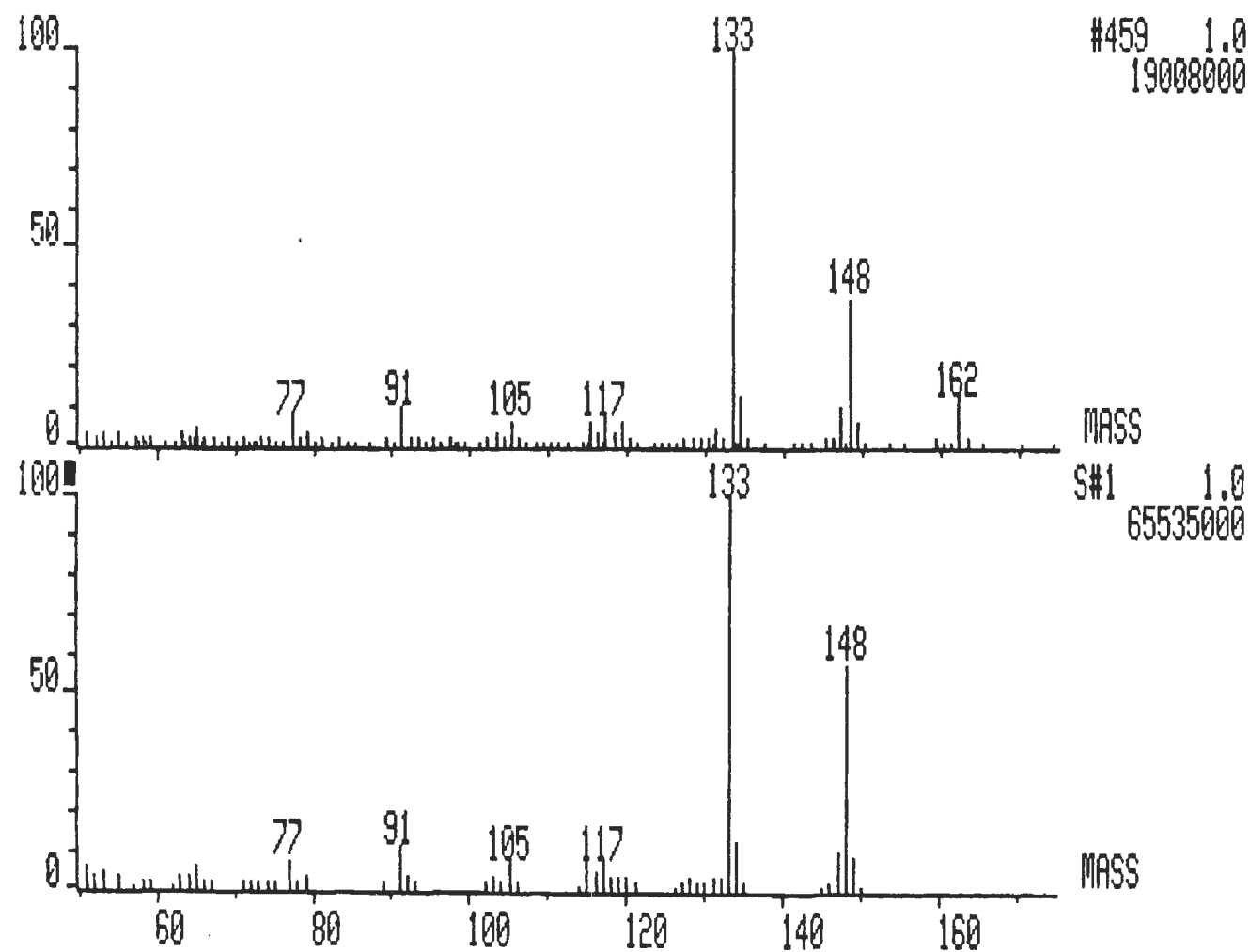
LIBFITS1#1* x1 Bgd=459 T1002201
BENZENE, PENTAMETHYL-
C11.H16.

+0:00:00

p848 M966 r863 RFN:700-12-9

Lib:NBS

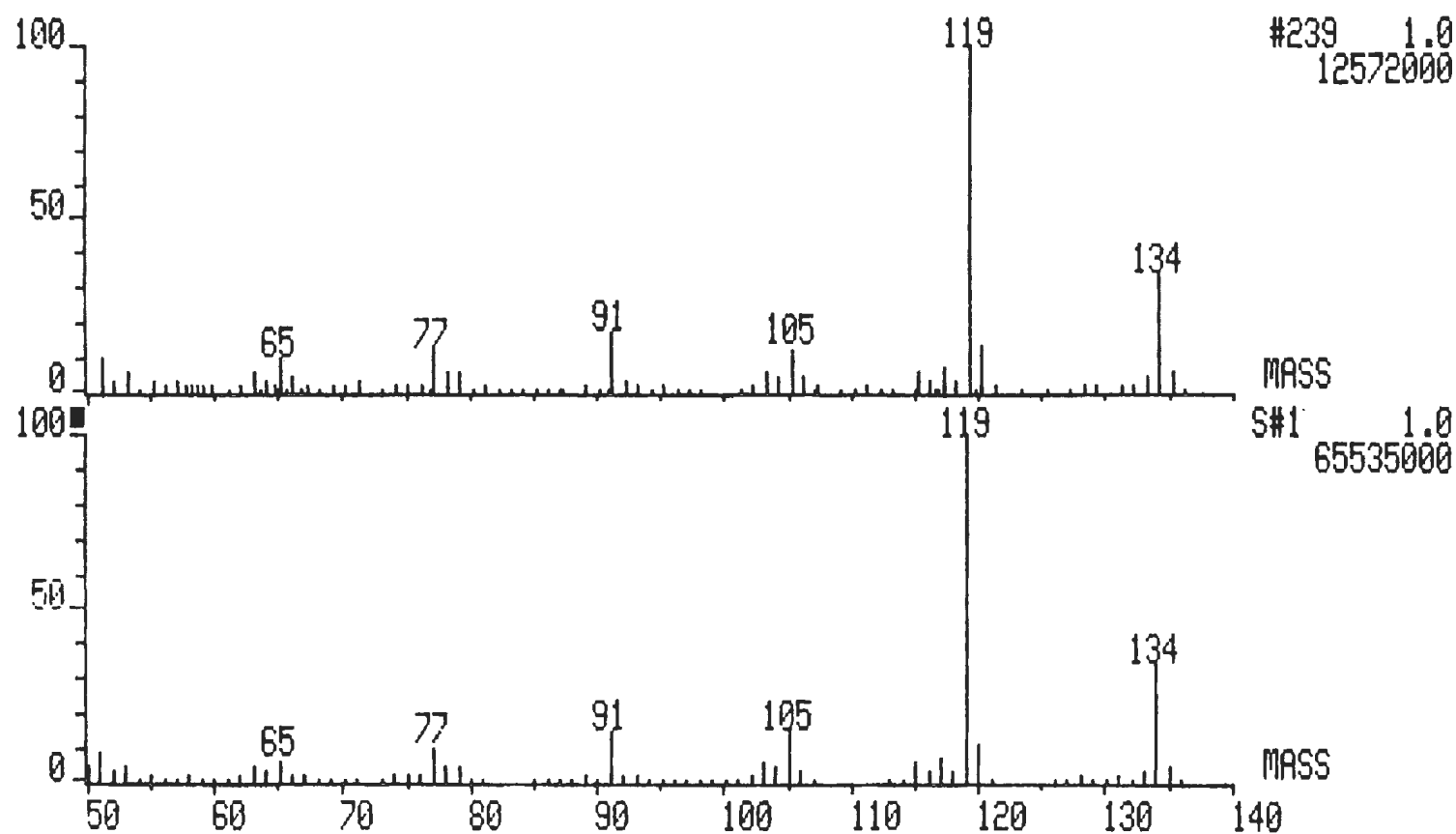
7169 Bpk: 133 Mwt: 148



LIBFITS2#1* x1 Bgd=239 T1002201
BENZENE, 2-ETHYL-1,4-DIMETHYL-
C10.H14.

+0:00:00

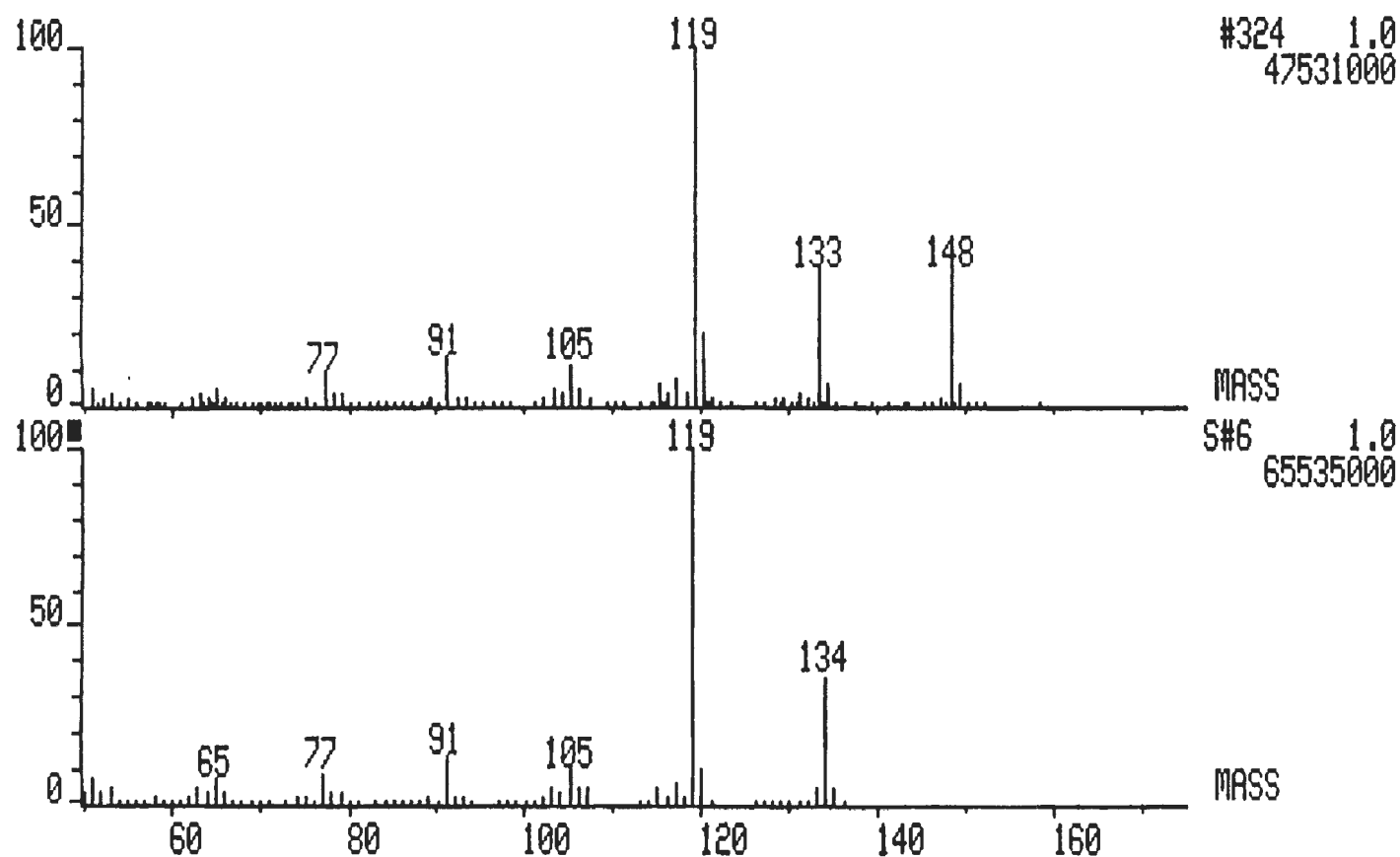
p925 M940 r961 RFN:1758-88-9
Lib:NBS 4787 Bpk: 119 Mwt: 134



LIBFITS2#6* x1 Bgd=324 T1002201
BENZENE, 1-ETHYL-3,5-DIMETHYL-
C10.H14.

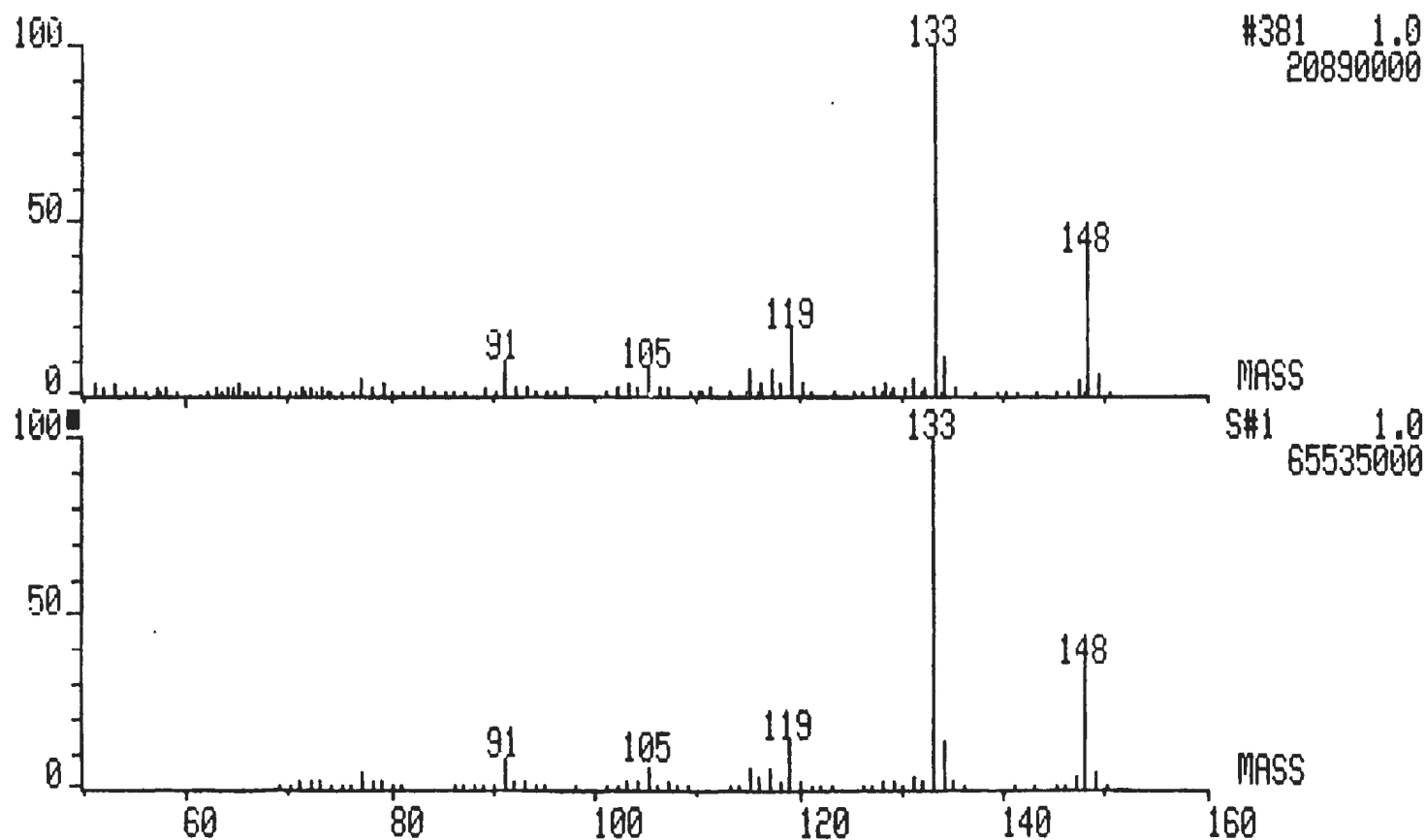
+0:00:00

Lib:NBS p656 M804 r679 RFN:934-74-7
4782 Bpk: 119 Mwt: 134



LIBFITS1#1* x1 Bgd=381 T1002201
BENZENE, ETHYL-1,2,4-TRIMETHYL-
C11.H16.

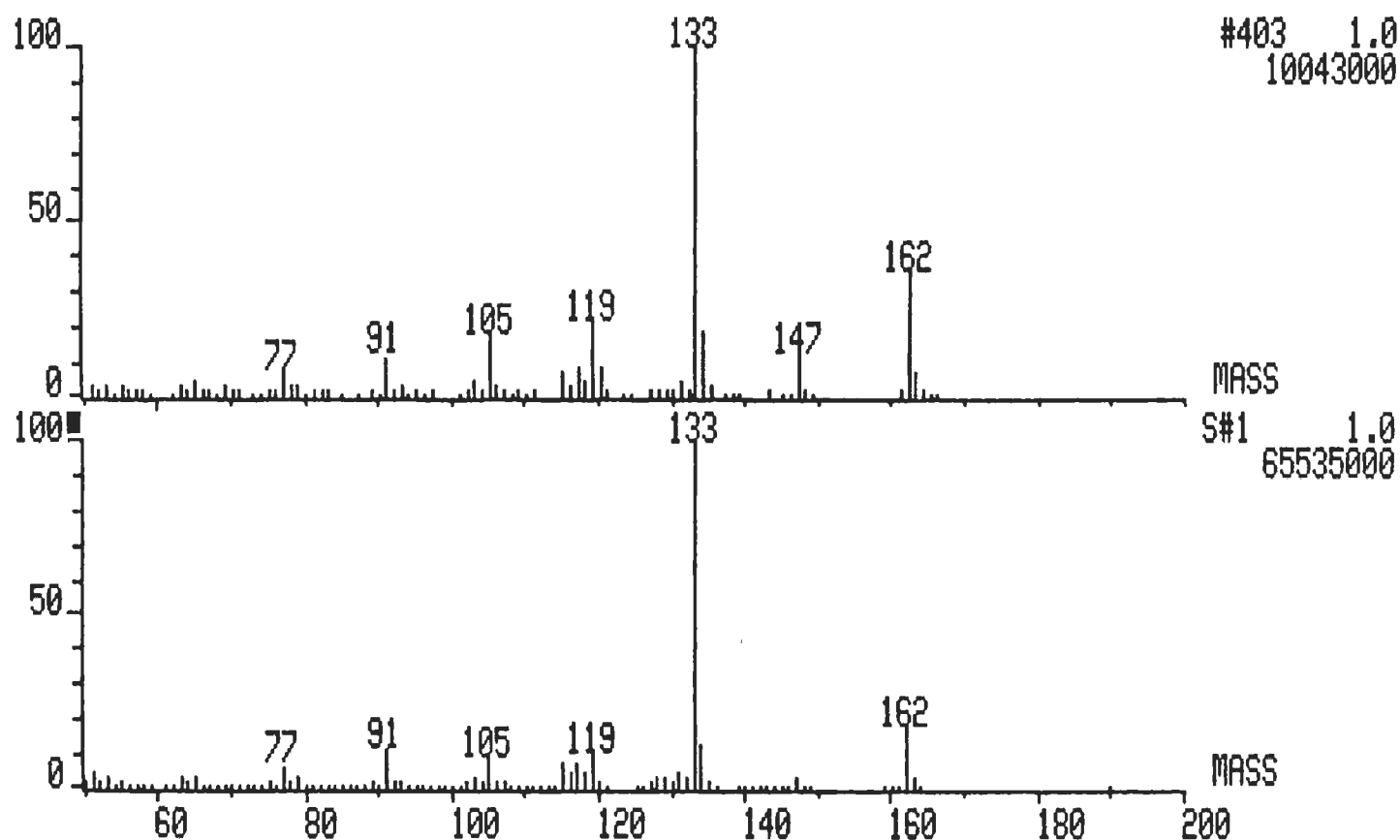
+0:00:00
p936 M989 r938 RFN:54120-62-6
Lib:NBS 7190 Bpk: 133 Mwt: 148



LIBFITS2#1* x1 Bgd=403 T1002201
BENZENE, 2,4-DIMETHYL-1-(1-METHYLPROPYL)-
C12.H18.

+0:00:00

Lib:NBS p888 M896 r920 RFN:1483-60-9
9865 Bpk: 133 Mwt: 162



T1903202 #1-1400

19-MAR-92 09:59 TS250

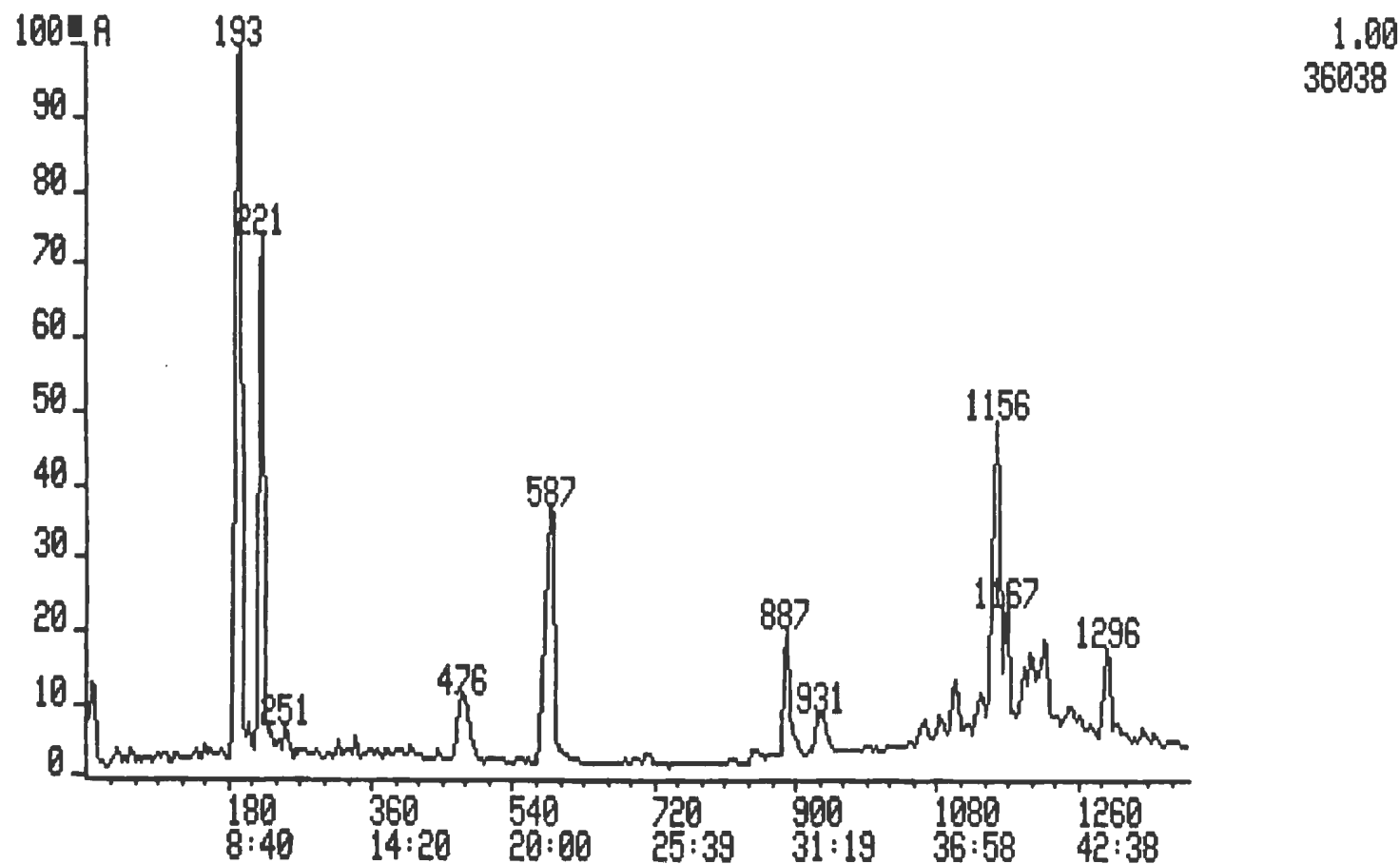
(EI+)

Sys:DEFAULTS

A:ATIC

Text:S-2-D-2

>



LIBFITS2#1* x1 Bgd=476 T1903202

+0:00:00

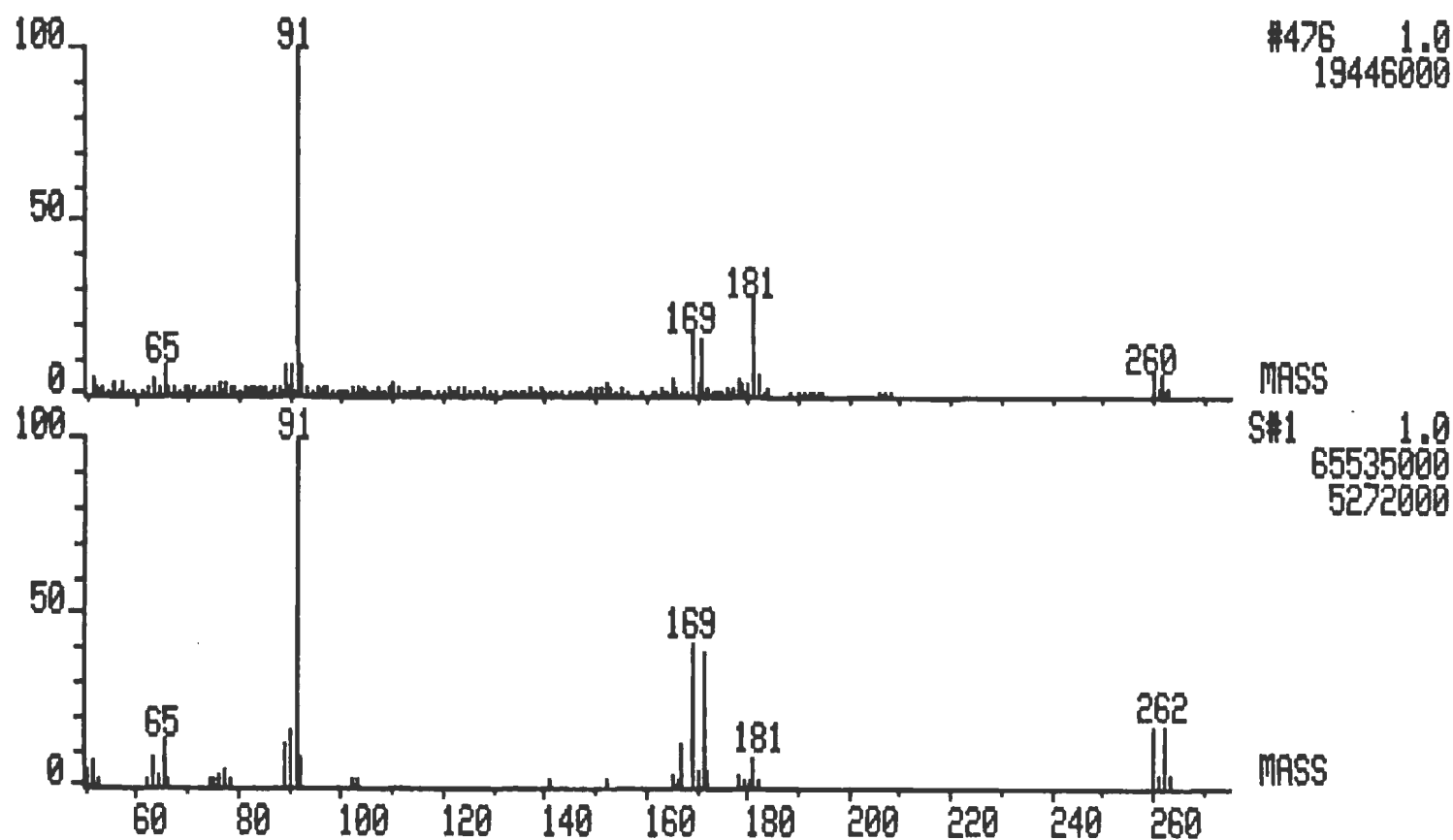
BENZENE, 1-BROMO-4-(2-PHENYLETHYL)-

p664 M845 r672 RFN:14310-24-8

C14.H13.BR.

Lib:NBS

24899 Bpk: 91 Mwt: 260

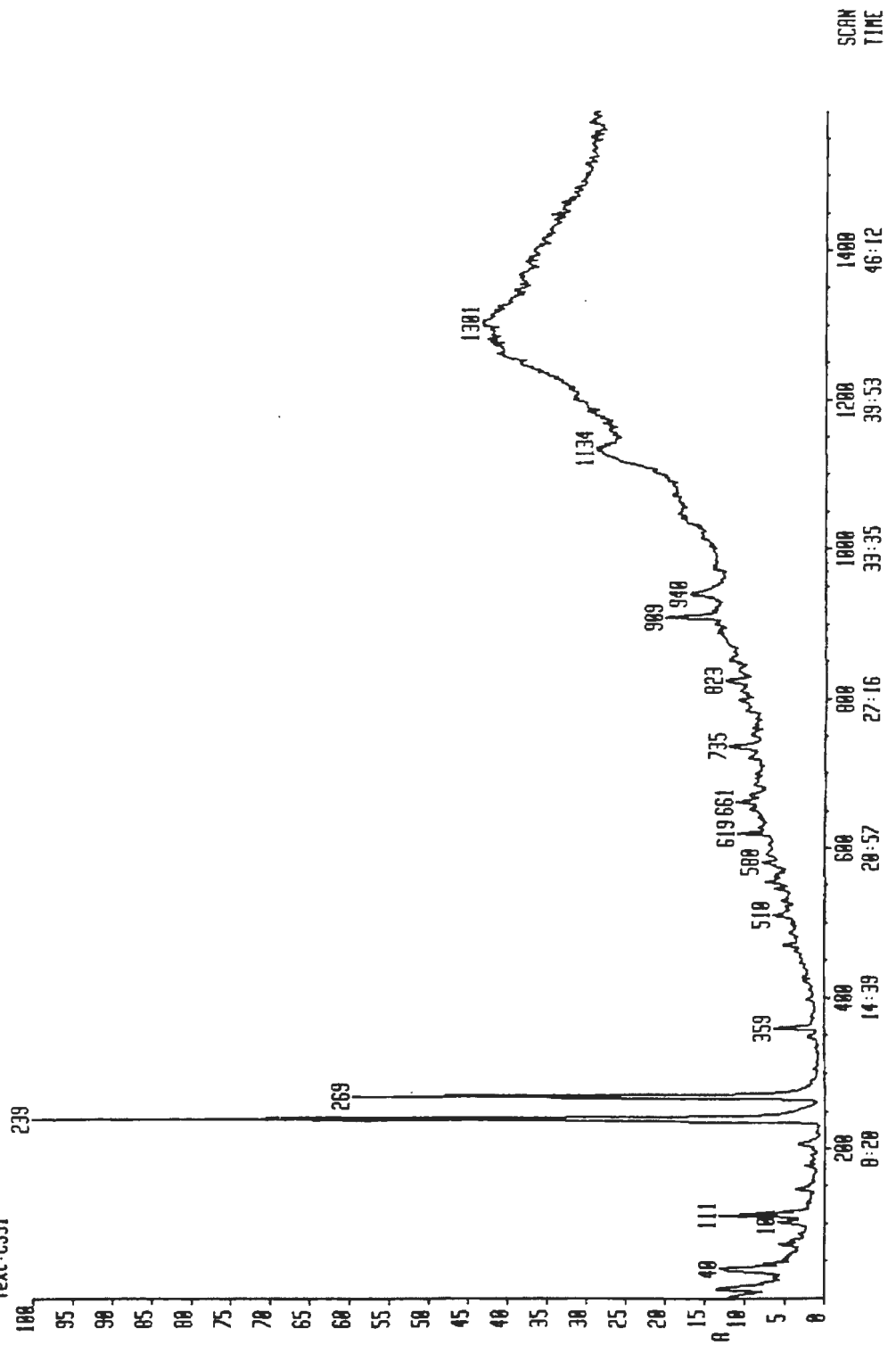


IHP
R: 4486

Sys:DEFAULTS

(E1+)

12107206 01-1585 21-JUL-92 19:33 IS250
Chromatogram Identifiers : A :ATIC
Text:CS31



T1407204 #1-1775

14-JUL-92 13:20 TS250

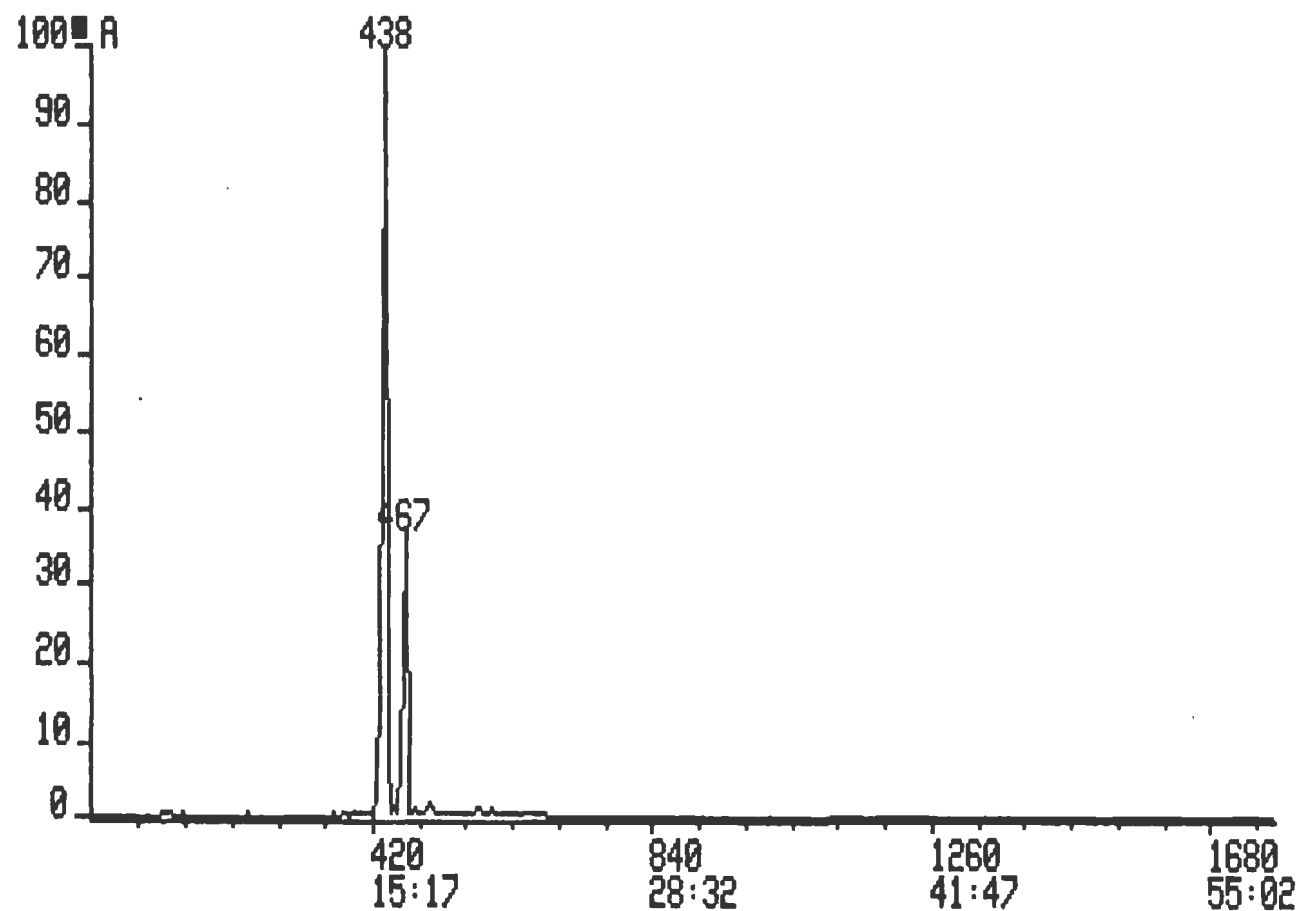
(EI+)

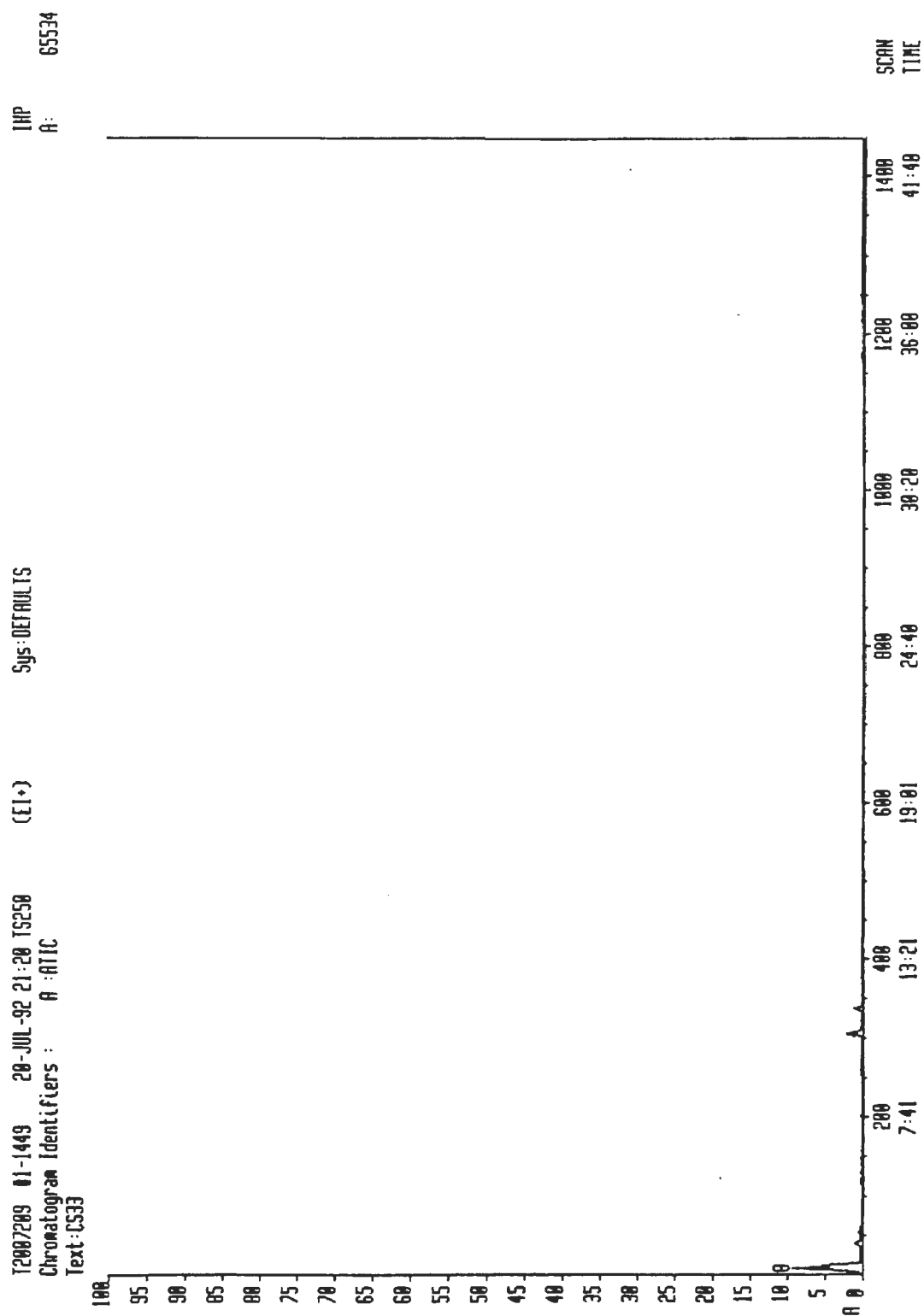
Sqs:DEFAULTS

A:ATIC

Text:CS-32

1.00
26528





IHP
R: 16349

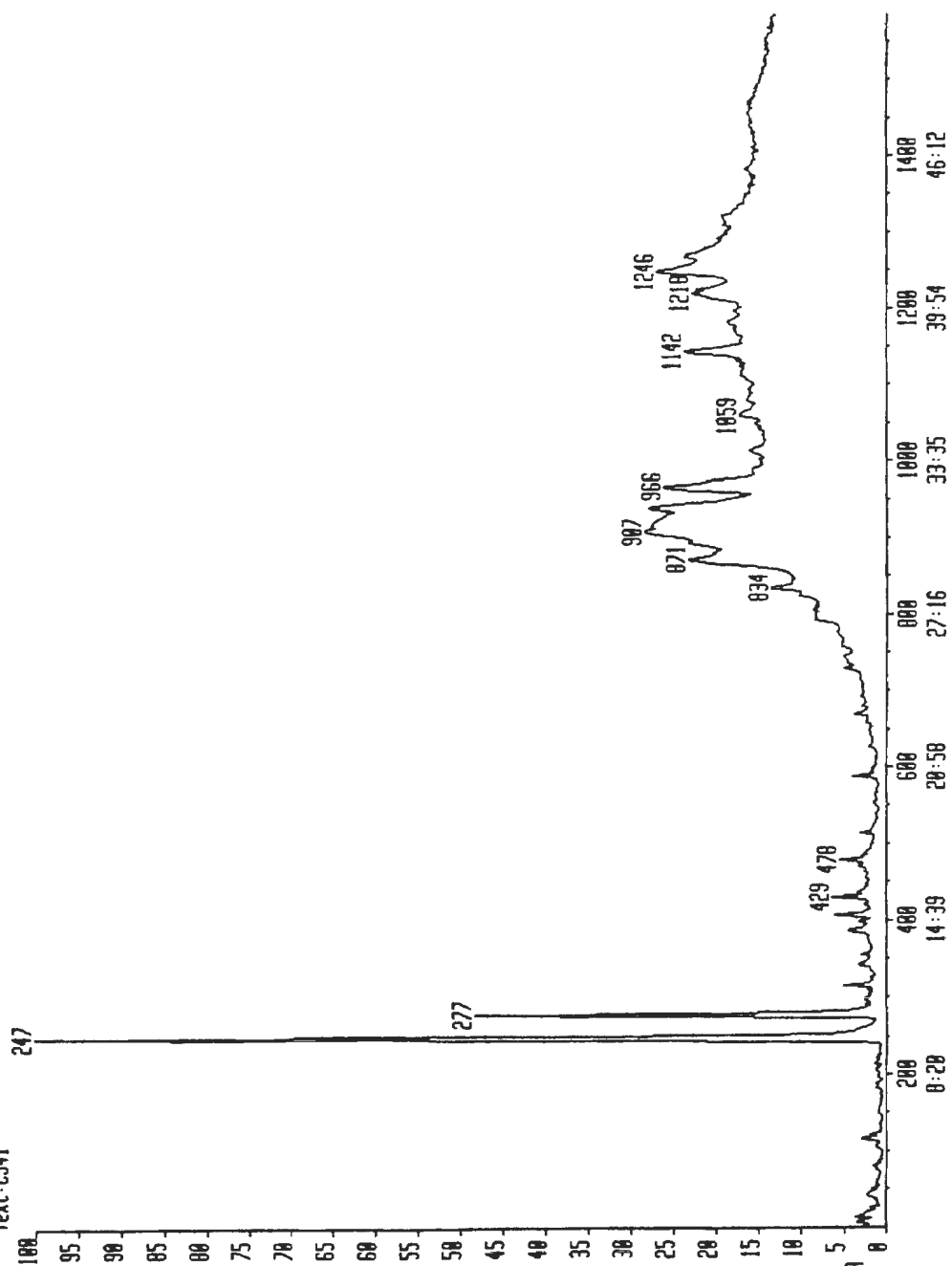
Sys:DEFAULTS

(E1+)

12107205 #1-1505 21-JUL-92 16:54 TS250

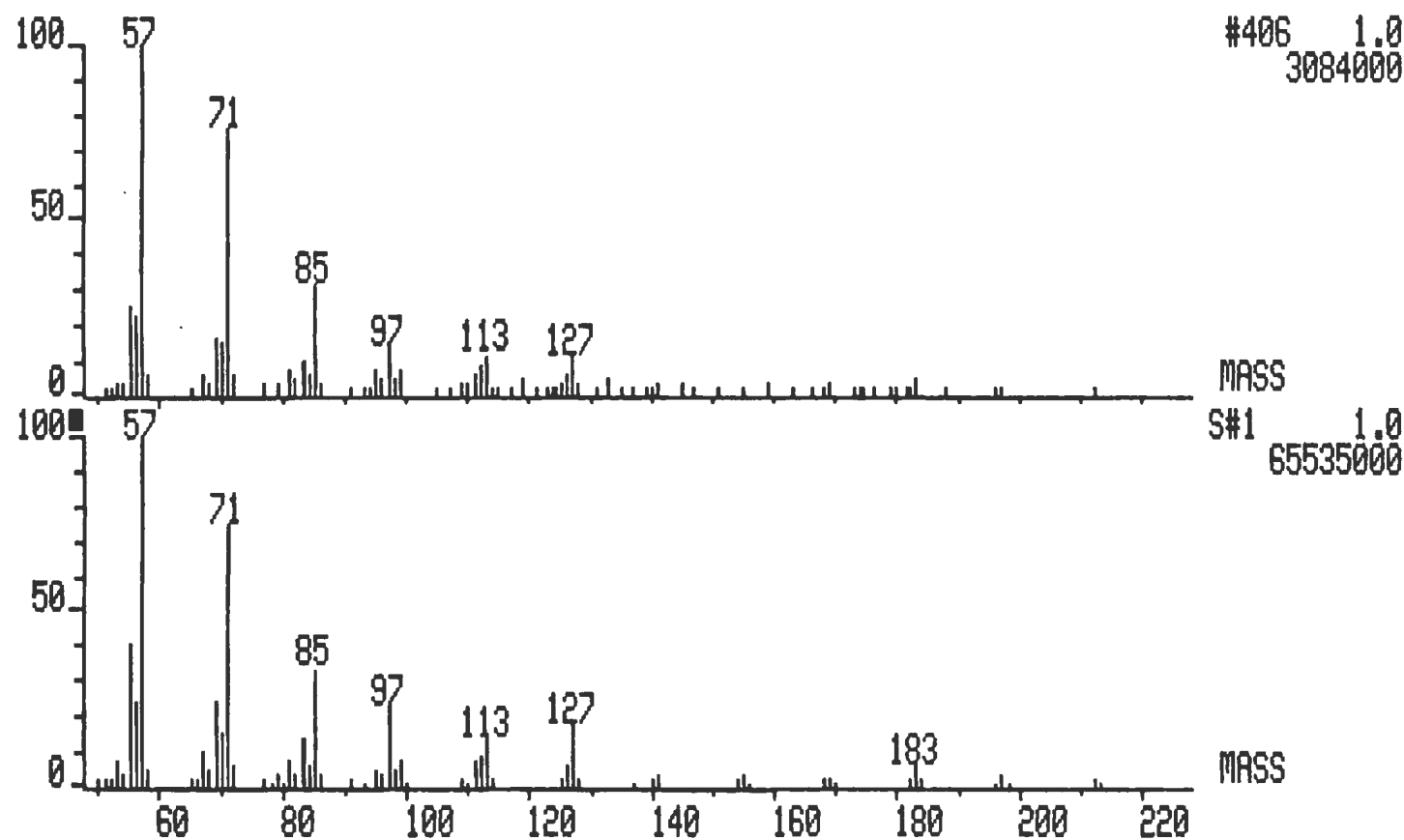
Chromatogram Identifiers : A :ATTIC

Text:CS41



LIBFITS2#1* x1 Bgd=406 T2107205
DODECANE, 2,6,10-TRIMETHYL-
C15.H32.

+0:00:00
p665 M793 r815 RFN:3891-98-3
Lib:NBS 18545 Bpk: 57 Mwt: 212



THP
A: 17395

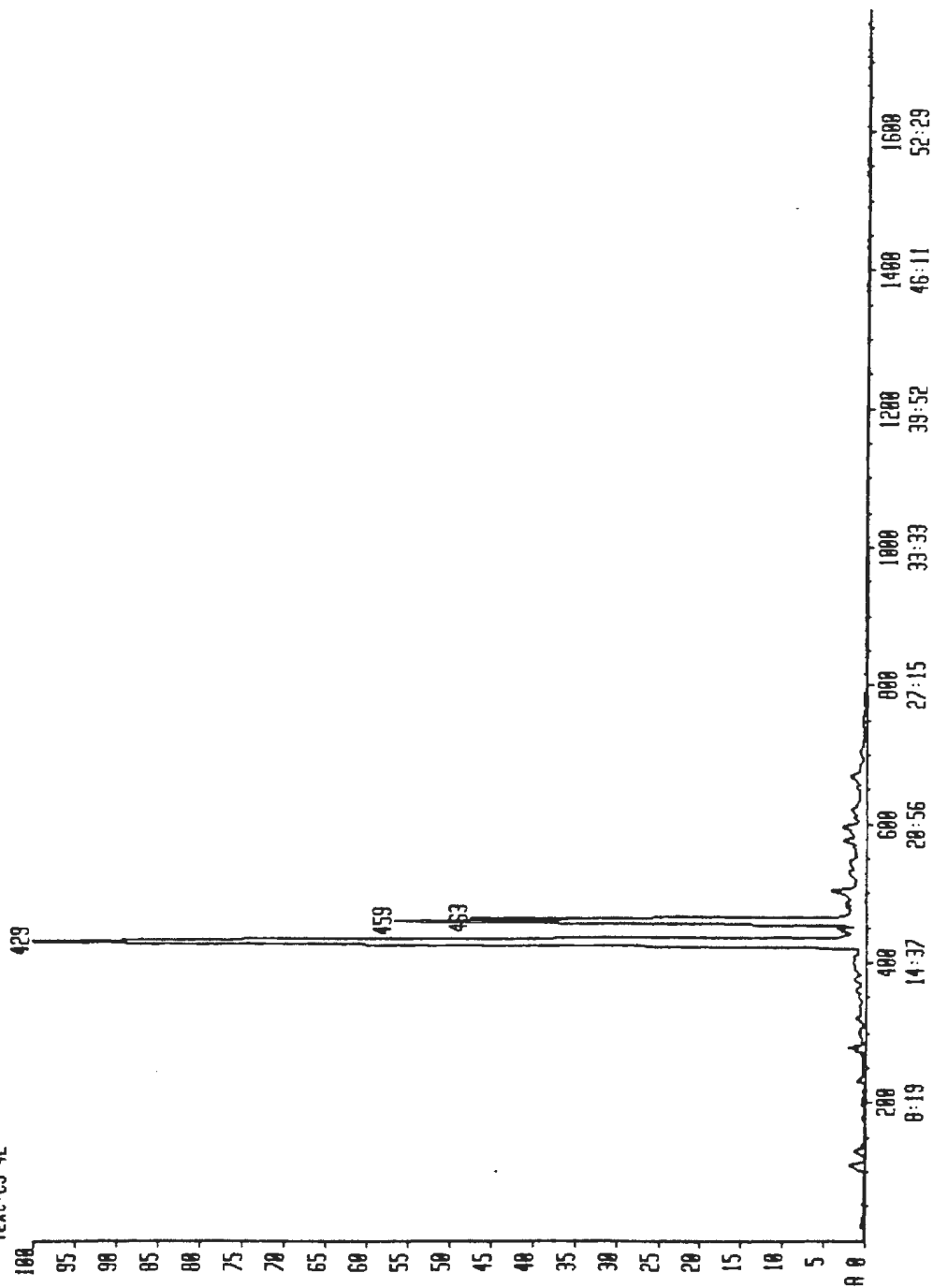
Sys: DEFAULTS

(E1+)

71507203 81-1776 15-JUL-92 11:30 TS250

Chromatogram Identifiers: A: ATIC

Text: CS 42



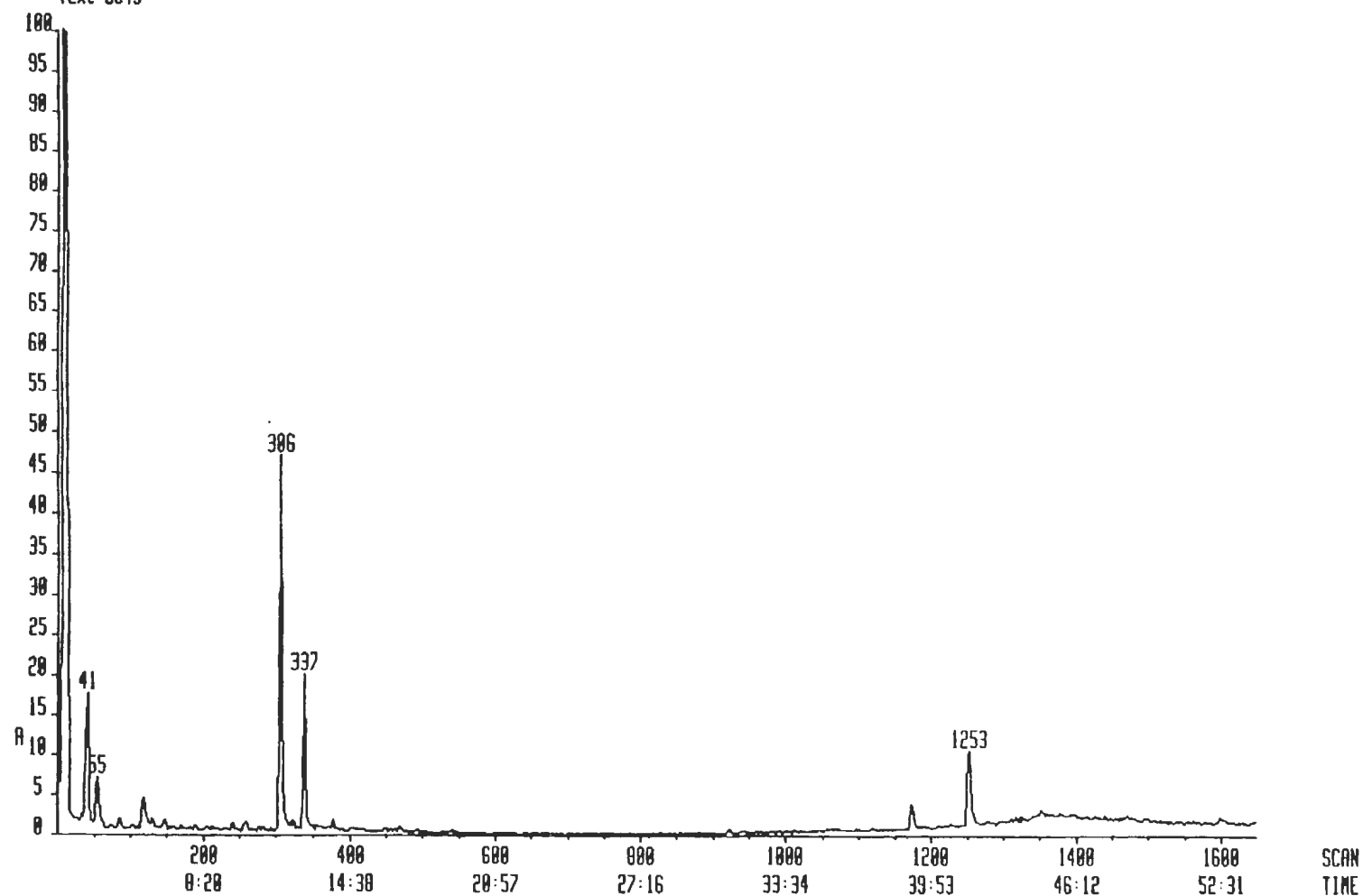
T2007200 11-1640 20-JUL-92 20:04 TS250
Chromatogram Identifiers : A : ATIC
Text: CS43

(EI+)

Sys: DEFAULTS

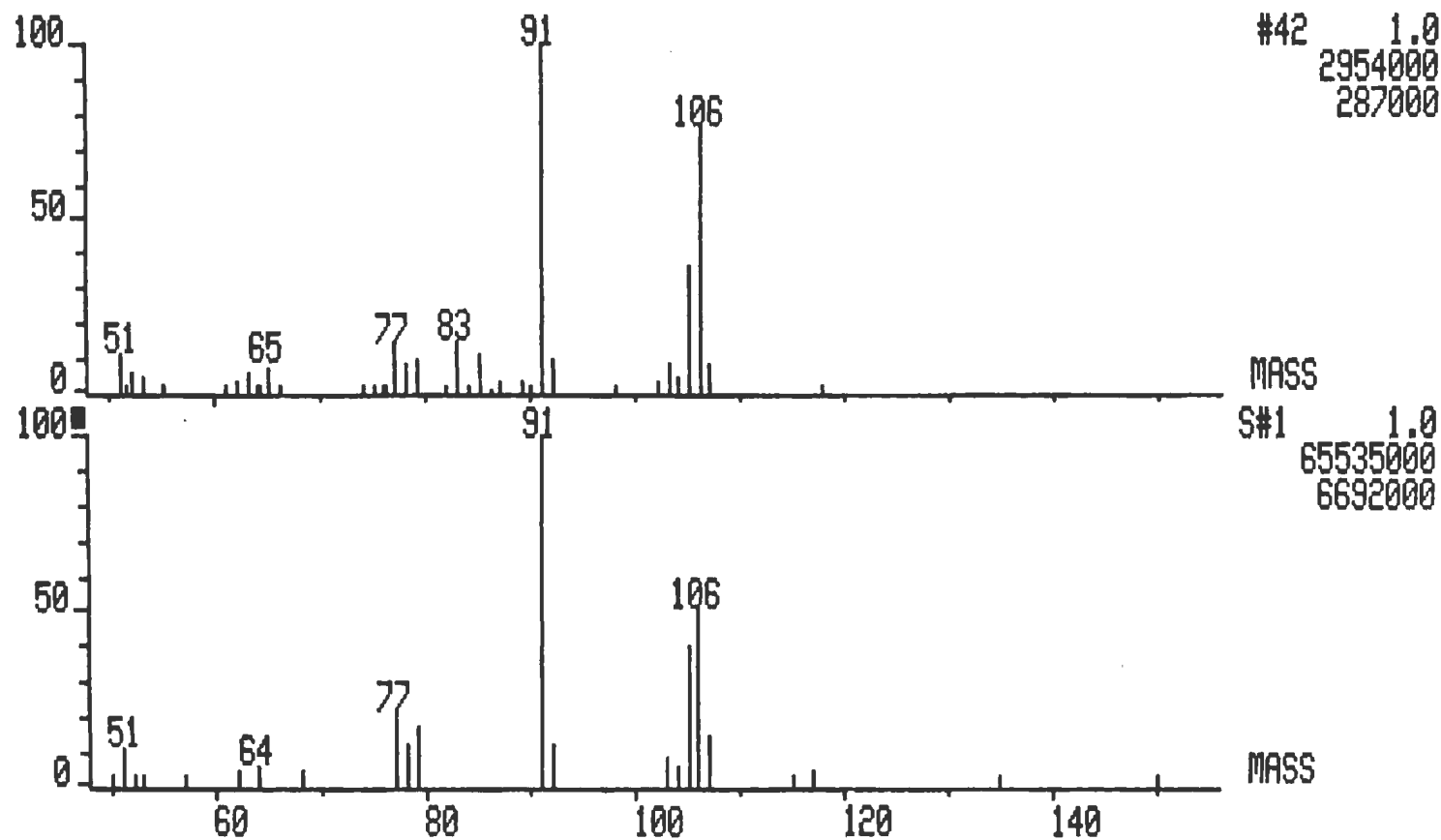
IHP

R: 6350



LIBFITS2#1* x1 Bgd=42 T2007208
BENZENEETHANOL, .ALPHA.,.BETA.-DIMETHYL-
C10.H14.O.

+0:00:00
p702 M840 r814 RFN:52089-32-4
Lib:NBS 7487 Bpk: 91 Mwt: 150



LIBFITS2#1* x1 Bgd=270 T1908101
BENZENE, 1-METHYL-2-(1-METHYLETHYL)-
C10.H14.

+0:00:00

p847 M847 r933 RFN:0-00-0

Lib:NBS

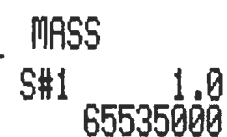
4777 Bpk: 119 Mwt: 134

100 119 #270 1.0
50 91 943000
0

100 119 MASS
50 S#1 1.0
0 65535000

100 119
50 79
0 MASS
50 100 150 200 250 300 350 400 450 500

p861 M861 r975 RFN:527-53-7
4776 Bpk: 119 Mwt: 134



APPENDIX G

CHROMATOGRAMS AND MASS SPECTRA

Okmulgee, Oklahoma

The following are chromatograms and mass spectra for water and sediment samples from Okmulgee. The accession labels are as follows:

Accession Label	Page
OKW-1 = Water from Station 1	232-233
OKW-2 = Water from Station 2	234
OKW-3 = Water from Station 3	235
OKS-1-1 = Sediment from Station 1, fraction #1	236-240
OKS-1-2 = Sediment From Station 1, fraction #2	241-248
OKS-1-3 = Sediment from Station 1, fraction #3	249
OKS-2-1 = Sediment from Station 2, fraction #1	250-251
OKS-2-2 = Sediment from Station 2, fraction #2	252
OKS-2-3 = Sediment from Station 2, fraction #3	253-254
OKS-3-1 = Sediment from Station 3, fraction #1	255-257
OKS-3-2 = Sediment from Station 3, fraction #2	258-259
OKS-3-3 = Sediment from Station 3, fraction #3	260

The mass spectra are presented as two spectra. The top spectra is that of the sample with the peak number in the upper right corner. The bottom spectra is that of the library match corresponding to the sample. The name and molecular formula of the compound are given at the top. Also, the CAS registry number is listed, if available, at the top right following the label "RFN". In the interest of being concise, spectra were only included once per occurrence.

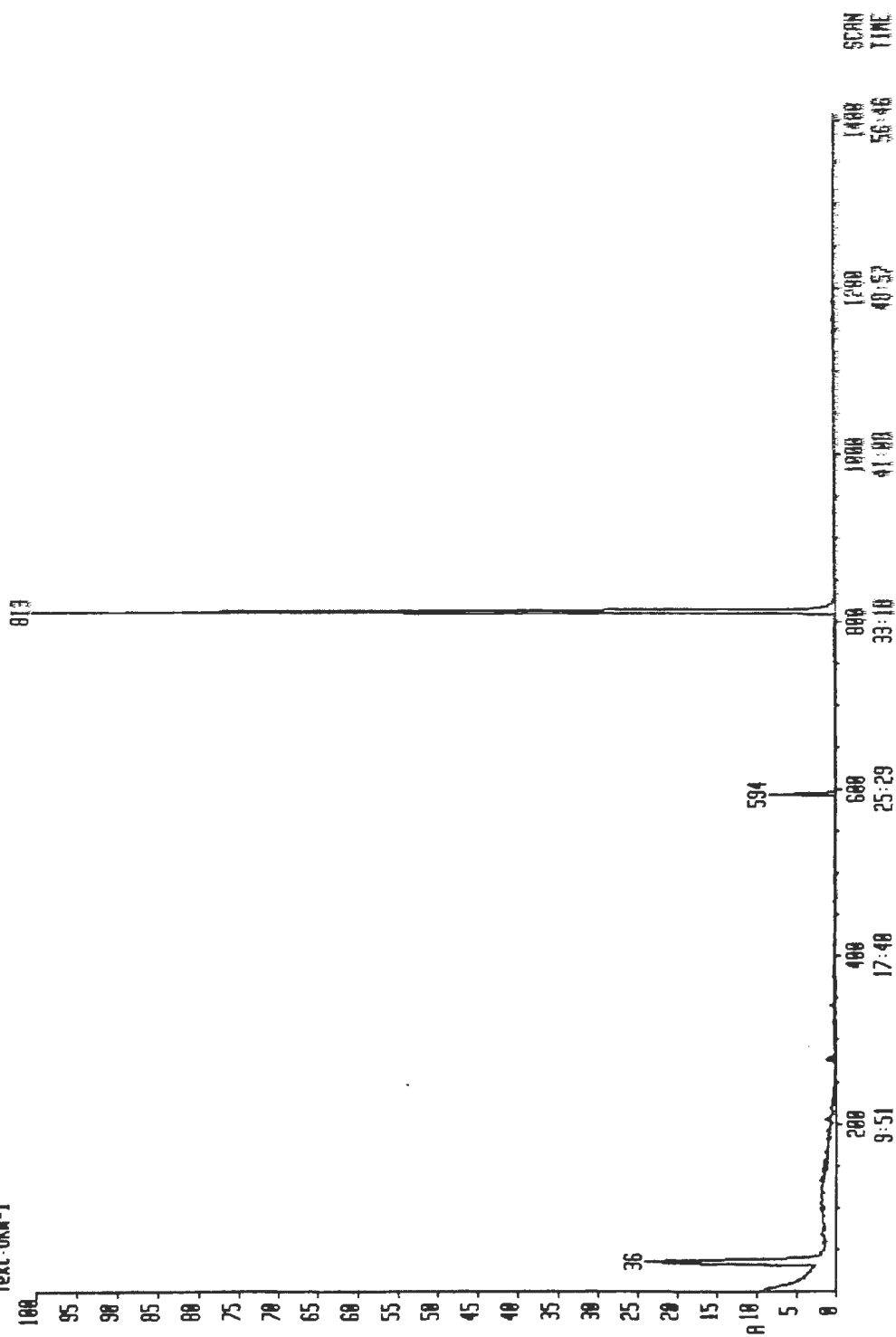
IMP
A: 1162

Sys: DEFAULTS

(E1+)

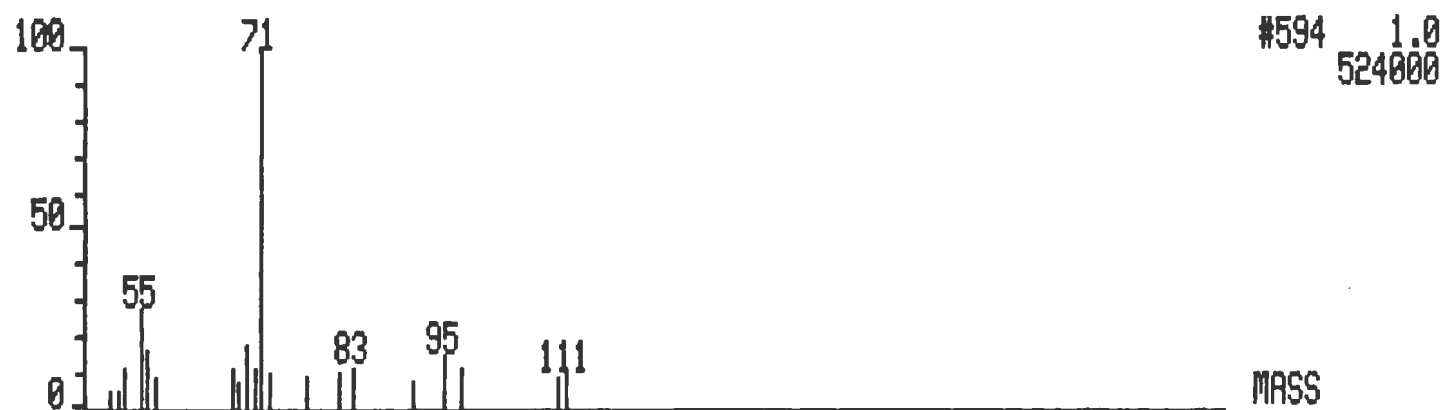
10083201 01-1407 0-SEP-92 09:31 TS250
Chromatogram Identifiers : R: ATIC

Text: OKM-1

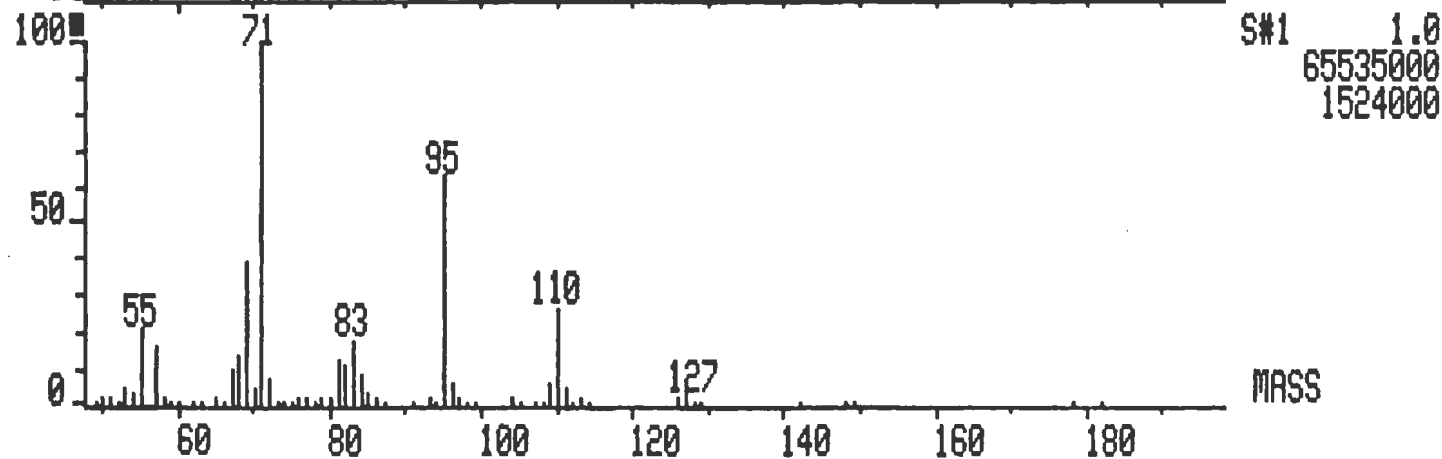


LIBFITS1#1* x1 Bgd=594 T0809201
 CYCLOHEXANOL, 3,5-DIMETHYL-
 C8.H16.O.

+0:00:00
 p611 M638 r800 RFN:5441-52-1
 Lib:NBS 4070 Bpk: 71 Mwt: 128



#594 1.0
 524000



MASS
 S#1 1.0
 65535000
 1524000

MASS

T0009202 01-1407 0-SEP-92 10:42 TS250 (E1+)

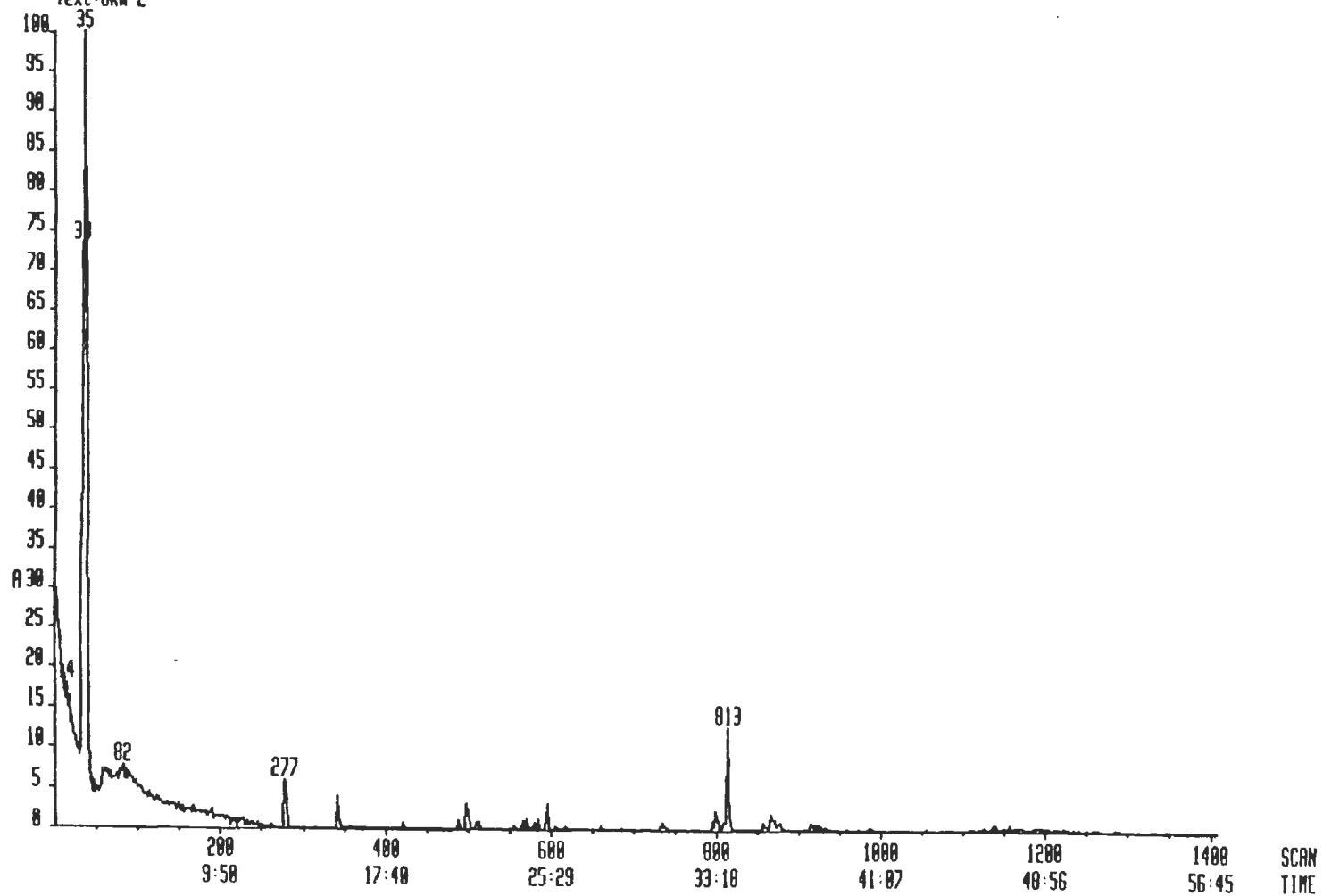
Sys:DEFAULTS

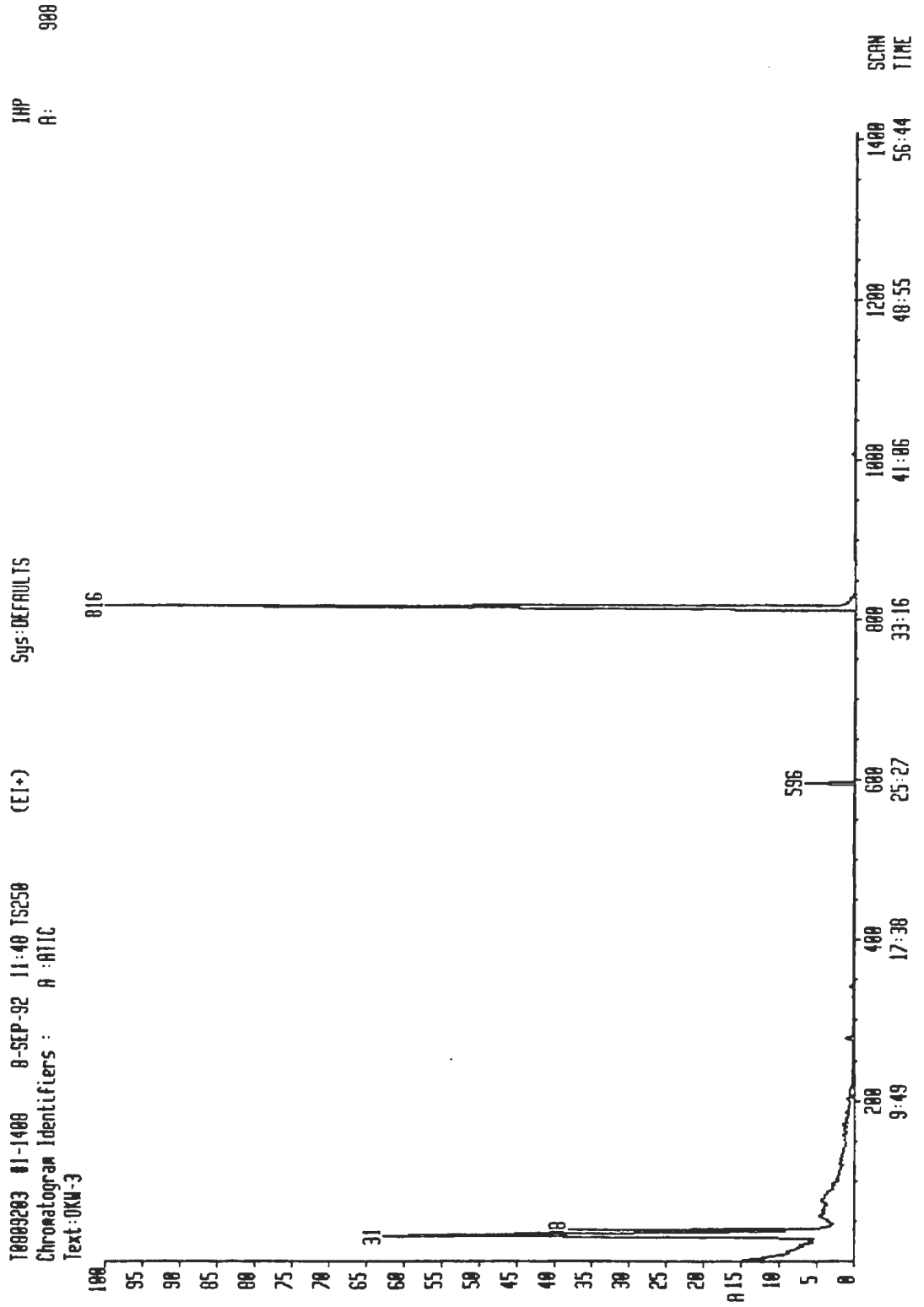
IHP

Chromatogram Identifiers : A :ATIC

A: 479

Text:OKW-2





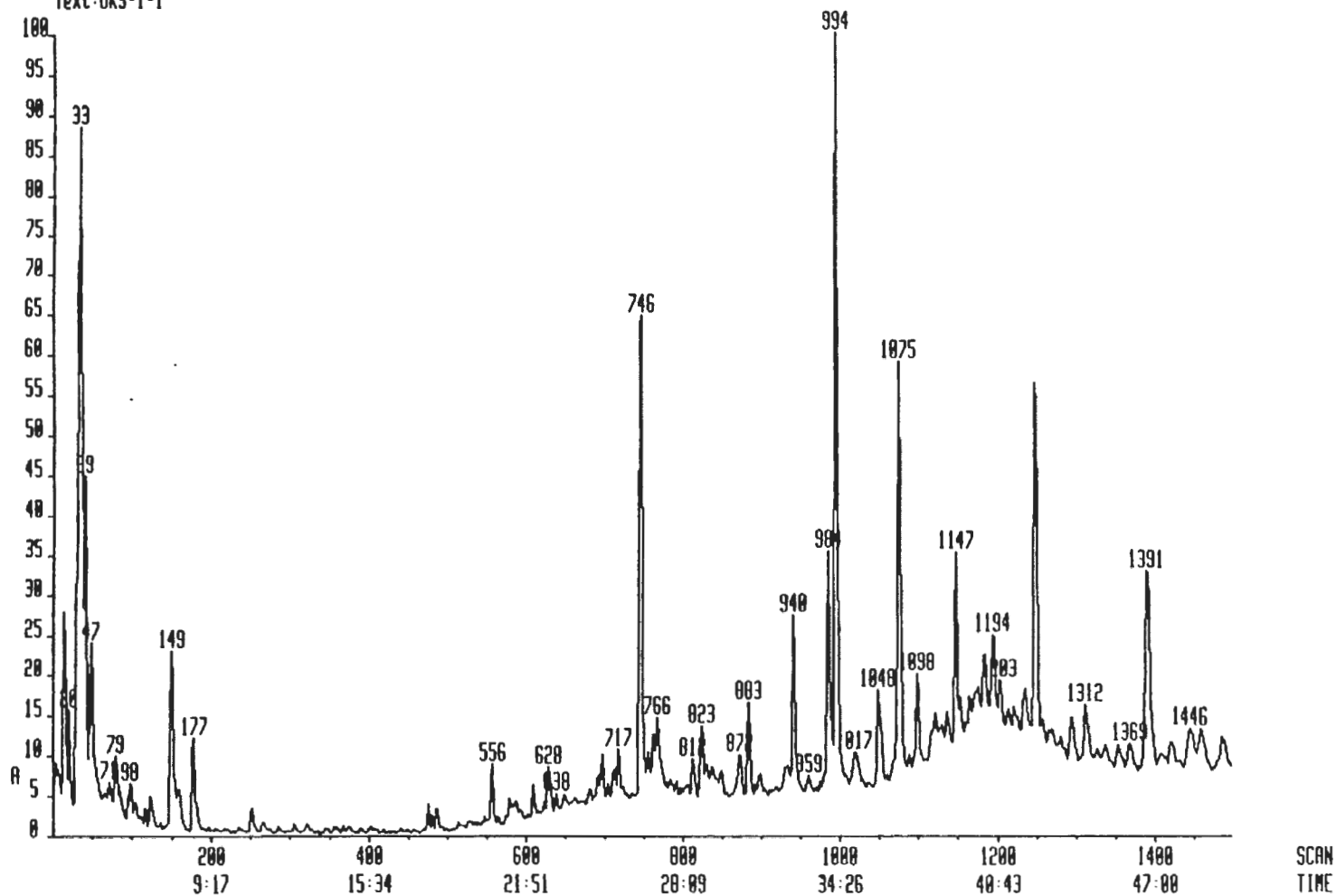
T0202301 01-1497 2-FEB-93 09:17 TS250
Chromatogram Identifiers : A : ATIC
Text:OKS-1-1

(E1+)

Sys:DEFAULTS

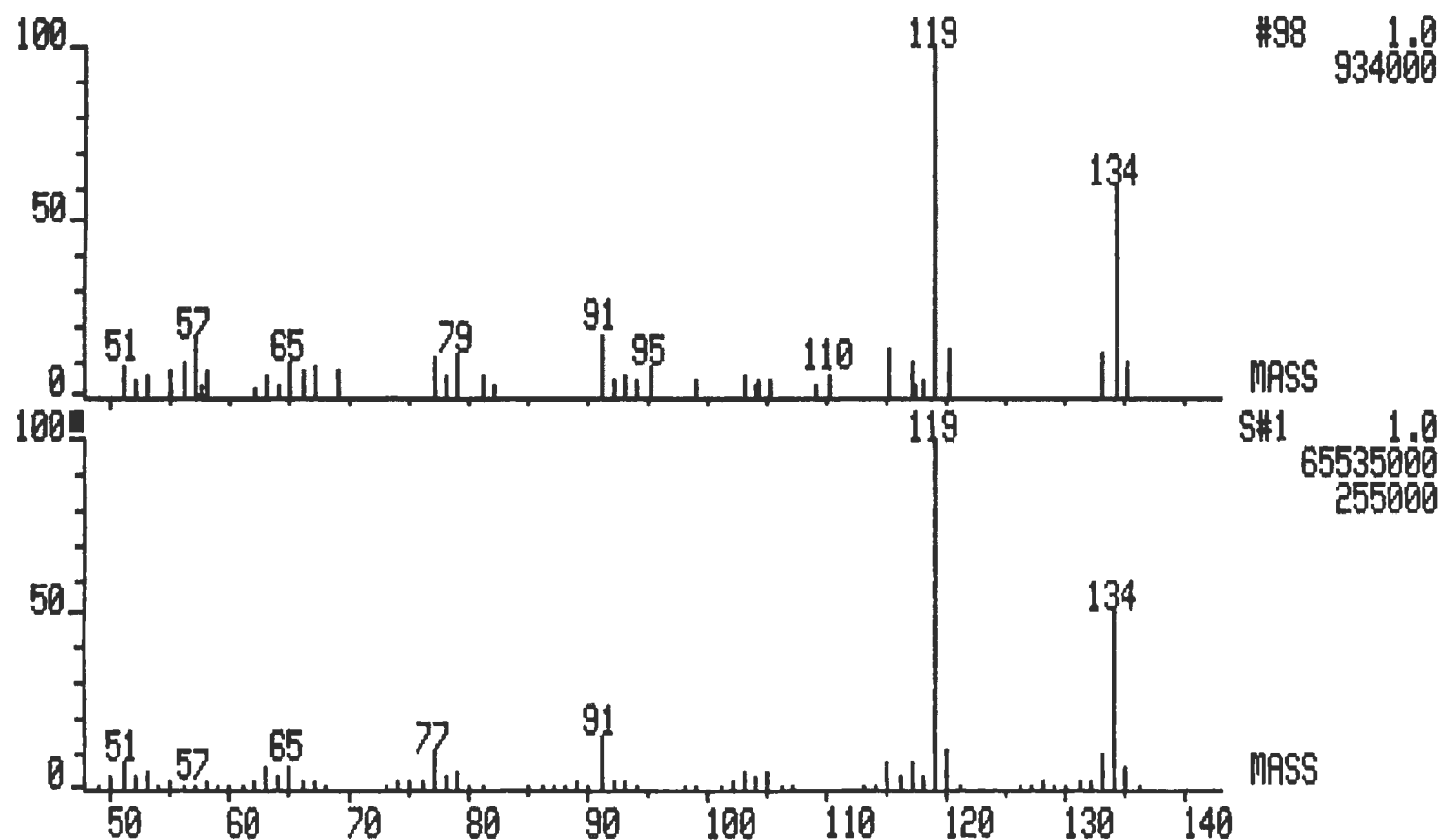
IHP

A: 3050



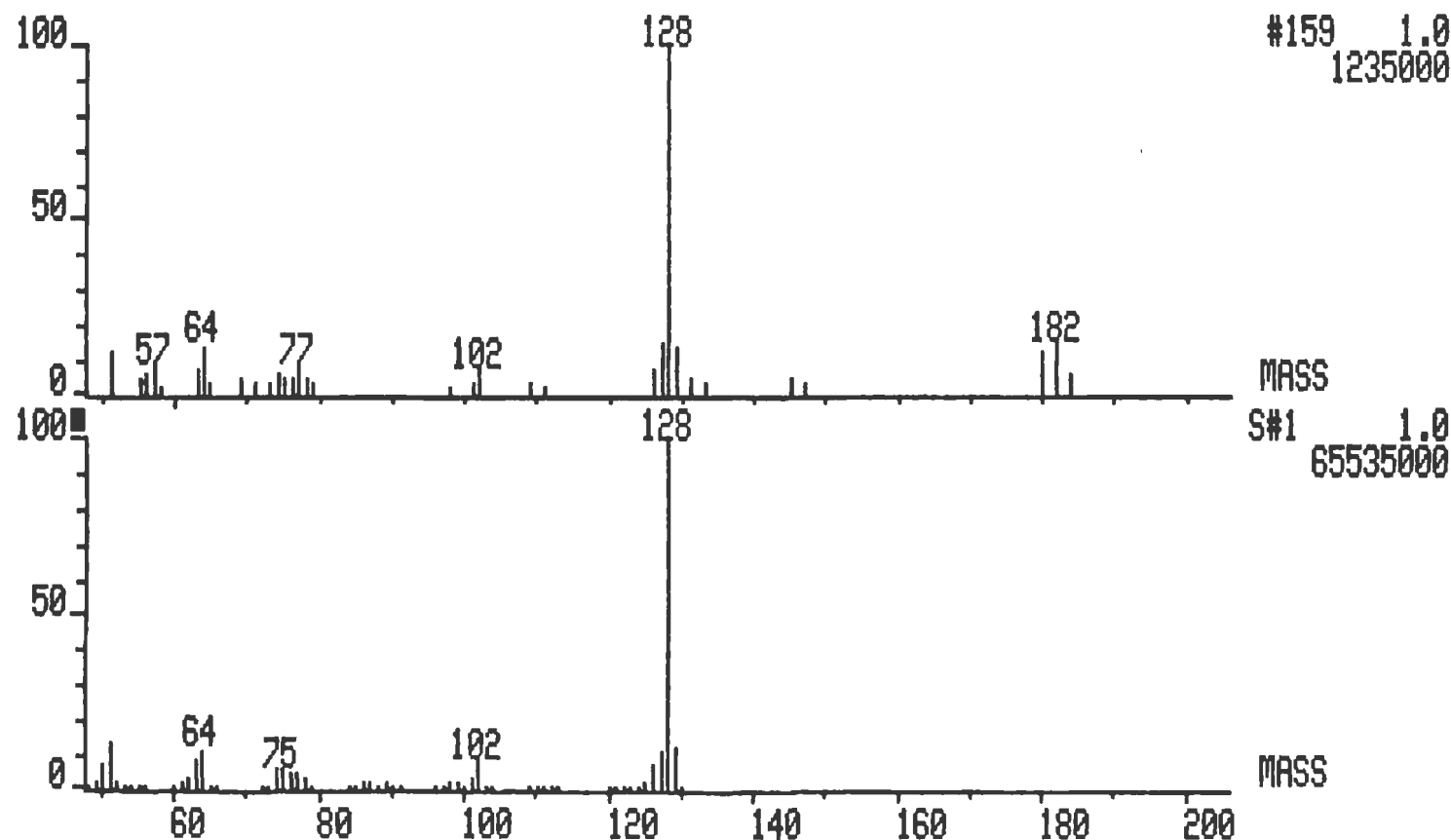
LIBFITS1#1* x1 Bgd=98 T0202301
BENZENE, 1,2,3,4-TETRAMETHYL-
C10.H14.

+0:00:00
p803 M848 r871 RFN:488-23-3
Lib:NBS 4775 Bpk: 119 Mwt: 134



LIBFITS1#1* x1 Bgd=159 T0202301
 NAPHTHALENE
 C10.H8.

+0:00:00
 p626 M866 r684 RFN:91-20-3
 Lib:NBS 3891 Bpk: 128 Mwt: 128



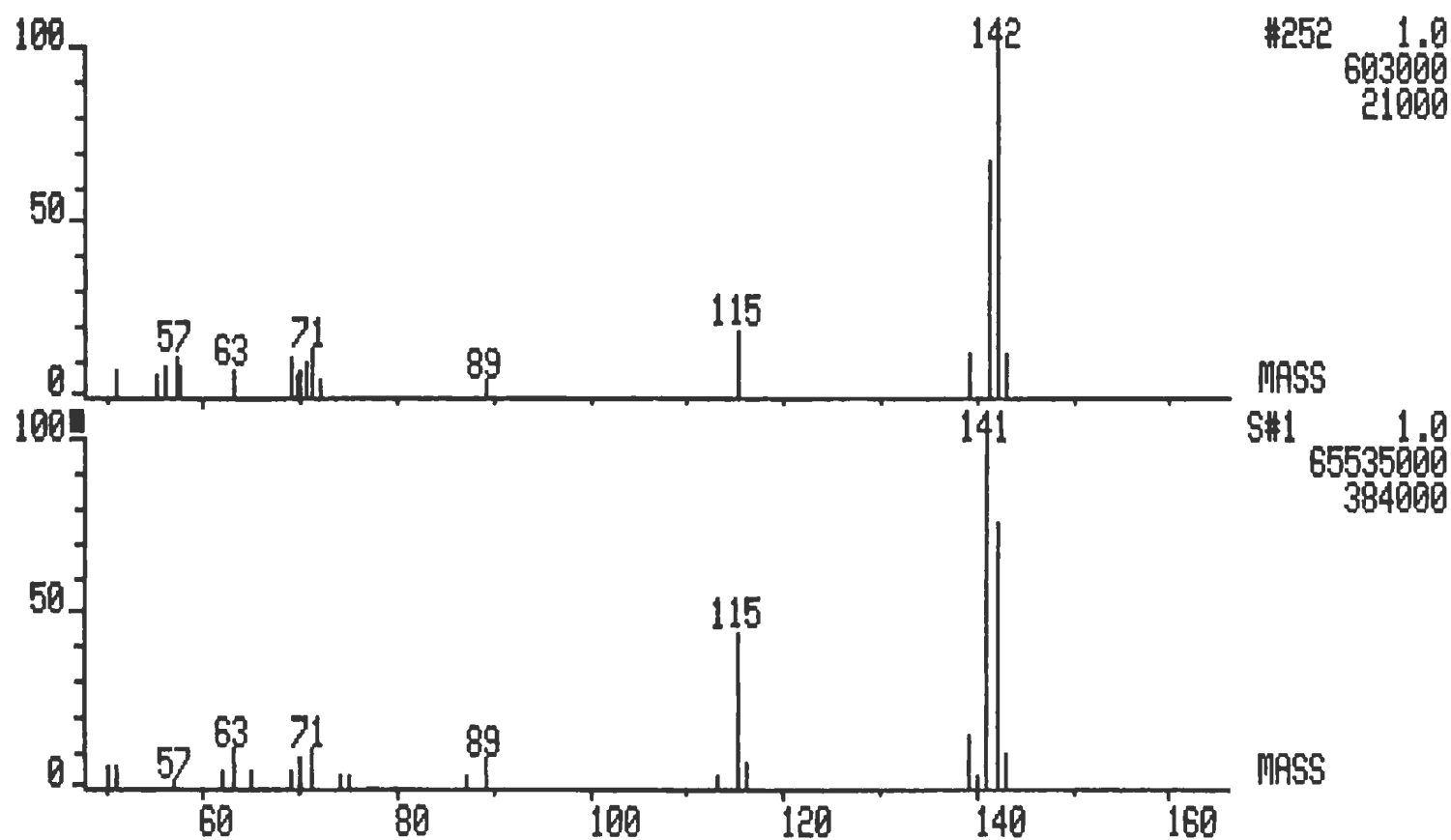
LIBFITS1#1* x1 Bgd=252 T0202301
 1H-INDENE, 1-ETHYLIDENE-
 C11.H10.

+0:00:00

p809 M862 r880 RFN:0-00-0

Lib:NBS

6113 Bpk: 141 Mwt: 142



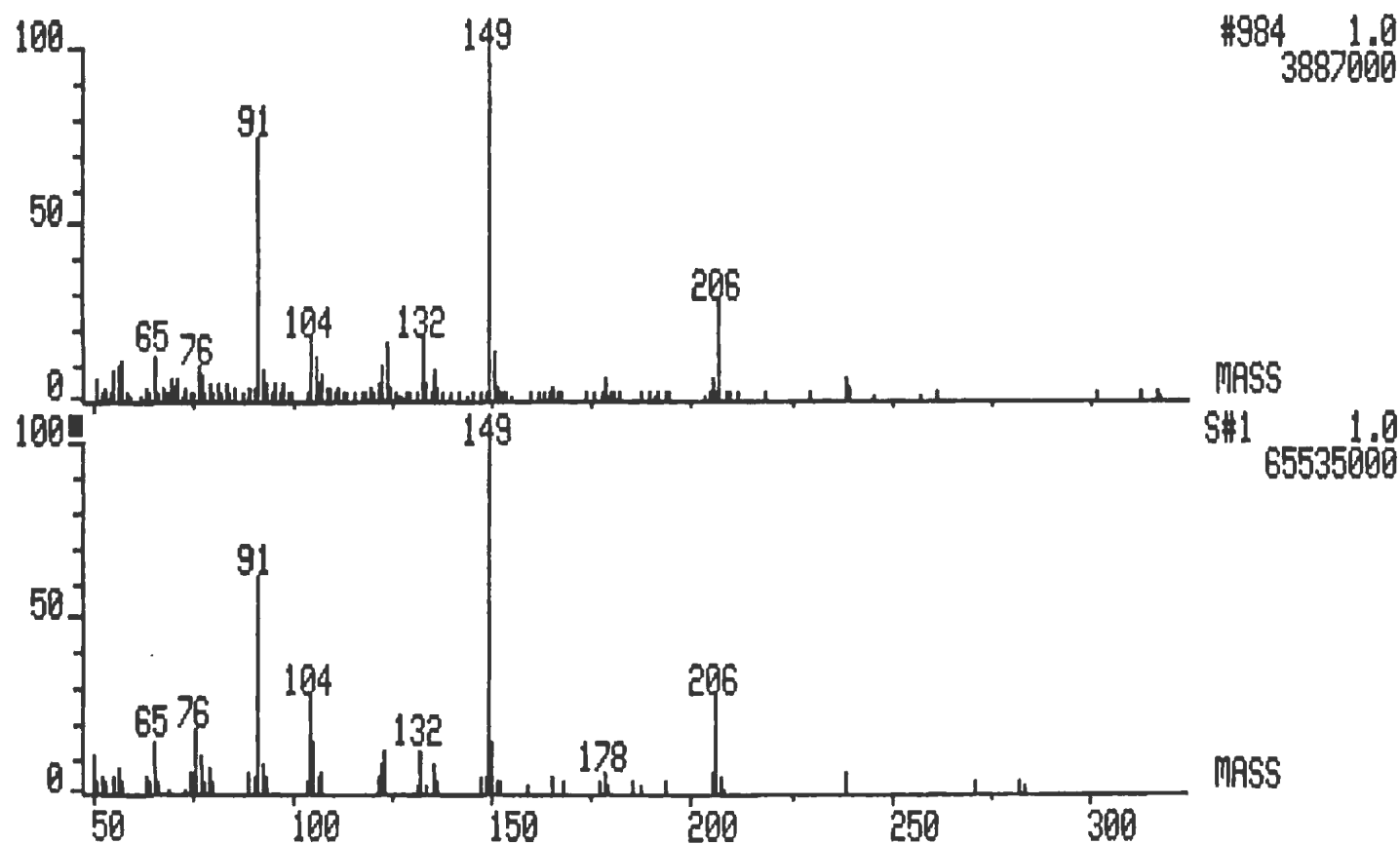
LIBFITS1#1* x1 Bgd=984 T0202301
BENZYL BUTYL PHTHALATE
C19.H20.04.

+0:00:00

p702 M889 r766 RFN:85-68-7

Lib:NBS

30320 Bpk: 149 Mwt: 312



T0402302 01-1719 4-FEB-93 10:32 TS250

(E1+)

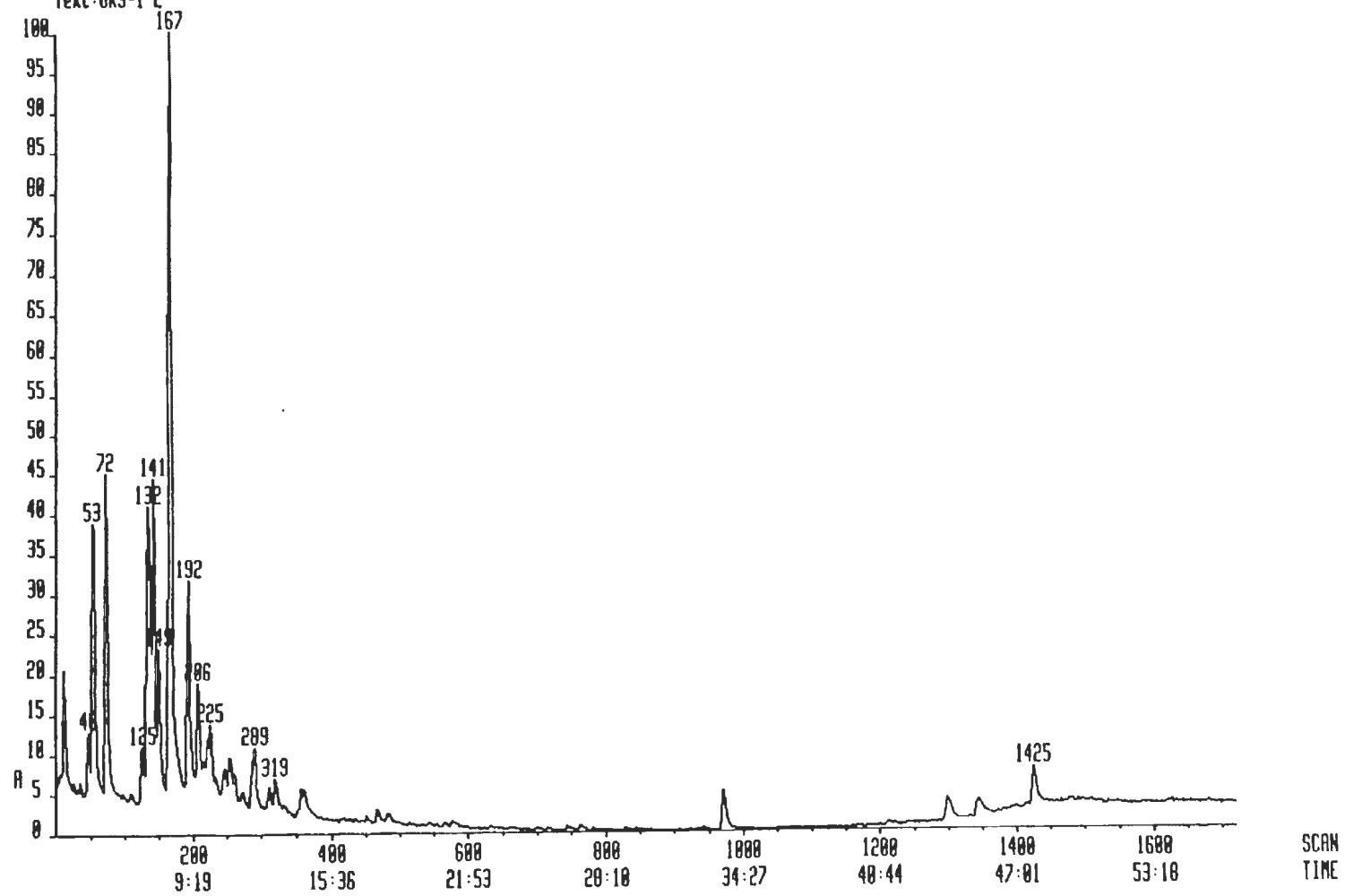
Sys:DEFAULTS

IHP

Chromatogram Identifiers : A:AT1C

A: 9231

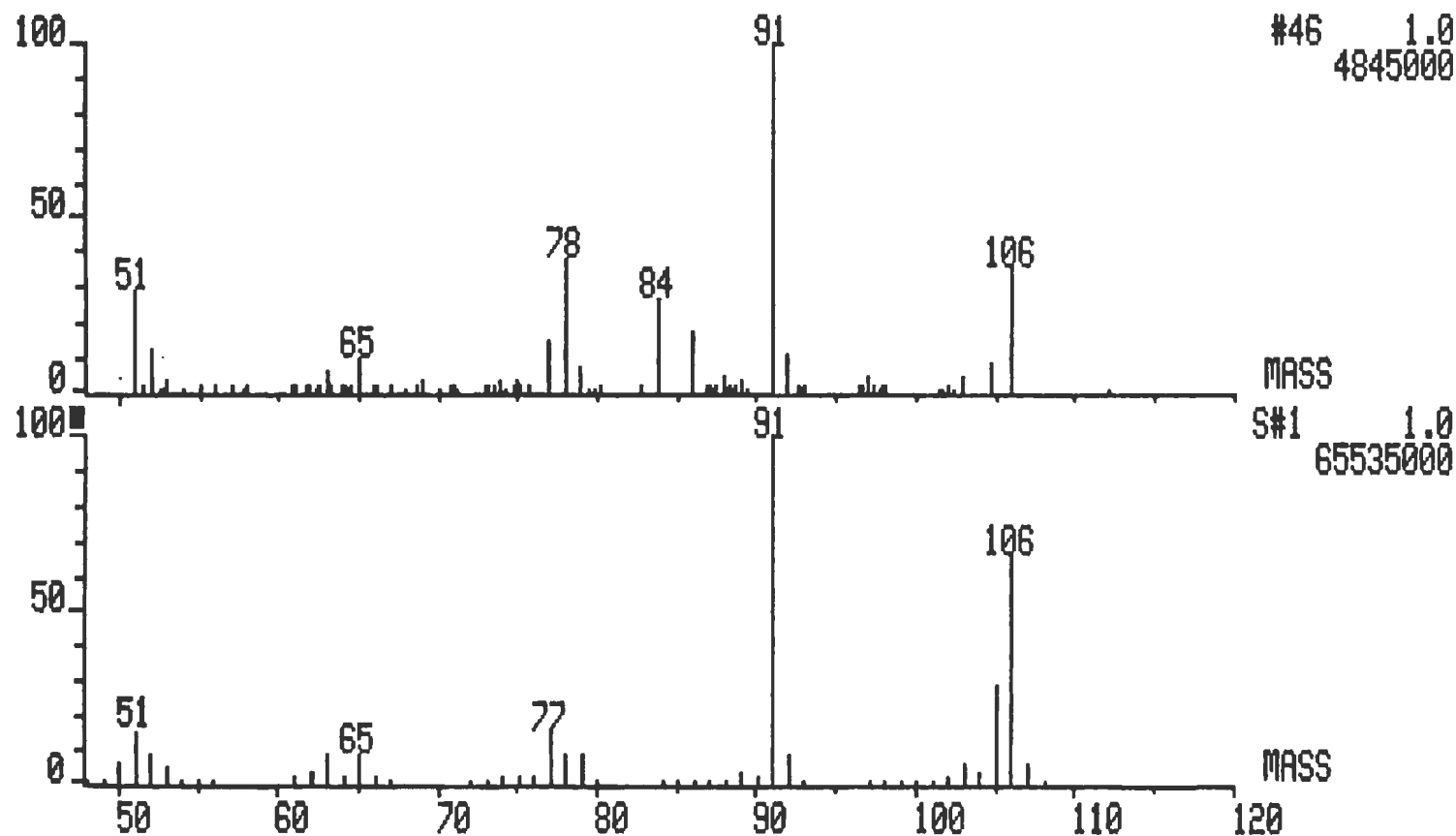
Text:OKS-1-2



LIBFITS1#1* x1 Bgd=46 T0402302
BENZENE, 1,3-DIMETHYL-
C8.H10:

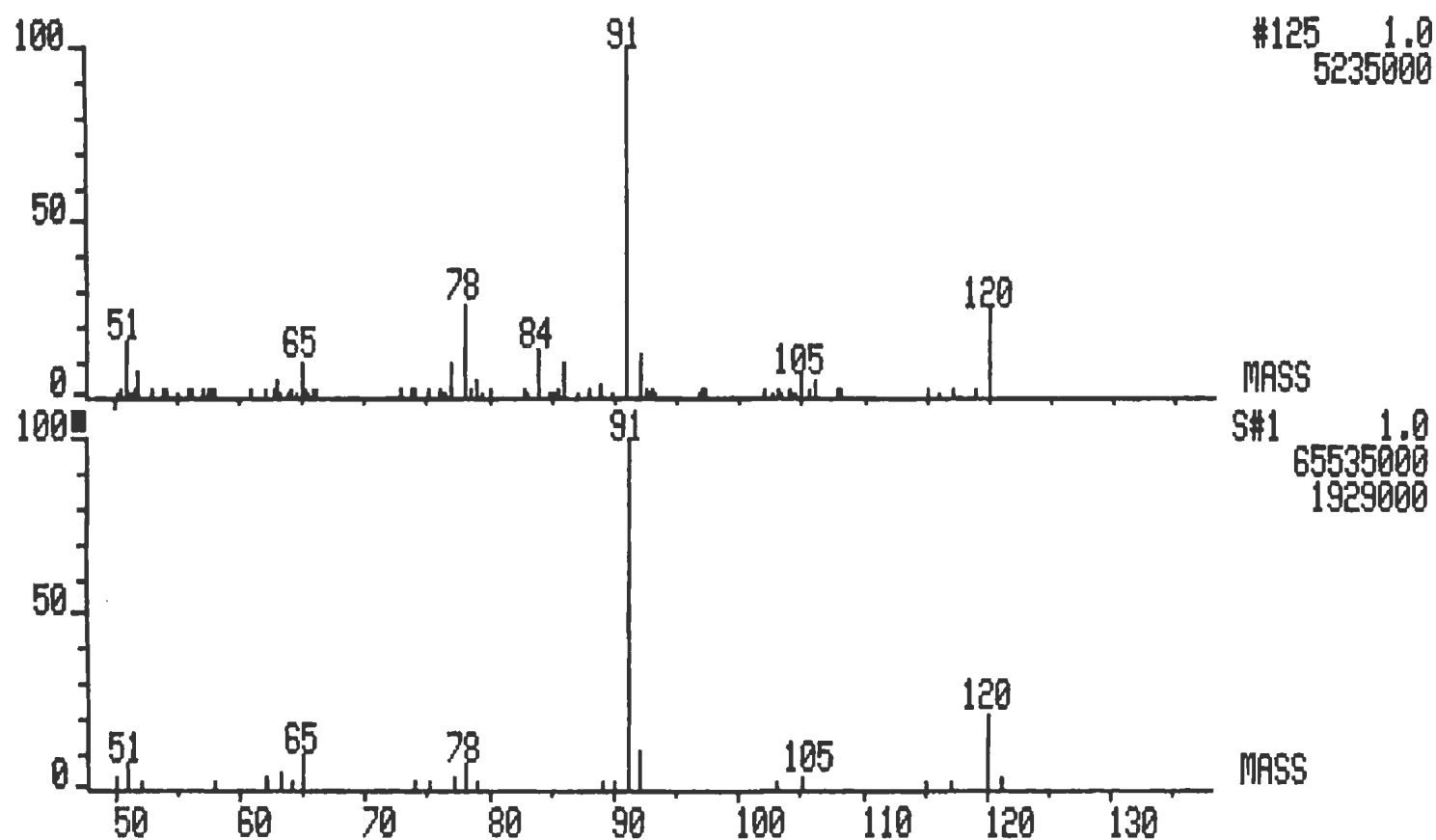
+0:00:00

p698 M720 r762 RFN:108-38-3
Lib:NBS 1731 Bpk: 91 Mwt: 106



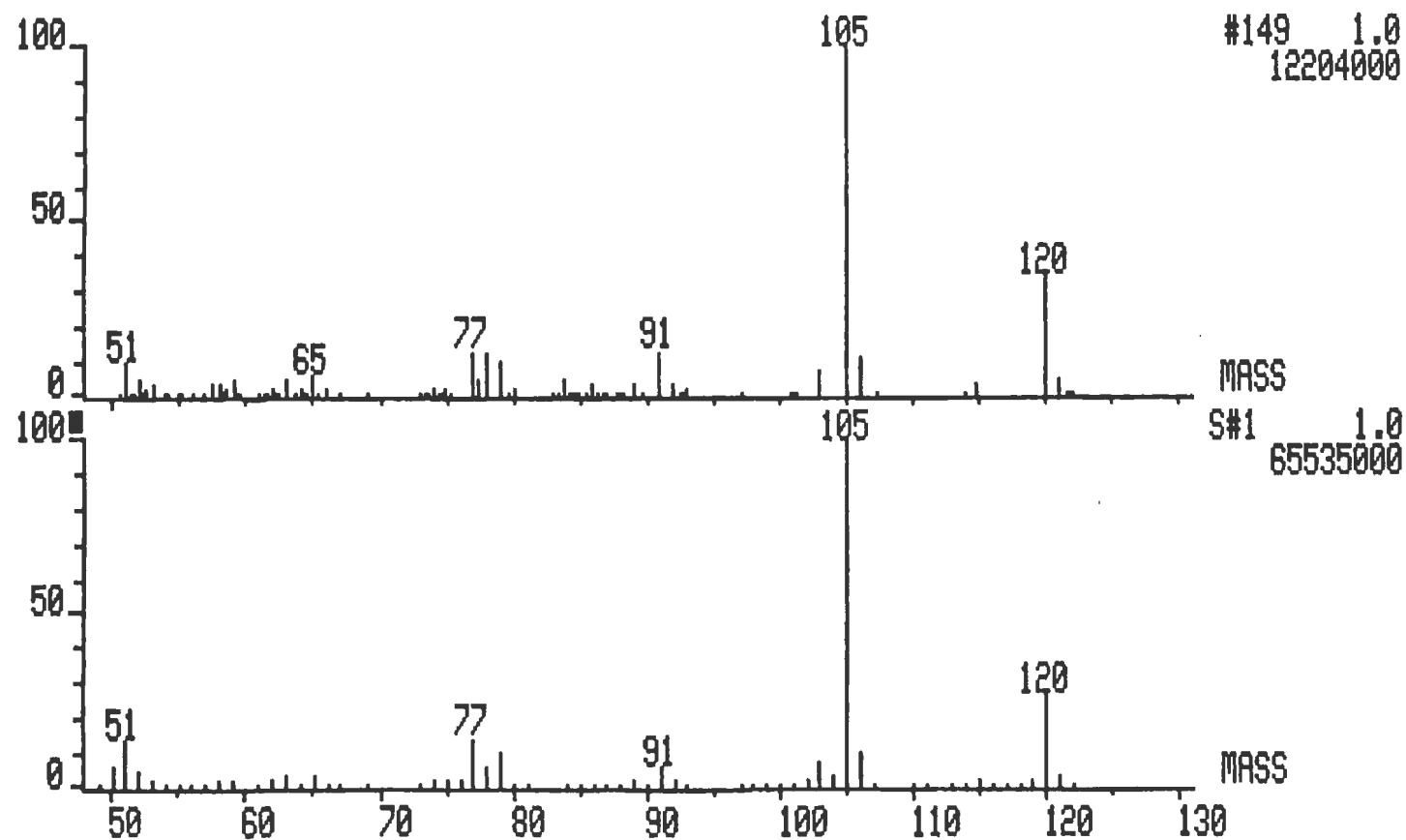
LIBFITS1#1* x1 Bgd=125 T0402302 +0:00:00
BENZENE, PROPYL-
C9.H12.

p716 M894 r755 RFN:103-65-1
Lib:NBS 3097 Bpk: 91 Mwt: 120



LIBFITS1#1* x1 Bgd=149 T0402302
BENZENE, (1-METHYLETHYL)-
C9.H12.

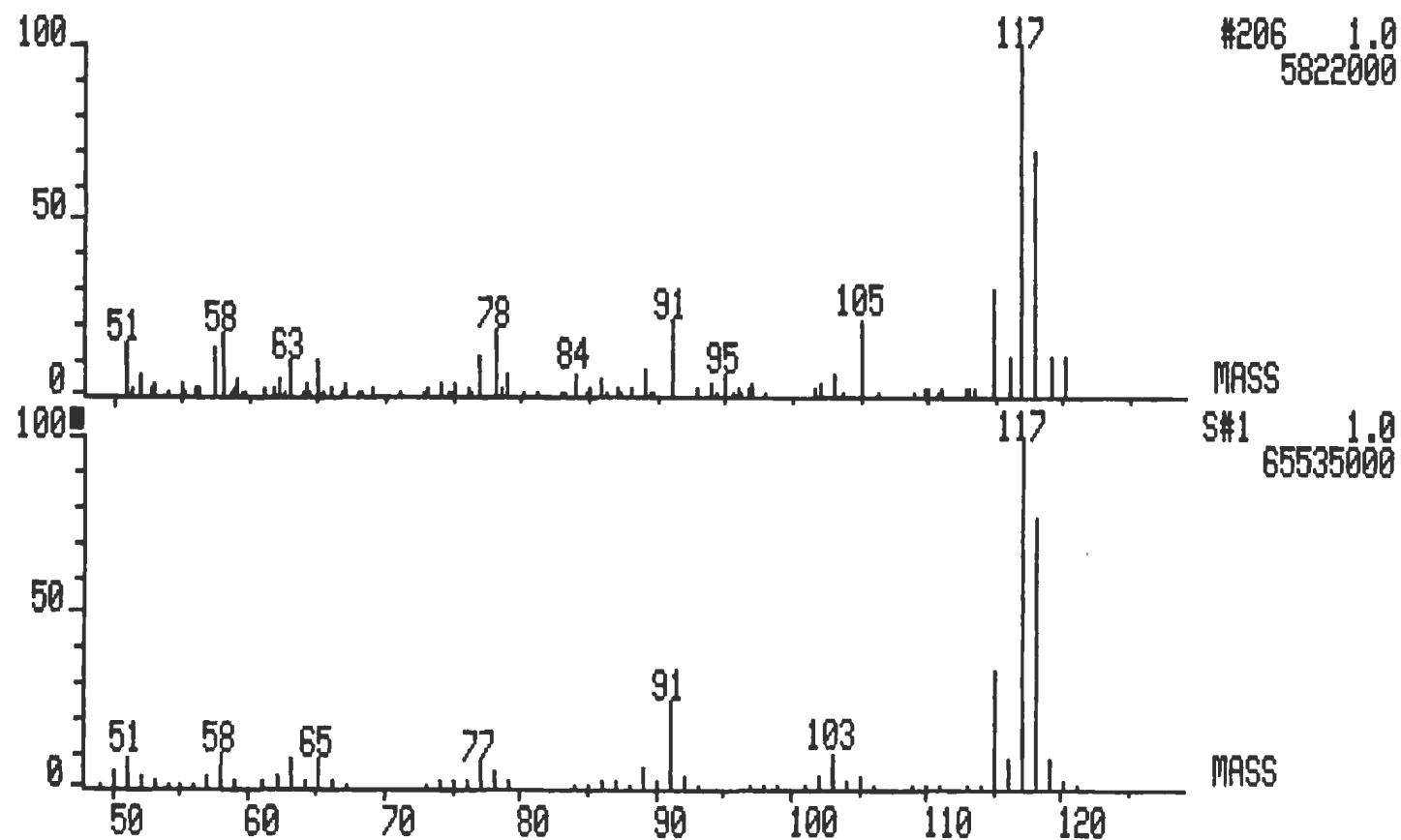
+0:00:00
p871 M873 r934 RFN:98-82-8
Lib:NBS 3096 Bpk: 105 Mwt: 120



LIBFITS1#1* x1 Bgd=206 T0402302
BENZENE, 1-ETHENYL-2-METHYL-
C9.H10.

+0:00:00

Lib:NBS p839 M869 r867 RFN:611-15-4
2969 Bpk: 117 Mwt: 118



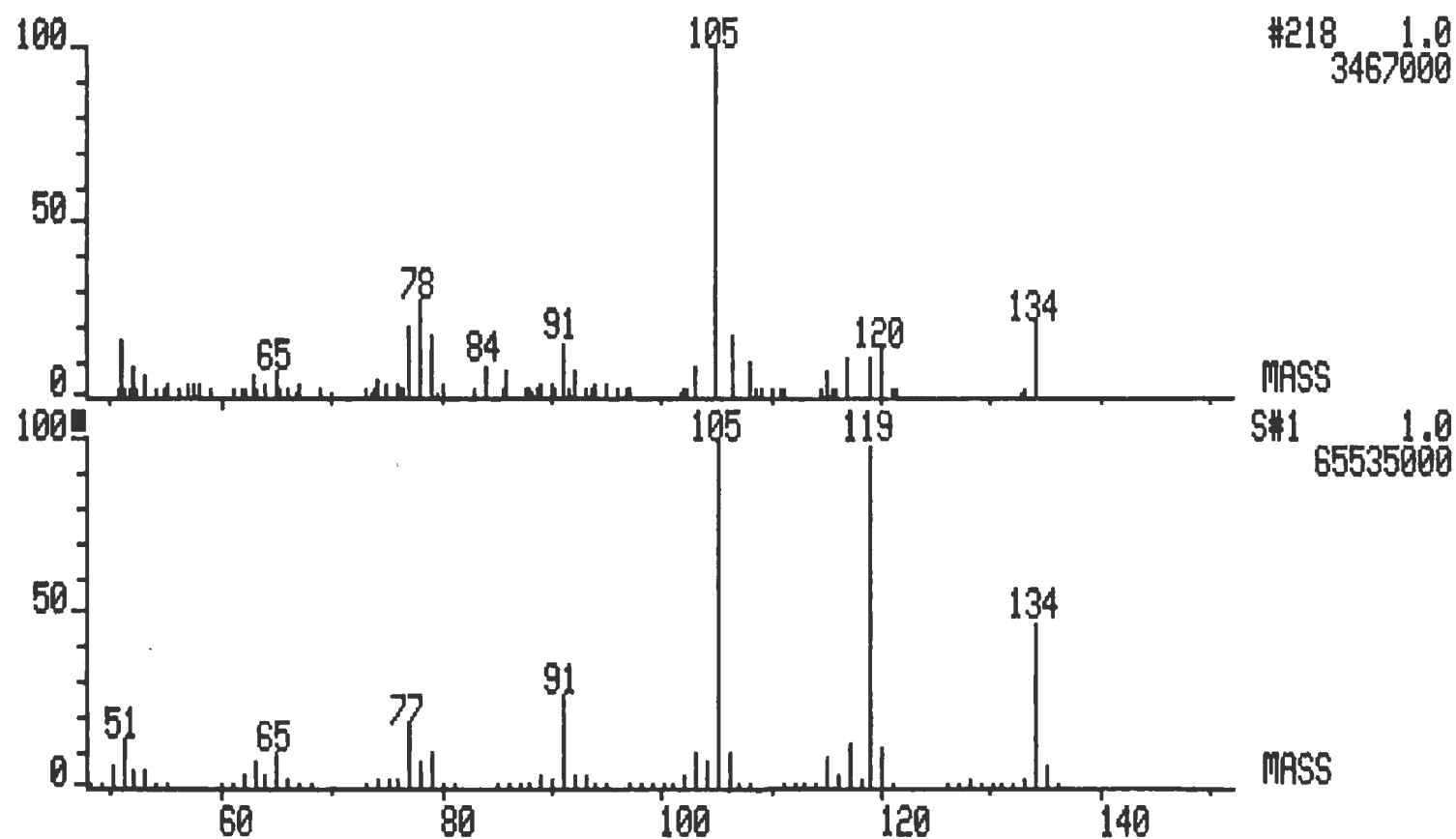
LIBFITS1#1* x1 Bgd=218 T0402302
BENZENE, 1,2-DIETHYL-
C10.H14.

+0:00:00

p671 M706 r727 RFN:0-00-0

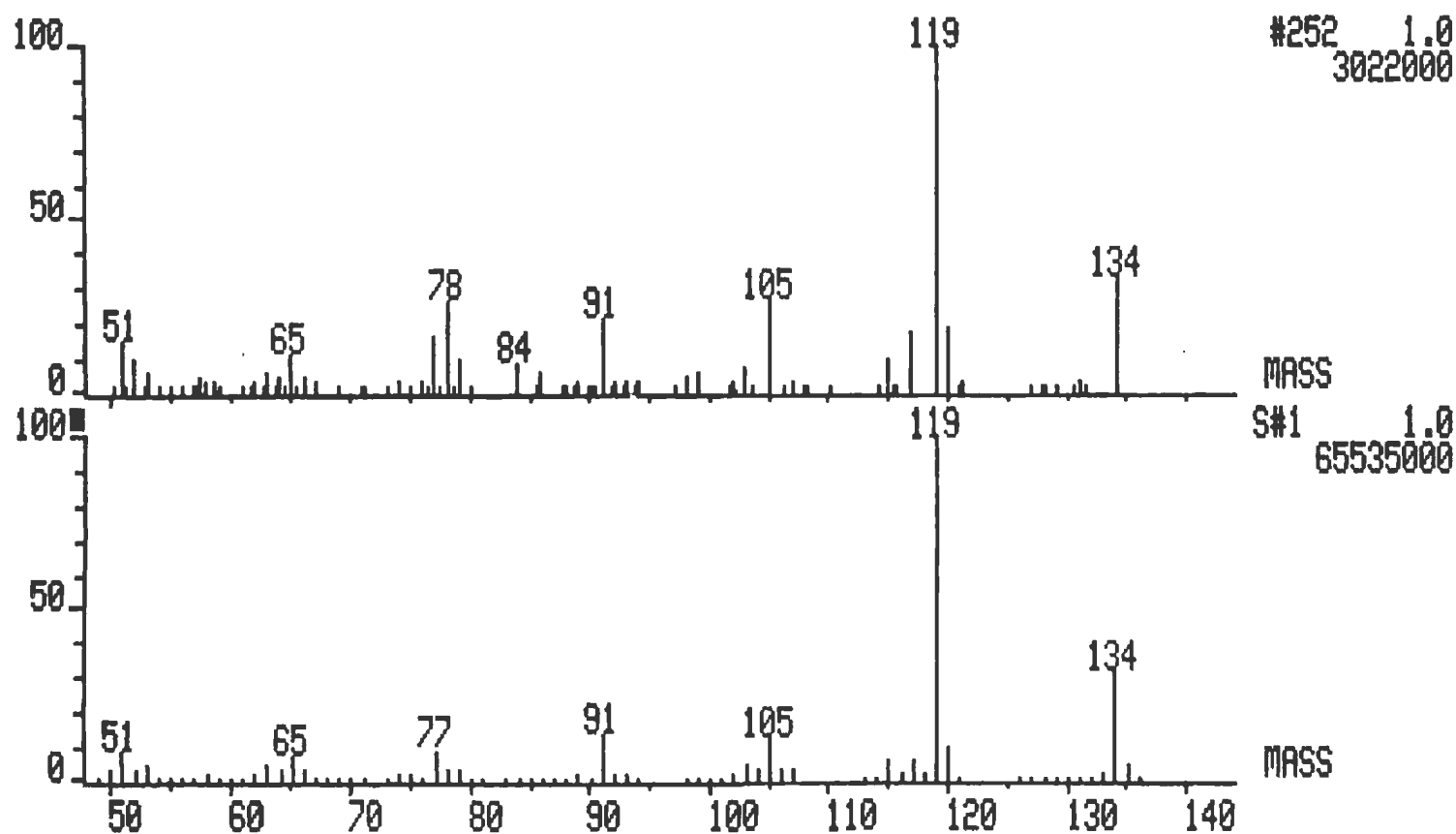
Lib:NBS

4772 Bpk: 105 Mwt: 134



LIBFITS1#1* x1 Bgd=252 T0402302
BENZENE, 1-ETHYL-2,3-DIMETHYL-
C10.H14.

+0:00:00
p803 M812 r861 RFN:933-98-2
Lib:NBS 4781 Bpk: 119 Mwt: 134



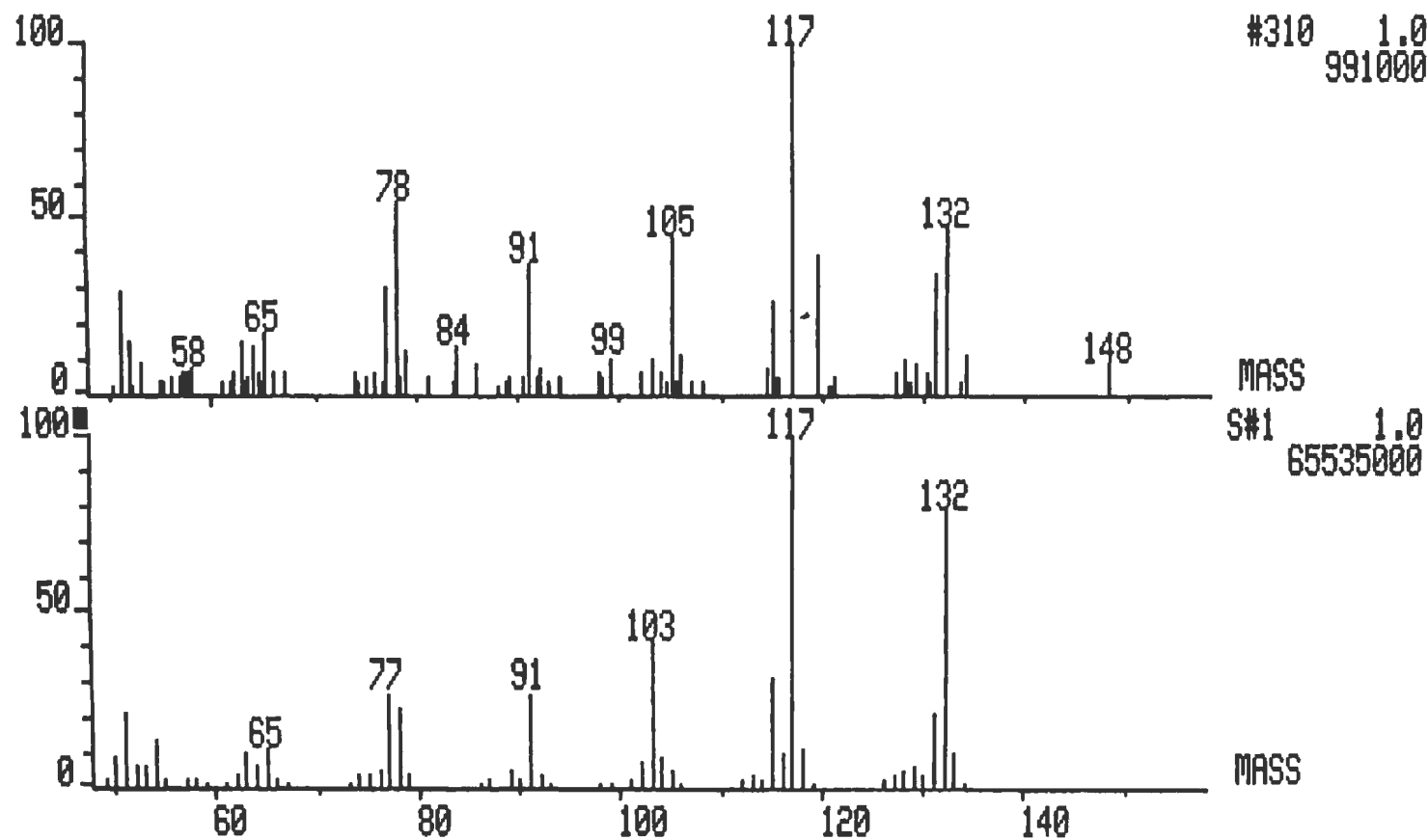
LIBFITS1#1* x1 Bgd=310 T0402302
BENZENE, (1-METHYLENEPROPYL)-
C10.H12.

+0:00:00

p671 M719 r719 RFN:2039-93-2

Lib:NBS

4536 Bpk: 117 Mwt: 132



T0302302 #1-1560 3-FEB-93 11:19 TS250

(EI+)

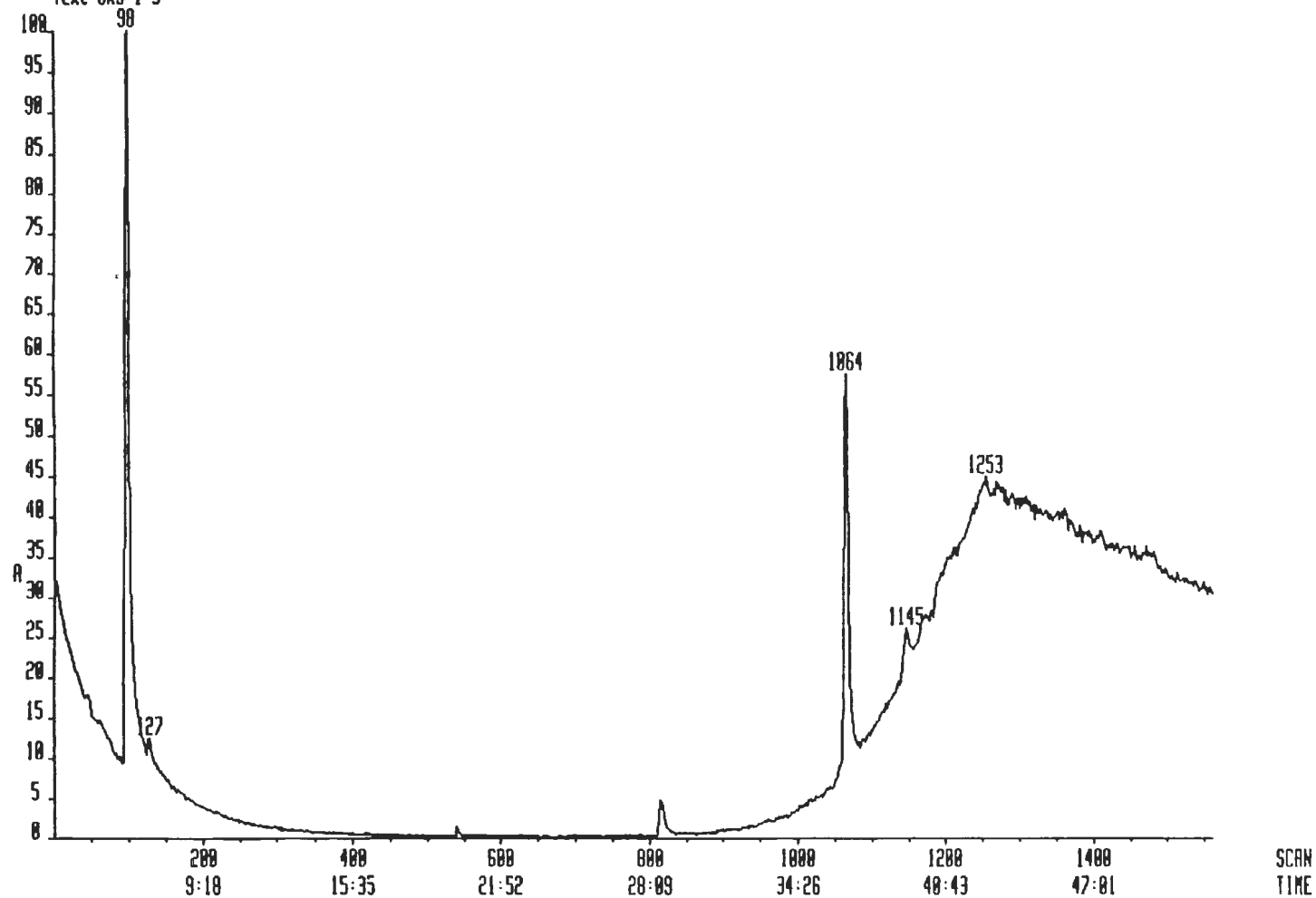
Sys:DEFAULTS

IHP

Chromatogram Identifiers : A:ATIC

A: 5404

Text:OKS-1-3



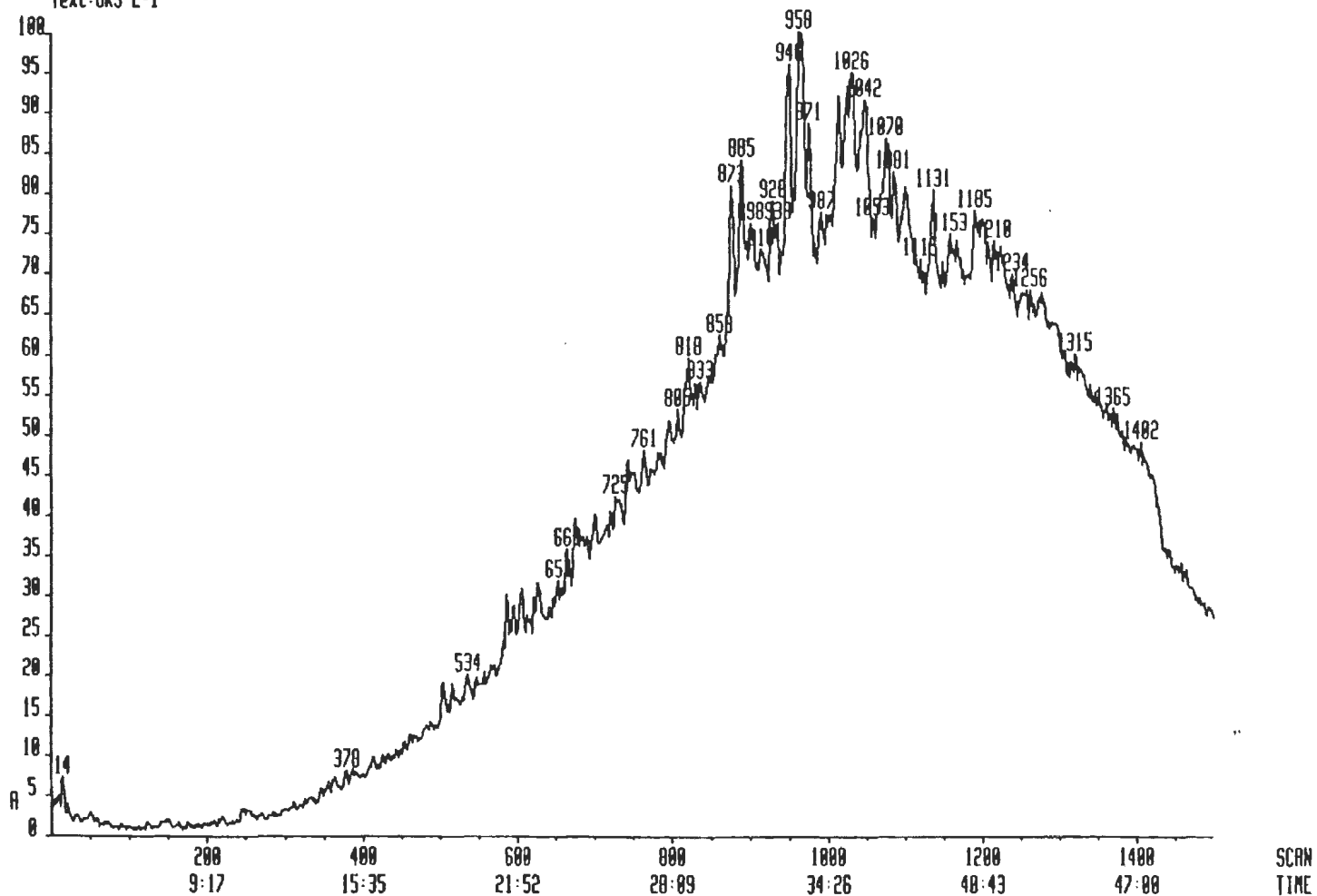
T0202303 01-1496 2-FEB-93 12:07 TS250
Chromatogram Identifiers : A : ATIC
Text:OKS-2-1

(E1+)

Sys:DEFAULTS

IHP

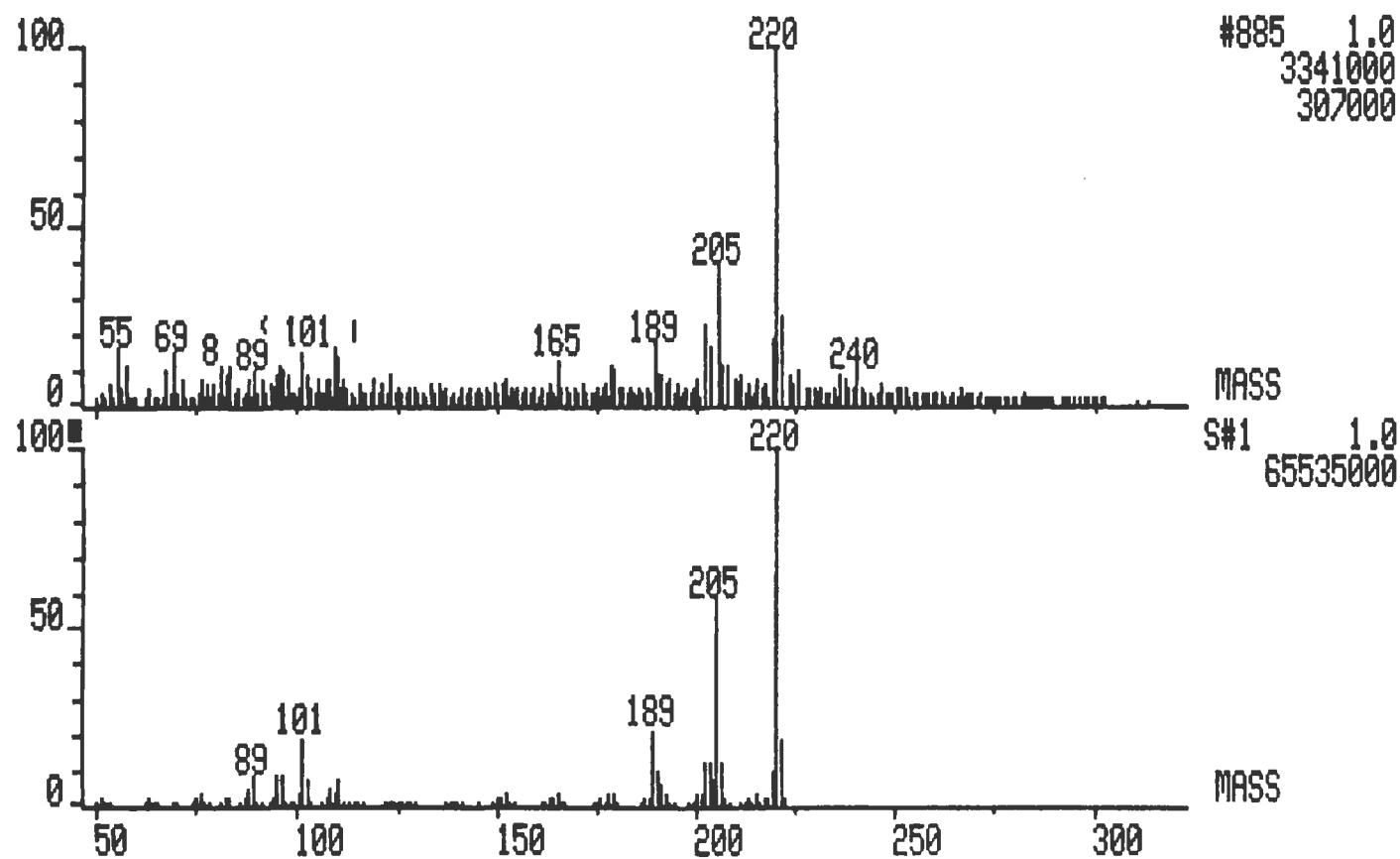
A: 3846



LIBFITS1#1* x1 Bgd=885 T0202303
PHENANTHRENE, 2,3,5-TRIMETHYL-
C17.H16.

+0:00:00

p445 M745 r466 RFN:3674-73-5
Lib:NBS 19656 Bpk: 220 Mwt: 220



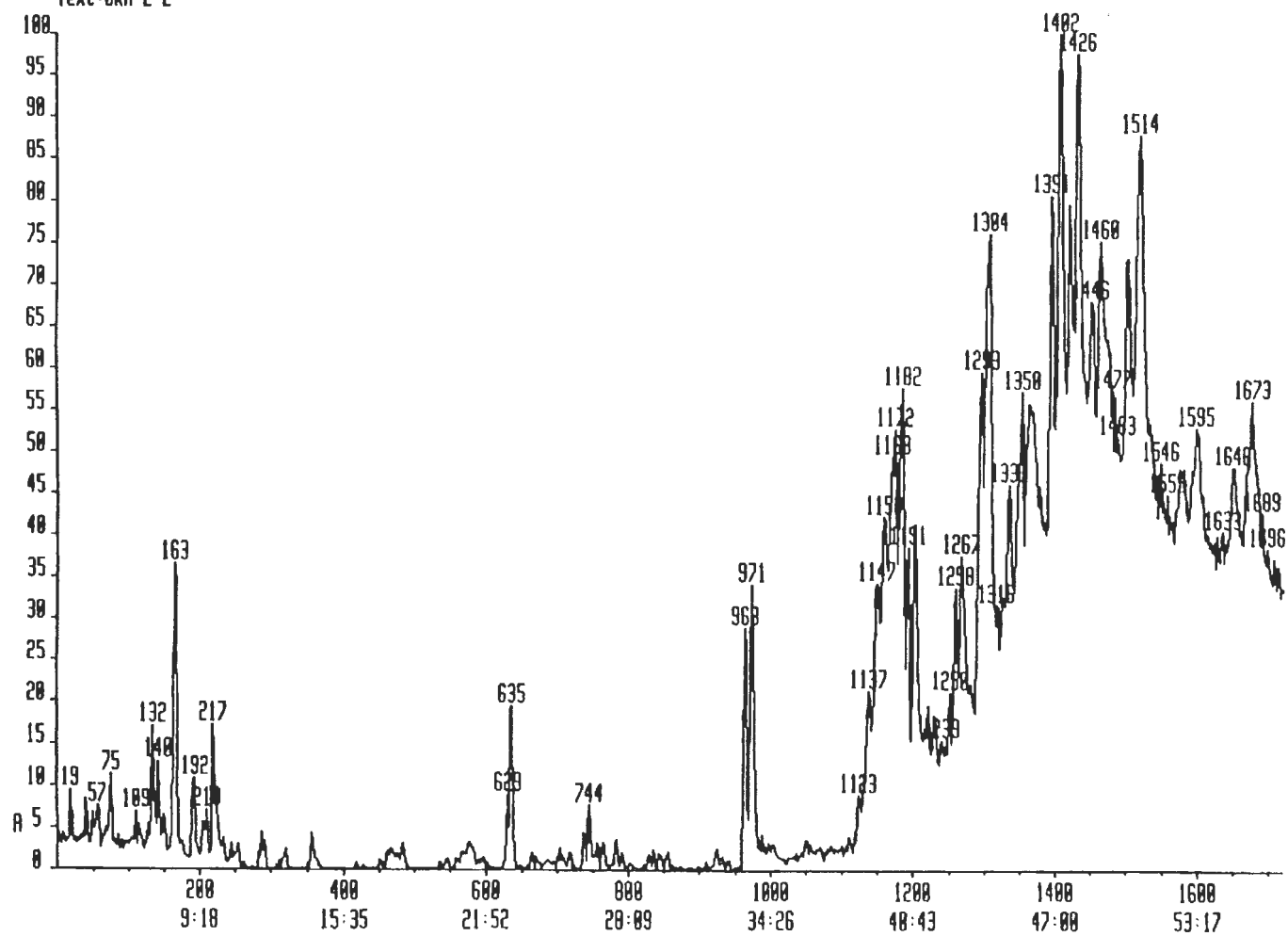
T0402303 01-1719 4-FEB-93 11:39 TS250
Chromatogram Identifiers : A : ATIC
Text:DKA-2-2

(E1+)

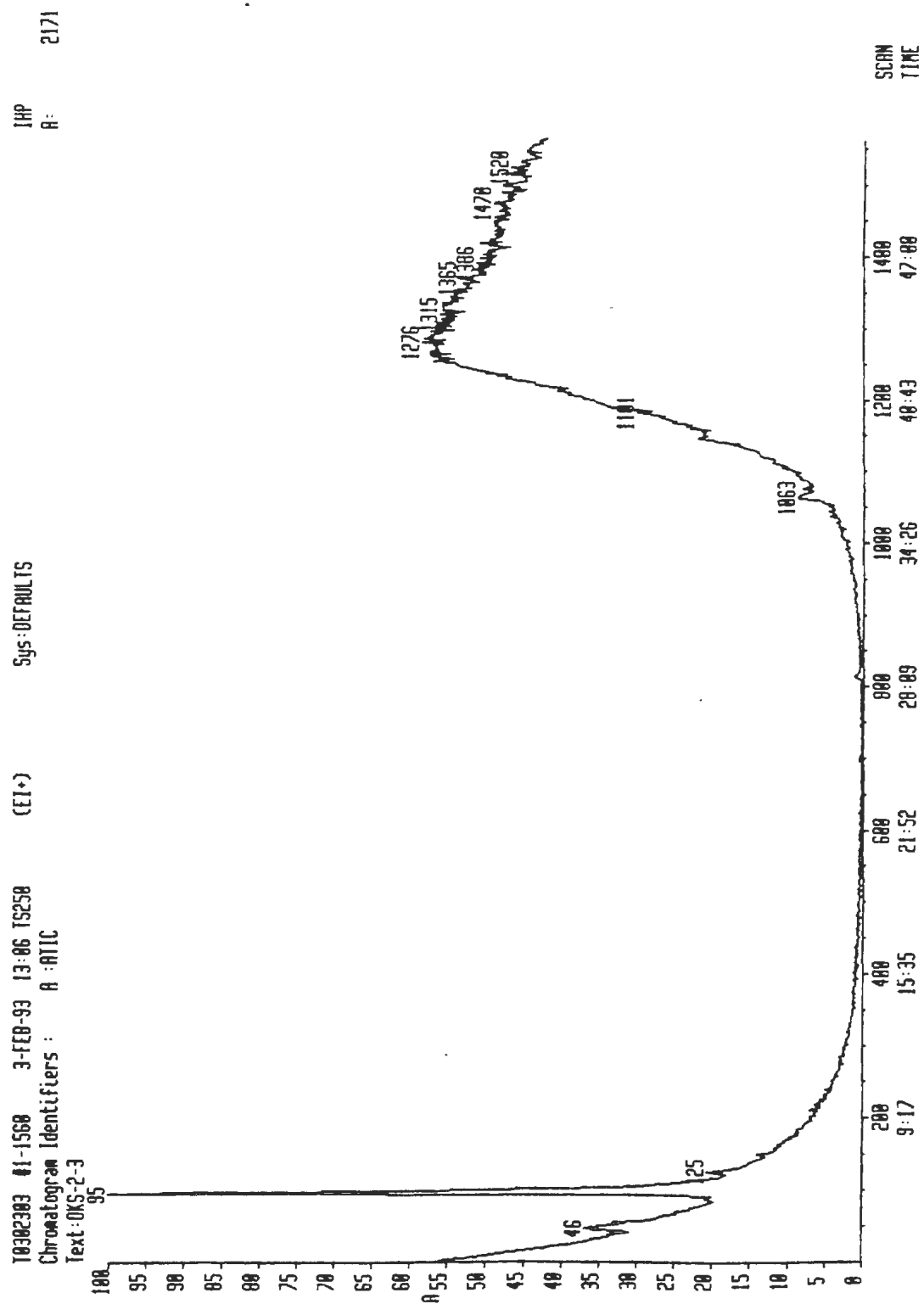
Sys:DEFAULTS

IHP

A: 572

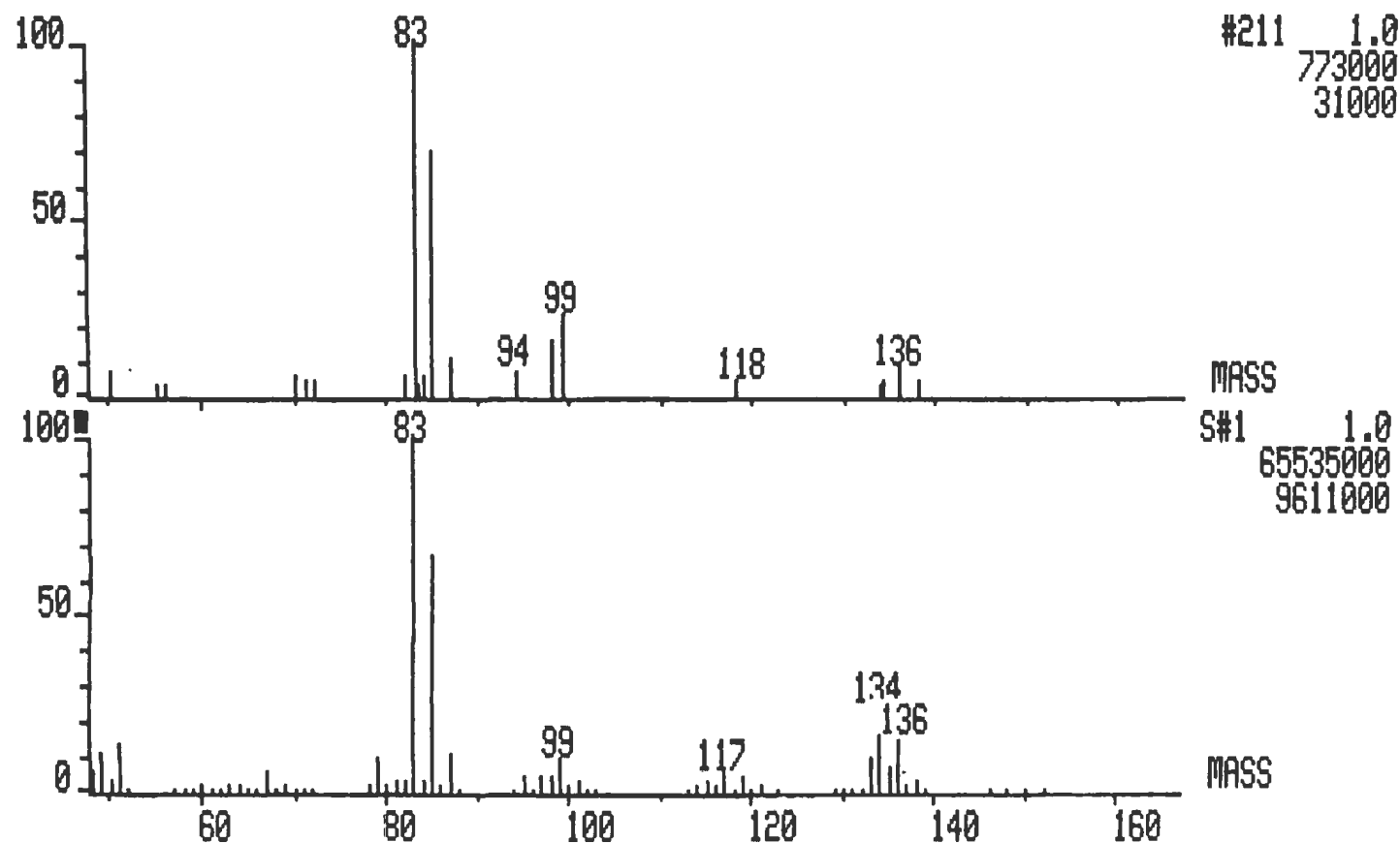


SCAN
TIME



LIBFITS1#1* x1 Bgd=211 T0302303
ETHANE, 1,1-DICHLORO-2,2-DIFLUORO-
C2.H2.CL2.F2.

+0:00:00
p560 M610 r793 RFN:471-43-2
Lib:NBS 4799 Bpk: 83 Mwt: 134



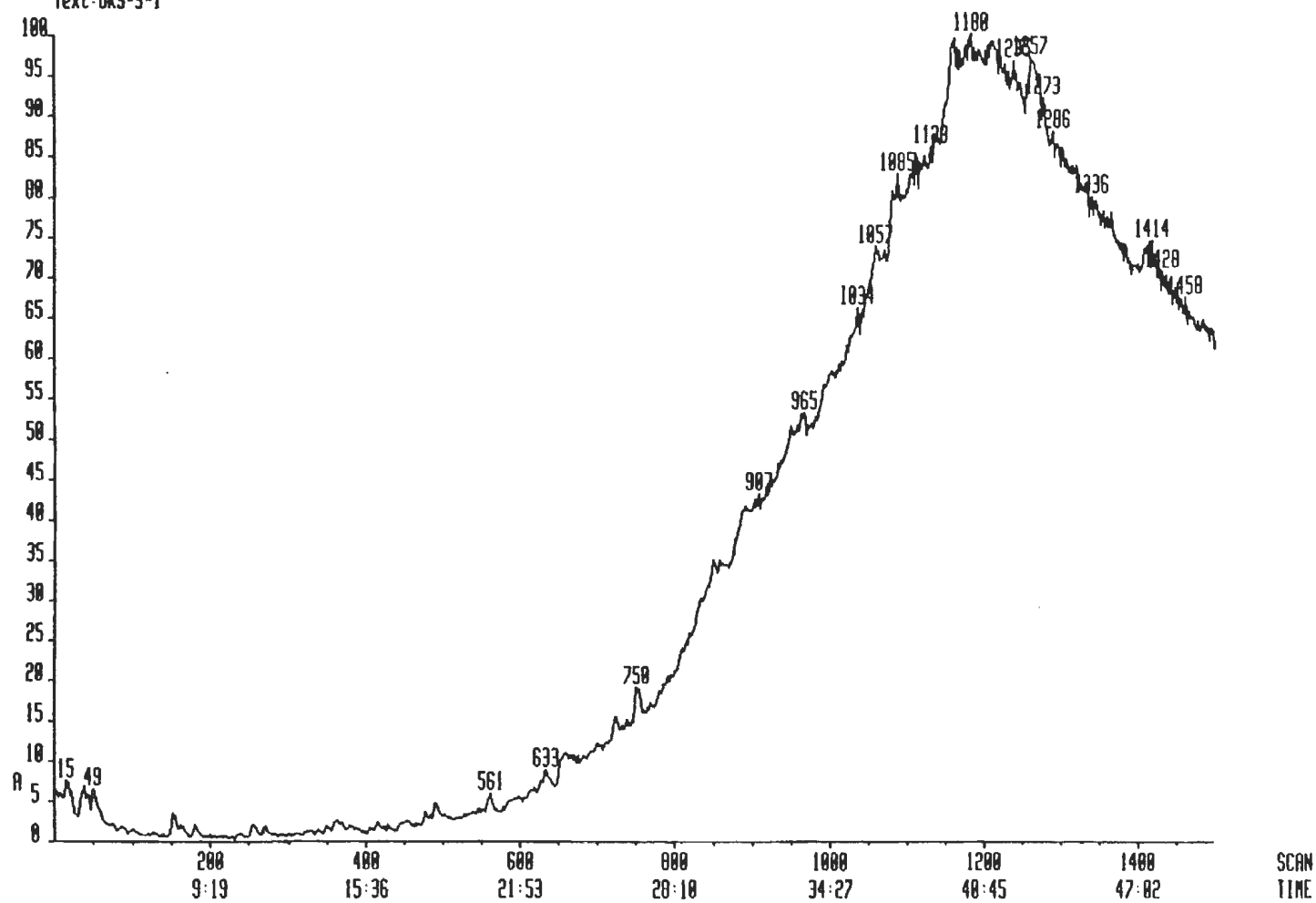
T0302301 01-1496 3-FEB-93 10:05 TS250
Chromatogram Identifiers : A :ATIC
Text:OKS-3-1

(E1+)

Sys:DEFAULTS

IMP
R:

5332



LIBFITS1#1* x1 Bgd=490 T0302301 +0:00:00

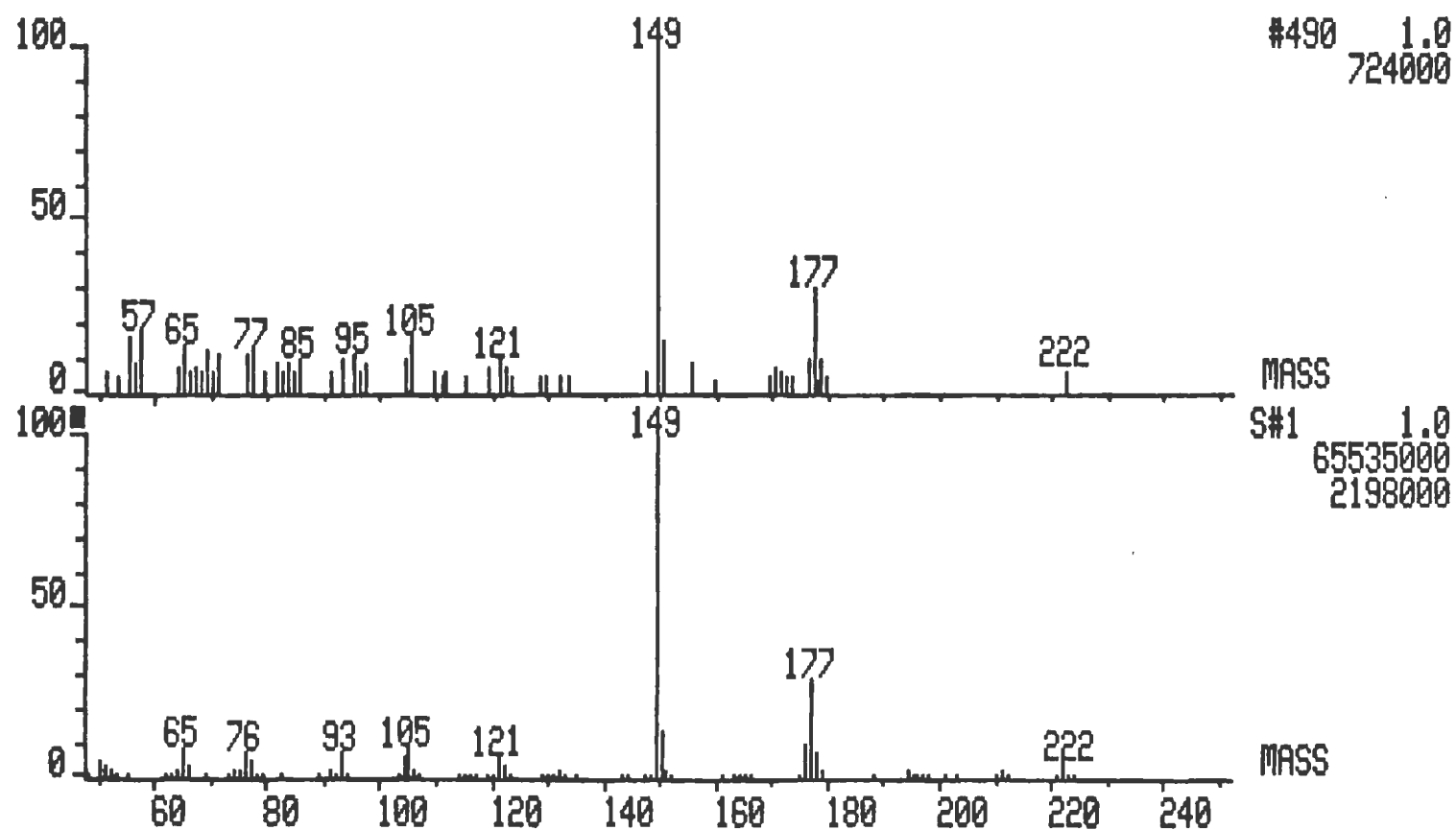
DIETHYL PHTHALATE

C12.H14.O4.

p573 M789 r629 RFN:84-66-2

Lib:NBS

19827 Bpk: 149 Mwt: 222



LIBFITS1#1* x1 Bgd=659 T0302301 +0:00:00

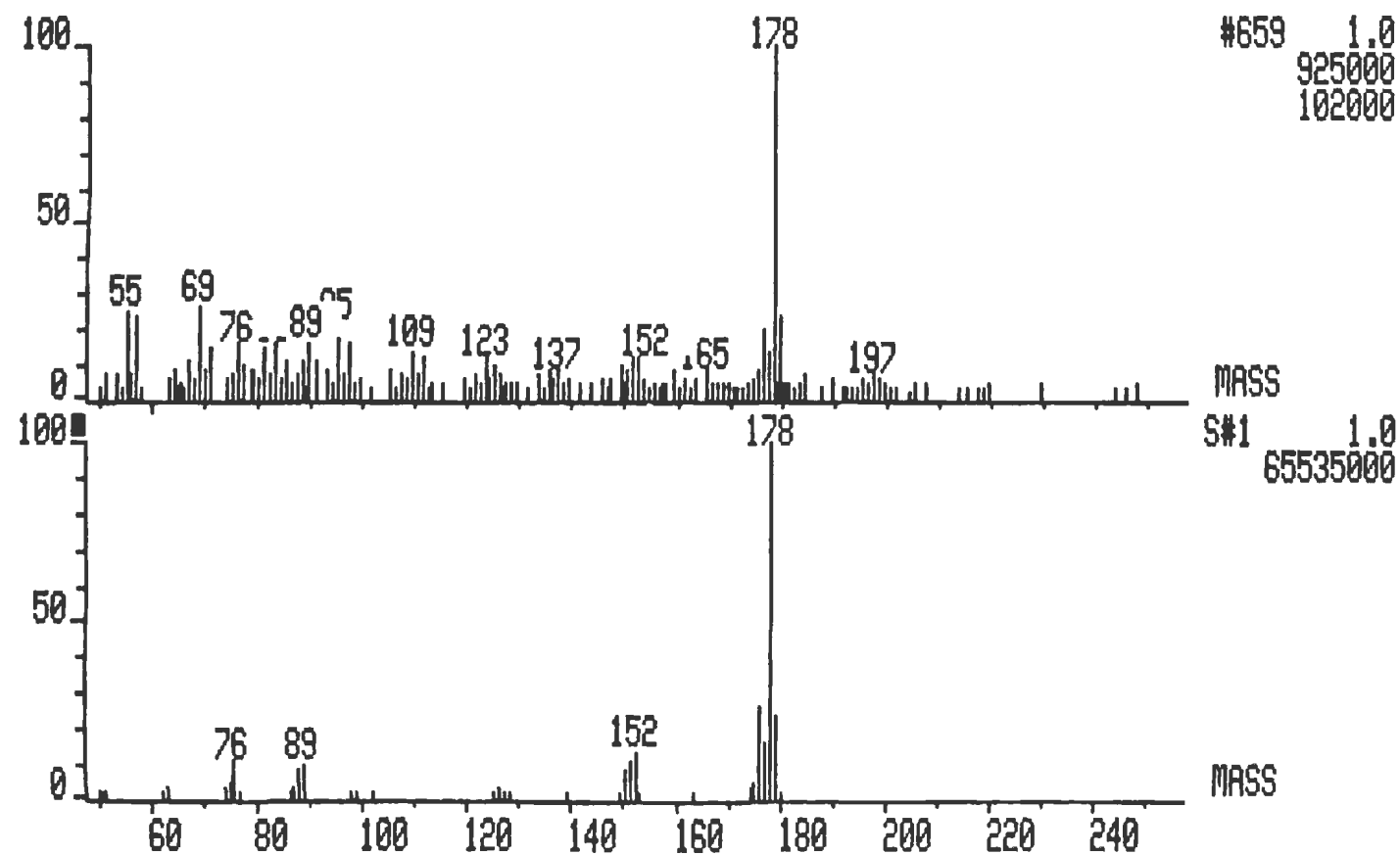
PHENANTHRENE

C14.H10.

p377 M917 r379 RFN:85-01-8

Lib:NBS

12787 Bpk: 178 Mwt: 178



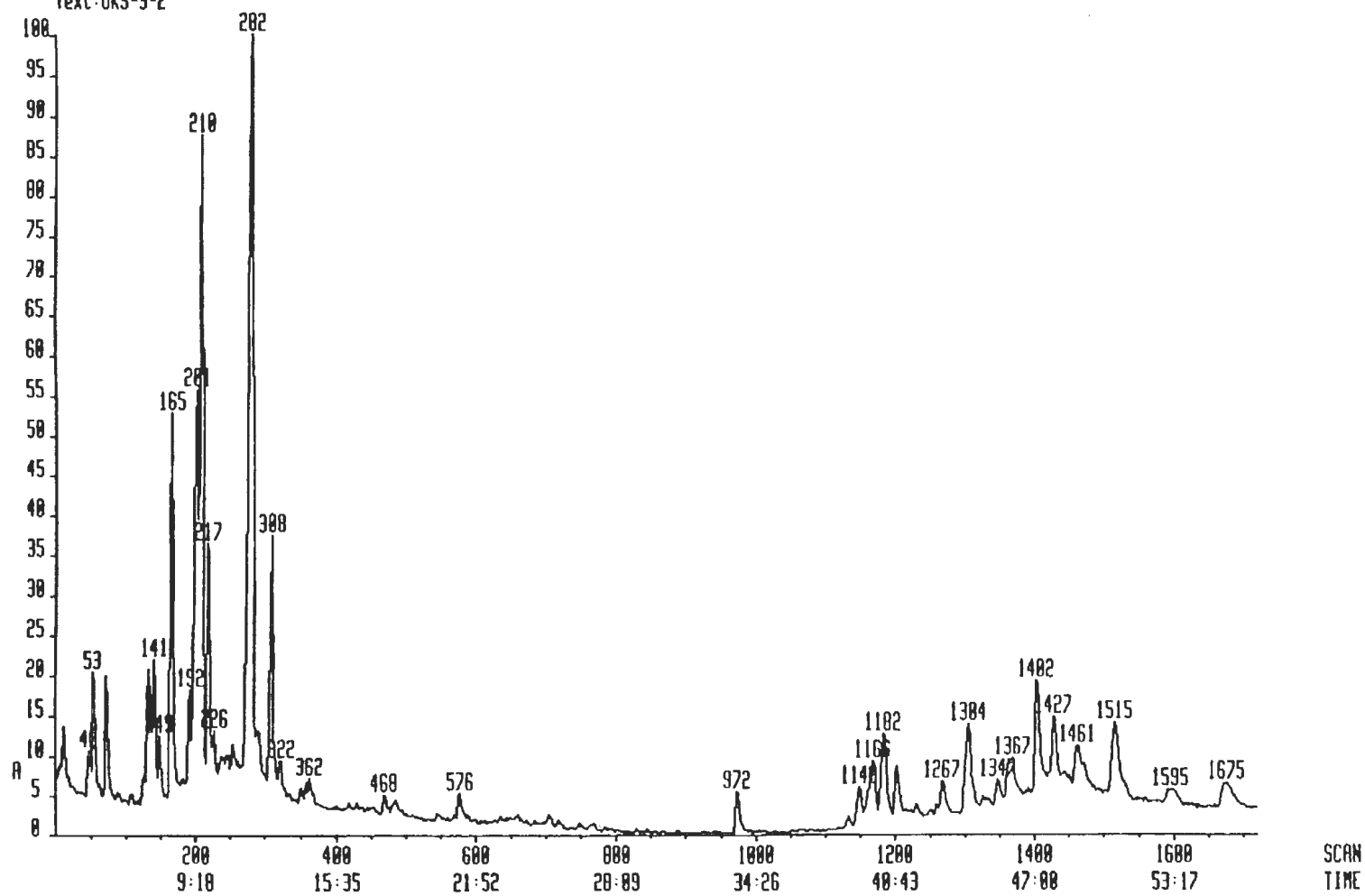
T0402304 01-1719 4-FEB-93 12:49 TS250
Chromatogram Identifiers : A : ATIC
Text:OKS-3-2

(E1+)

Sys:DEFAULTS

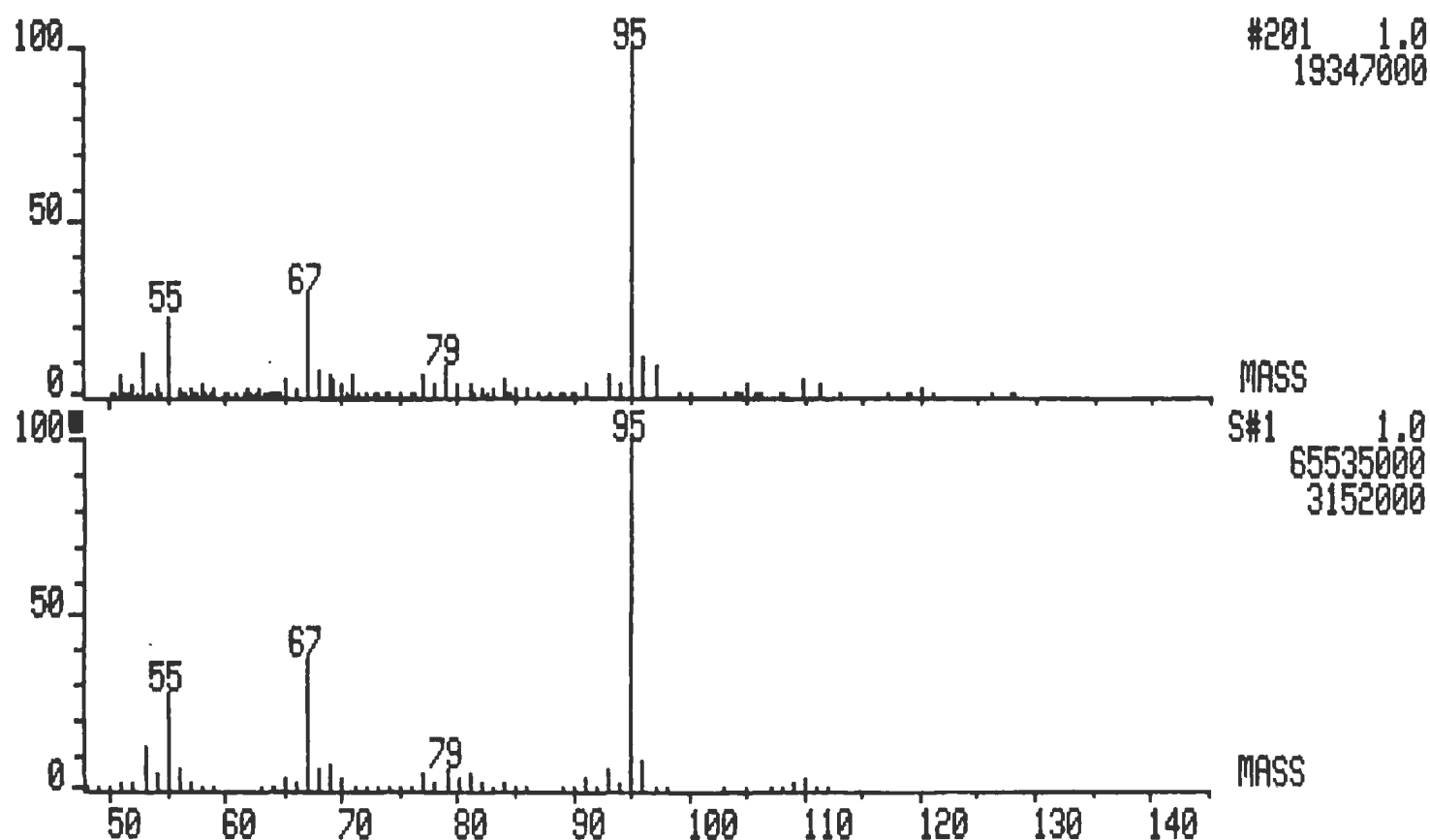
INP
A:

6279



LIBFITS1#1* x1 Bgd=201 T0402304
1,4-PENTADIENE, 2,3,4-TRIMETHYL-
C8.H14.

+0:00:00
p781 M806 r892 RFN:72014-90-5
Lib:NBS 2024 Bpk: 95 Mwt: 110



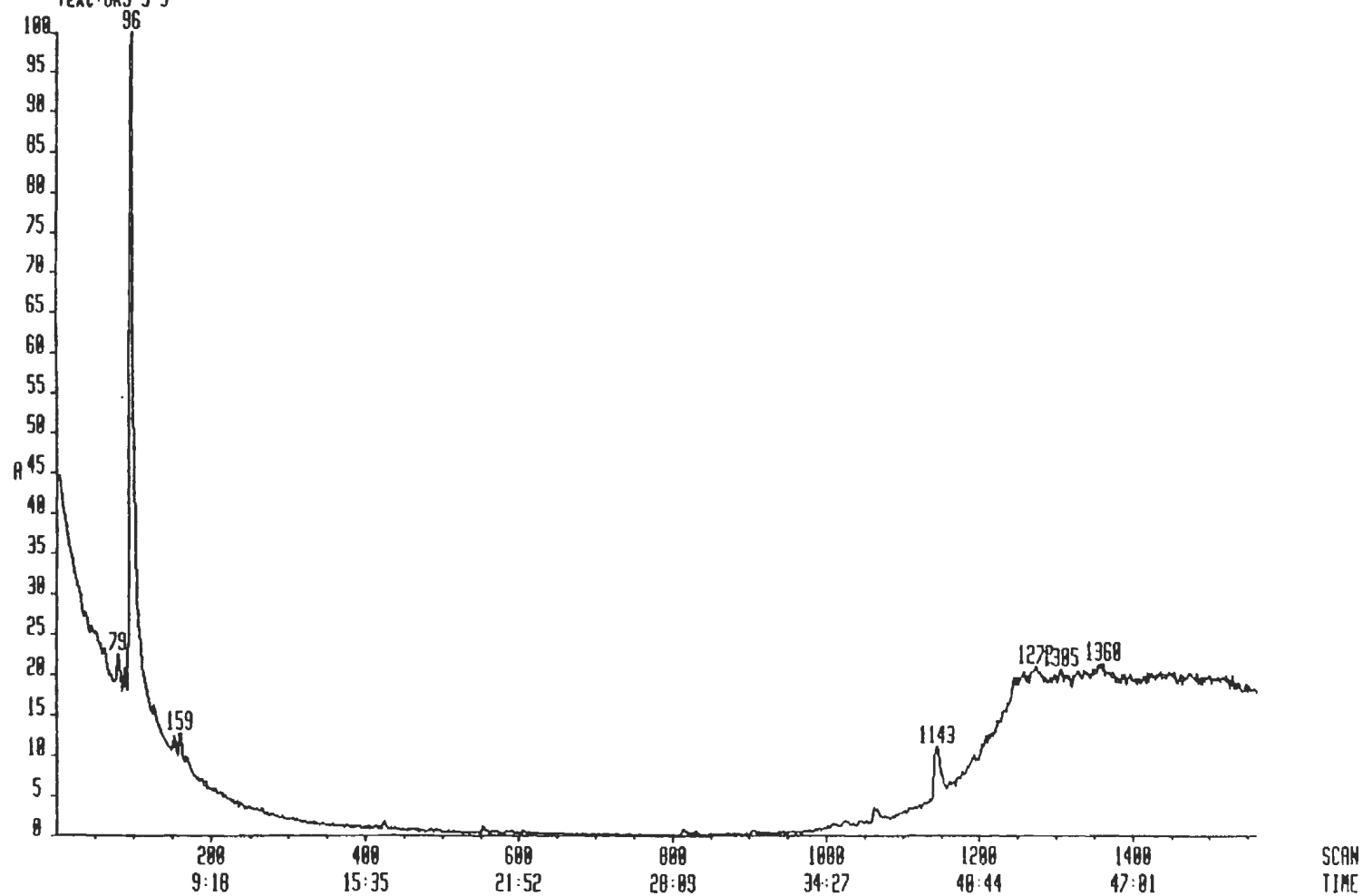
T0302304 #1-1560 3-FEB-93 14:10 TS250
Chromatogram Identifiers : A : ATIC
Text: OKS-3-3

(E1+)

Sys: DEFAULTS

IHP

A: 3550



APPENDIX H
CHEMICALS, REFERENCE NUMBERS,
AND SPECTRA RATINGS

ARBW-1

All Compounds Below Detection Limits

ARBW-2

All Compounds Below Detection Limits

ARBW-3

All Compounds Below Detection Limits

ARBS-1

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
12	1-Tridecanol	112-70-8			X		
17	Heptyl hexyl ether	7289-40-9			X		
71	3,5,24-Trimethyl-tetracontane	55162-61-3			X		
240	1,2,3-Trichlorobenzene	87-61-6	X				X
270	1,2,3-Trichlorobenzene	87-61-6	X				X
399	2,6,10-Trimethyldodecane	3891-98-3			X		
471	1,1'-Oxybisdecane	2456-28-2				X	
580	2-Methyl-1-(1,1-dimethyl)-propanoic acid	0-00-0	X				
661	2-Methyl-(S)-1-dodecanol	57289-26-6				X	
853	Butyl 2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5			X		X
911	Sulfur (molecular)	10544-50-0				X	

Peak #	Library Match	Reference #	Exc	Good	Poor	Artifact
7	1-Ethenyl-3-methylene-cyclopentene	61142-07-2			X	X
12	1-Ethenyl-3-methylene-cyclopentene	61142-07-2		X		X
20	1-Ethenyl-3-methylene-cyclopentene	61142-07-2			X	X
23	1,3-Dimethylbenzene	108-38-3		X		X
51	1-Chlorododecane	112-52-7			X	
55	1-Ethyl-3-methylbenzene	620-14-4		X		X
61	1-Ethyl-3-methylbenzene	620-14-4		X		X
79	1,3,5-Trimethylbenzene	108-67-8		X		X
89	2,6,10,13-Tetramethyl-pentadecane	17081-50-4			X	X
92	2,6,10,13-Tetramethyl-pentadecane	17081-50-4			X	X
105	1-Pentadecanol	629-76-5			X	
132	Cyclododecanol	1724-39-6			X	
139	8,4-Dimethyl-trans-cyclohexaneethanol	5113-94-0			X	
146	8,4-Dimethyl-trans-cyclohexaneethanol	5113-94-0			X	
167	4,5-Dimethylnonane	17302-23-7			X	
178	Decahydro-2-methyl-naphthalene	2958-76-1		X		X
241	1,2,3-Trichlorobenzene	87-61-6	X			X
273	1,2,3-Trichlorobenzene	87-61-6	X			X
379	7-Cyclohexyl-7-cyclohexyl-tridecane	13151-92-3			X	X
385	3,7,11-Trimethyl-1-dodecanol	6750-34-1			X	

ARBS-2 cont.

264

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
391	2-Bromo-5-ethylnonane	55162-38-4			X		
471	4-Methylpentadecane	2801-87-8			X		
1098	Diocetyl ester hexanedioate	123-79-5		X			X

ARBS-3

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
4	Tetrachloroethene	127-18-4		X			X
8	Tetrachloroethene	127-18-4			X		X
33	1,3-Dimethylbenzene	108-38-3			X		X
298	1,2,3-Trichlorobenzene	87-61-6		X			X
1166	Diocetyl ester hexanedioate	123-79-5		X			X
1243	Bis(2-ethylhexyl) phthalate	117-81-7	X				X
1269	Bis(2-ethylhexyl) phthalate	117-81-7		X			X
1290	Bis(2-ethylhexyl) phthalate	117-81-7		X			X
1299	Bis(2-ethylhexyl) phthalate	117-81-7		X			X
1344	Bis(2-ethylhexyl) phthalate	117-81-7		X			X

TW-1

265

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
54	Tetrachloroethene	127-18-4			X		X
281	2-Chloro-trans-cyclohexanol	6628-80-4			X		X
336	4,5-Dimethylnonane	17302-23-7		X			
780	2-Methyl-1-(1,1-dimethylethyl)propanoic acid	0-00-0	X				
790	Diethyl phthalate	84-06-2	X				
1051	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5	X				X

TW-2

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
51	Tetrachloroethene	127-18-4	X				X
280	Trans-2-chlorocyclohexanol	6628-80-4		X			X
780	2-Methyl-1-(1,1-dimethylethyl)propanoic acid	0-00-0	X				
793	Diethyl phthalate	84-06-2		X			

TW-3

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
54	Tetrachloroethene	127-18-4	X				X
277	Trans-2-chlorocyclohexanol	6628-80-4			X		X
356	Decamethylcyclopentasiloxane	541-02-6		X			
778	2-Methyl-1-(1,1-dimethylethyl)propanoic acid	0-00-0	X				
1048	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5	X				X

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
701	2-Propenylhydrazonepropanal	19031-78-8			X		
975	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5			X		X

TS-1-1

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
3	Didecyl ester decanedioate	2342-89-5			X		X
248	1,2,3-Trichlorobenzene	87-61-6	X				X
279	1,2,3-Trichlorobenzene	87-61-6	X				X
407	3,7,11-Trimethyl-1-dodecanol	6750-34-1			X		
430	2-Methyl-1-hexadecanol	2490-48-4				X	
479	3,7,11-Trimethyl-1-dodecanol	6750-34-1			X		
514	3,7,11-Trimethyl-1-dodecanol	6750-34-1			X		
554	2-Bromo-5-ethylnonane	55162-38-4				X	
774	Phenanthrene	85-01-8				X	
809	Isoheptadecanol	57289-07-3			X		
1003	Pyrene	129-00-0			X		
1201	Diisooctyl ester 1,2- benzenedicarboxylate	27554-26-3			X		X

TS-1-2

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
279	Cis-2-chlorocyclohexanol	16536-58-6				X	
411	1,2,3-Trichlorobenzene	87-61-6	X				X
422	1,3,5-Trichlorobenzene	108-70-3		X			X
428	1,2,3-Trichlorobenzene	87-61-6	X				X
1369	Bis(2-ethylhexyl) phthalate	117-81-7		X			X
1495	Diisononyl ester 1,2- benzenedicarboxylate	28553-12-0	X				X

TS-1-3

267

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
9	Tetrachloroethene	127-18-4	X				X
42	α,β -Dimethylbenzeneethanol	52089-32-4		X			
54	α,β -Dimethylbenzeneethanol	52089-32-4		X			
117	Phenol	108-95-2		X			
306	1,2,3-Trichlorobenzene	87-61-6	X				X
337	1,2,3-Trichlorobenzene	87-61-6	X				X
1173	Diioctyl ester hexanedioate	123-79-5		X			X
1253	Diisooctyl ester 1,2-benzene dicarboxylate	28553-12-0	X				X
1379	Diisooctyl ester 1,2-benzene dicarboxylate	28553-12-0	X				X

TS-2-1

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
5	6-Methylheptyl ester 2-propanoate	54774-91-3				X	
249	1,2,3-Trichlorobenzene	87-61-6	X				X
258	Naphthalene	91-20-3	X				
279	1,2,3-Trichlorobenzene	87-61-6	X				X
359	1-Methylnaphthalene	90-12-0		X			
456	1,5-Dimethylnaphthalene	571-61-9		X			
373	2-Methylnaphthalene	91-57-6	X				
445	1,2-Dimethylnaphthalene	573-98-8			X		
467	2,3-Dimethylnaphthalene	581-40-8			X		
471	1,2-Dimethylnaphthalene	573-98-8			X		
538	(1-Methylethyl)naphthalene	29253-36-9			X		

TS-2-1 cont.

268

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
764	Anthracene	120-12-7		X			
769	9-Octadecen-1-ol	143-28-2		X			
955	Pyrene	129-00-0			X		
998	Pyrene	129-00-0		X			
1196	Naphthacene	92-24-0			X		

TS-2-2

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
112	1-Ethenyl-3-methylene-cyclopentene	61142-07-2			X		X
133	1,2-Dimethylbenzene	95-47-6	X				X
233	1,3,5-Trimethylbenzene	108-67-8		X			X
429	1,2,3-Trichlorobenzene	87-61-6	X				X
463	1,2,3-Trichlorobenzene	87-61-6	X				X
578	7-Cyclohexyl-7-cyclohexyl-tridecane	13151-92-3			X		X
598	2,6,10-Trimethyldodecane	3891-98-3		X			

TS-2-3

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
9	Tetrachloroethene	127-18-4		X			X
5	Tetrachloroethene	127-18-4		X			X
306	1,2,3-Trichlorobenzene	87-61-6	X				X
336	1,2,3-Trichlorobenzene	87-61-6		X			X
937	Ethyl ester hexadecanoate	628-97-7		X			X
1173	Diethyl ester hexanedioate	123-79-5		X			X
1251	Diisooctyl ester 1,2-benzenedicarboxylate	27554-26-3	X				X

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
248	1,2,3-Trichlorobenzene	87-61-6	X				X
278	1,2,3-Trichlorobenzene	87-61-6	X				X
359	1-Methylnaphthalene	90-12-0		X			
407	2,6,10-Trimethyldodecane	6750-34-1		X			
430	2-Methyl-(S)-1-dodecanol	57289-26-6			X		
455	1,5-Dimethylnaphthalene	571-61-9			X		
523	1,2-Dihydroacenaphthylene	83-32-9			X		
605	9H-Fluorene	86-73-7			X		
668	3,7,11-Trimethyl-1-dodecanol	6750-34-1			X		
465	1,5-Dimethylnaphthalene	571-61-9			X		
479	2,6,10,15-Tetramethyl-heptadecane	54833-48-6			X		
738	Dibenzothiophene	132-65-0			X		
760	Phenanthrene	85-01-8		X			
805	Dodecyl isopropyl ether	29379-42-8				X	
842	1-Methylphenanthrene	832-69-9			X		
854	4-Methylphenanthrene	832-64-4			X		
895	9,10-Anthracenedione	84-65-1			X		
952	Pyrene	129-00-0		X			
987	Pyrene	129-00-0		X			
1056	7H-Benzo[c]fluorene	205-12-9				X	
1187	Triphenylene	217-59-4			X		
1357	Benzo[a]pyrene	50-32-8		X			

TS-3-2

270

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
17	Methylbenzene	108-88-3	X				
111	1-Ethenyl-3-methylene-cyclopentene	61142-07-2			X		X
132	1-Ethenyl-3-methylene-cyclopentene	61142-07-2			X		X
198	1-Ethyl-3-methylbenzene	620-14-4		X			X
232	1,3,5-Trimethylbenzene	108-67-8			X		X
429	1,2,3-Trichlorobenzene	87-61-6	X				X
451	1-Tridecanol	26248-42-0			X		
466	1,2,3-Trichlorobenzene	87-61-6	X				X

TS-3-3

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
118	Phenol	108-95-2			X		
307	1,2,3-Trichlorobenzene	87-61-6	X				X
339	1,2,3-Trichlorobenzene	87-61-6	X				X
1179	Diethyl ester hexanedioate	123-79-5	X				X
1253	Diisooctyl ester 1,2-benzenedicarboxylate	27554-26-3		X			X

TS-4-1

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
52	1,3,5-Trimethylbenzene	108-67-8		X			X
78	3,4-Dimethyl-methyl-, ester 2-cyclopentenecarboxylate	62185-63-1			X		X
789	12-(Acetyloxy)-methyl ester 9-octadecenoate	140-03-4		X			
818	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5		X			X

TS-4-2

271

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
56	1-Ethenyl-3-methylene-cyclopentene	61142-07-2			X		X
75	1-Ethenyl-3-methylene-cyclopentene	61142-07-2			X		X
134	(1-Methylethyl)benzene	98-82-8			X		
137	(1-Methylethyl)benzene	98-82-8			X		
143	1,2,3-Trimethylbenzene	526-73-8			X		X
151	(1-Methylethyl)benzene	98-82-8		X			
167	1,2,4-Trimethylbenzene	0-00-0		X			X
195	1,2,3-Trimethylbenzene	526-73-8		X			X
976	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5		X			X

TS-4-3

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
1068	Diethyl ester hexanedioate	123-79-5		X			X
1253	Diisooctyl ester 1,2-benzenedicarboxylate	27554-26-3		X			X

ARDW-1

272

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
35	Tetrachloroethene	127-18-4		X			X
815	Bis(2-methylethyl) ester 1,2-benzenedicarboxylate	117-82-8		X			X
1007	Benzyl butyl phthalate	85-68-7		X			

ARDW-2

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
37	Tetrachloroethene	127-18-4		X			X

ARDW-3

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
10	Tetrachloroethene	127-18-4		X			X
700	2-1,1-(1,1-dimethylethyl)- propanoic acid	0-00-0		X			
927	2,2-Dimethyl-1,2-diphenyl- ethanone	24650-42-8		X			
979	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5		X			X
1216	Benzyl butyl phthalate	85-68-7	X				
1252	2-Ethylhexyldiphenyl ester phosphate	1241-94-7			X		

ARDS-1-1

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
15	1,3,5-Trimethylbenzene	108-67-8	X				X
49	2,4-Diethyl-1,3,2- dioxaborolane	57633-63-3			X		
83	Decamethylcyclopenta- siloxane	541-02-6			X		
113	1,2,3-Trichlorobenzene	87-61-6		X			X

ARDS-1-1 cont.

273

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
178	1,2,3-Trichlorobenzene	87-61-6		X			X
476	2-Methyl-1-(1,1-dimethyl-ethyl)propanoic acid	0-00-0			X		
995	Diioctyl ester hexanedioate	123-79-5			X		X
1073	Diisooctyl ester 1,2-benzene dicarboxylate	27554-26-3		X			X
1099	2,6,10,14-Tetramethyl-hexadecane	638-36-8		X			
1249	Dotriacontane	544-85-4			X		

ARDS-1-2

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
53	1,3-Dimethylbenzene	108-38-3	X				
72	1,3-Dimethylbenzene	108-38-3	X				
124	Propylbenzene	103-65-1		X			
131	(1-Methylethyl)benzene	98-82-8		X			
140	1,3,5-Trimethylbenzene	108-67-8		X			X
148	(1-Methylethyl)benzene	98-82-8		X			
165	1,3,5-Trimethylbenzene	108-67-8		X			X
191	1-Ethyl-3-methylbenzene	620-14-4		X			
252	1,4-Diethylbenzene	105-05-5		X			
289	2-Ethyl-1,4-dimethylbenzene	1758-88-9		X			
971	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5			X		X

ARDS-1-3

274

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
99	2,4-Diethyl-1,3,2-dioxoborolane	57633-63-3			X		
1067	Dioctyl ester hexanedioate	123-79-5		X			X
1147	3-Nitro-1,2-benzene-dicarboxylic acid	605-11-2		X			X
1170	3-Nitro-1,2-benzene-dicarboxylic acid	605-11-2		X			X
1201	3-Nitro-1,2-benzene-dicarboxylic acid	605-11-2		X			X

ARDS-2-1

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
2	1-Ethyl-3-methylbenzene	620-14-4		X			X
5	1,3,5-Trimethylbenzene	108-67-8			X		X
20	1,3,5-Trimethylbenzene	108-67-8		X			X

ARDS-2-2

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
59	1-Ethenyl-3-methylene-cyclopentene	61142-07-2		X			X
78	1,3-Dimethylbenzene	108-38-3	X				
139	1-Ethyl-3-methylbenzene	620-14-4	X				X
147	1-Ethyl-3-methylbenzene	620-14-4	X				X
155	1-Ethyl-2-methylbenzene	611-14-3		X			X
172	1,3,5-Trimethylbenzene	108-67-8		X			X
217	2,3,4-Trimethyl-1,4-pentadiene	72014-90-5		X			
299	2,3,3-Trimethyl-1,4-pentadiene	756-02-5		X			
980	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5		X			X
1309	Diisooctyl ester 1,2-benzene dicarboxylate	27554-26-3		X			X

ARDS-2-3

275

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
1068	Diioctyl ester hexanedioate	123-79-5		X			X
1172	Diisooctyl ester 1,2-benzene dicarboxylate	27554-26-3		X			X
1205	Diisooctyl ester 1,2-benzene dicarboxylate	27554-26-3		X			X

ARDS-3-1

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
17	1,3,5-Trimethylbenzene	108-67-8		X			X
85	Decamethylcyclopentasiloxane	541-02-6		X			
826	Sulfur (molecular)	10544-50-0			X		

ARDS-3-2

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
54	1-Ethenyl-3-methylene-cyclopentene	61142-07-2		X			X
73	1,3-Dimethylbenzene	108-38-3		X			
75	1,3-Dimethylbenzene	108-38-3		X			
135	(1-Methylethyl)benzene	98-82-8		X			
142	1-Ethyl-3-methylbenzene	620-14-4		X			X
151	(1-Methylethyl)benzene	98-82-8		X			
167	1,3,5-Trimethylbenzene	108-67-8		X			X
194	1,2,3-Trimethylbenzene	526-73-8		X			X
227	1,2-Diethylbenzene	135-01-3			X		
276	2,3,4-Trimethyl-1,4-pentadiene	72014-90-5		X			
984	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5		X			X

ARDS-3-3

All Compounds Below Detection Limits

CW-1A

276

All Compounds Below Detection Limits

CW-2A

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
217	2,4-Dimethyl-2,3-heptadien-5-yne	41898-89-9		X			

CW-2A-2

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
216	1,3,5-Trimethylbenzene	108-67-8		X			X
270	1-Methyl-2-(1-methylethyl)-benzene	0-00-0		X			
306	1,2,3,4-Tetramethylbenzene	488-23-3	X				
434	1,2,3,5-Tetramethylbenzene	527-53-7		X			
652	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5		X			X

CW-2A-3

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
232	1,3,5-Trimethylbenzene	108-67-8		X			X
237	1,2,3-Trimethylbenzene	108-67-8		X			X
264	1,2,3-Trimethylbenzene	108-67-8		X			X
286	1,2,3,4-Tetramethylbenzene	488-23-3		X			
301	1,2,3,4-Tetramethylbenzene	488-23-3		X			
321	1,2,3,4-Tetramethylbenzene	488-23-3		X			
338	1,2,3,4-Tetramethylbenzene	488-23-3		X			
359	1,3-Diethyl-5-methylbenzene	2050-24-0			X		
363	Diethylmethylbenzene	25550-13-4			X		
409	1-Ethylidene-1H-indene	0-00-0			X		
417	1-Methylnaphthalene	90-12-0		X			

CW-2A-3 cont.

277

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
461	1,6-Dimethylnaphthalene	575-43-9			X		
469	1,5-Dimethylnaphthalene	571-61-9		X			
509	7-(1H-imidazol-2-yl)-bicyclo-[4,2,0]octan-7-ol	69393-32-4				X	
646	1,3,5-Trimethylbenzene	108-67-8				X	X
666	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5		X			X

CW-3-A

All Compounds Below Detection Limits

CW-4-A

All Compounds Below Detection Limits

CS-1A-1

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
217	1,2,3-Trichlorobenzene	87-61-6	X				X
220	1,2,3-Trichlorobenzene	87-61-6		X			X
248	1,2,3-Trichlorobenzene	87-61-6	X				X
532	5,6,7,7a-Tetrahydro-4,7,7a-trimethyl-(S)-2(4H)-benzofuranone	17092-92-1	X				
1352	1-Dotriacontanol	6624-79-9			X		X

CS-1A-2

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
84	1-Ethenyl-3-methylene-cyclopentene	61142-07-2			X		X
105	1-Ethenyl-3-methylene-cyclopentene	61142-07-2			X		X
172	1-Ethyl-3-methylbenzene	620-14-4		X			X

CS-1A-2 cont.

278

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
207	1,3,5-Trimethylbenzene	108-67-8		X			X
399	1,2,3-Trichlorobenzene	87-61-6	X				X
425	1-Tridecanol	112-70-8			X		
436	1,2,3-Trichlorobenzene	87-61-6	X				X
449	Diethyl(1-ethyl-2-methyl-1-butenyl)-(E)-borane	61204-98-6			X		
453	2-Cyclohexyl-2-cyclohexyl-dodecane	13151-82-1		X			X

CS-1A-3

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
9	1,3-Dimethylbenzene	108-38-3	X				
85	4-Hydroxybenzenesulfonic acid	98-67-9	X				
277	1,3,5-Trichlorobenzene	108-70-3	X				X
309	1,2,3-Trichlorobenzene	87-61-6	X				X

CS-1B-1

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
91	Cis-2-chloro-cyclohexanol	16536-58-6			X		X
221	1,2,3-Trichlorobenzene	87-61-6	X				X

CS-1B-2

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
19	Ethylcyclopentane	1640-89-7			X		
37	Methylbenzene	108-88-3	X				
105	Ethylbenzene	100-41-4		X			
115	Ethylbenzene	100-41-4		X			
136	1-Ethenyl-3-methylene-cyclopentene	61142-07-2			X		X

CS-1B-2 cont.

279

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
235	1,3,5-Trimethylbenzene	108-67-8		X			X
435	1,2,3-Trichlorobenzene	87-61-6	X				X
471	1,2,3-Trichlorobenzene	87-61-6	X				X

CS-1B-3

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
5	Tetrachloroethene	127-18-4		X			X
38	(Nitromethyl)benzene	622-42-4			X		
55	5-(Benzylamino)thiazolo[5,4-d]pyrimidine	19835-22-4			X		
305	1,2,3-Trichlorobenzene	87-61-6		X			X
336	1,2,3-Trichlorobenzene	87-61-6	X				X
1011	3,7-Dimethylpropanoate-6-octen-1-ol	141-14-0			X		

CS-2-1

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
155	1,3,5-Trimethylbenzene	108-67-8		X			X
182	1,3,5-Trimethylbenzene	108-67-8			X		X
210	1,3,5-Trimethylbenzene	108-67-8		X			X
239	2-Ethyl-1,4-dimethylbenzene	1758-88-9	X				
302	1,2,3,4-Tetramethylbenzene	488-23-3	X				
324	1-Ethyl-3,5-dimethylbenzene	934-74-7		X			
337	1,2,3,4-Tetramethylbenzene	488-23-3		X			
369	Diethylmethylbenzene	25550-13-4		X			
381	Ethyl-1,2,4-trimethylbenzene	54120-62-6		X			
403	2,4-Dimethyl-1-(1-methylpropyl)-benzene	1483-60-9		X			

CS-2-1 cont.

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
420	1,4-Dimethyl-2-(2-methyl-propyl)benzene	55669-88-0			X		
459	Pentamethylbenzene	700-12-9		X			
475	1-Ethylidene-1H-indene	0-00-0			X		
491	1-Methylnaphthalene	90-12-0		X			
513	1,2,3,4-Tetrahydro-1,5,8-trimethylnaphthalene	21693-51-6		X			
568	1-Ethyl-naphthalene	1127-76-0		X			
574	1,5-Dimethylnaphthalene	571-61-9		X			
588	1,6-Dimethylnaphthalene	575-43-9	X				
592	1,6-Dimethylnaphthalene	575-43-9		X			
611	N,N-Dimethyl-N',N'-dimethyl-p-phenylenediamine	5775-53-1				X	
923	1-(2,3-xylyl)-1-(3,4-xylyl)-ethane	2816-98-0			X		
967	7,8,9,10-Tetrahydro-6-methylbenzo[b]naphtho-[2,3-d]-thiophene	24964-06-5				X	
1080	4[[4-(Dimethylamino)phenyl]imino-2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol-3-one	1456-89-9				X	
1100	Bis(3,5-dimethylbenzyl)sulfide	0-00-0				X	

CS-2-2

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
6	1-Ethenyl-3-methylene-cyclopentene	61142-07-2	X				X
187	1,2,3-Trichlorobenzene	87-61-6	X				X
218	1,2,3-Trichlorobenzene	87-61-6	X				X
251	3,7,11-Trimethyl-1-dodecanol	6750-34-1			X		
338	3,7,11-Trimethyl-1-dodecanol	6750-34-1				X	

CS-2-2 cont.

281

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
476	1-Bromo-4-(2-phenylethyl)-benzene	14310-24-8			X		
587	1-Bromo-4-(2-phenylethyl)-benzene	14310-24-8		X			

CS-2-3

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
100	Tetrachloroethene	127-18-4			X		X

CS-3-1

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
111	Cis-2-chloro-cyclohexanol	16536-58-6			X		X
116	Cis-2-chloro-cyclohexanol	16536-58-6				X	X
239	1,2,3-Trichlorobenzene	87-61-6		X			X
269	1,2,3-Trichlorobenzene	87-61-6	X				X
359	Methyl ester decanoic acid	110-42-9			X		X
510	2,2-Dimethyl-3-(2-methylpropyl)ethyl ester cyclopropane carboxylate	33419-38-4			X		X
823	2-[[[2-[[[2-[[2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl ester cyclopropanebutanoate	56051-53-7			X		X

CS-3-2

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
114	Ethylbenzene	100-41-4	X				
135	Ethylbenzene	100-41-4	X				
234	1,3,5-Trimethylbenzene	108-67-8		X			X
433	1,2,3-Trichlorobenzene	87-61-6		X			X
468	1,2,3-Trichlorobenzene	87-61-6		X			X
599	2,6,10-Trimethyldodecane	3891-98-3				X	

CS-3-3

282

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
8	Tetrachloroethene	127-18-4	X				X
37	1,3-Dimethylbenzene	108-38-3			X		X
53	1,3-Dimethylbenzene	108-38-3		X			X
305	1,2,3-Trichlorobenzene	87-61-6	X				X
337	1,2,3-Trichlorobenzene	87-61-6	X				X

CS-4-1

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
75	Didecyl ester decanedioate	2432-89-5				X	X
116	Cis-2-chlorocyclohexanol	16536-58-6				X	X
247	1,2,3-Trichlorobenzene	87-61-6	X				X
277	1,2,3-Trichlorobenzene	87-61-6	X				X
406	2,6,10-Trimethyldodecane	3891-98-3			X		
429	3,5,24-Trimethyltetracontane	0-00-0				X	
513	1,3,5-Trimethylbenzene	108-67-8		X			X
587	2-Methyl-1-(1,1-dimethyl-ethyl)-propanoic acid	0-00-0			X		
907	[[1,2-Ethanediy]bis[carbamo-dithioato]]-2-manganese	12427-38-2			X		

CS-4-2

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
109	1-Ethenyl-3-methylene-cyclopentene	61142-07-2			X		X
130	1,2-Dimethylbenzene	95-47-6		X			X
231	1,3,5-Trimethylbenzene	108-67-8	X				X
279	Cis-2-chlorocyclohexanol	16536-58-6			X		X
427	1,2,3-Trichlorobenzene	87-61-6	X				X
459	1,2,3-Trichlorobenzene	87-61-6	X				X

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
9	Tetrachloroethene	127-18-4	X				X
42	α ,8-Dimethylbenzeneethanol	52089-32-4		X			
55	1,2-Dimethylbenzene	95-47-6		X			X
119	4-Hydroxybenzenesulfonic acid	98-67-9			X		
306	1,2,3-Trichlorobenzene	87-61-6	X				X
337	1,2,3-Trichlorobenzene	87-61-6	X				X
1172	Diethyl ester hexanedioate	123-79-5			X		X
1253	Diisooctyl ester 1,2-benzenedicarboxylate	27554-26-3	X				X

OKW-1

284

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
34	Tetrachloroethene	127-18-4		X			X
36	Tetrachloroethene	127-18-4		X			X
594	3,5-Dimethylcyclohexanol	5441-52-1			X		
813	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5		X			X

OKW-2

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
33	Tetrachloroethene	127-18-4		X			X
35	Tetrachloroethene	127-18-4		X			X
37	Tetrachloroethene	127-18-4		X			X

OKW-3

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
31	Tetrachloroethene	127-18-4		X			X
38	Tetrachloroethene	127-18-4		X			X
596	3,5-Dimethylcyclohexanol	5441-52-1		X			
813	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5		X			X

OKS-1-1

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
2	1-Ethyl-3-methylbenzene	620-14-4		X			X
13	1,3,5-Trimethylbenzene	108-67-8	X				X
20	1,3,5-Trimethylbenzene	108-67-8		X			X
47	2,4-Diethyl-1,3,2-dioxaborolane	57633-63-3			X		
71	1-Methyl-1-(1-methylethyl)-benzene	0-00-0			X		

OKS-1-1 cont.

285

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
98	1,2,3,4-Tetramethylbenzene	488-23-3		X			
159	Naphthalene	91-20-3		X			
252	1-ethylidene-1H-indene	0-00-0		X			
487	Diethyl phthalate	84-66-2		X			
717	Methyl ester hexadecanoate	112-39-0			X		
746	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5		X			X
984	Benzyl butyl phthalate	85-68-7		X			
994	Dioctyl ester hexanedioate	123-79-5		X			X
1075	Butylcyclohexyl ester 1,2- benzenedicarboxylate	84-64-0			X		X

OKS-1-2

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
46	1,3-Dimethylbenzene	108-38-3		X			
53	1-Ethenyl-3-methylenecyclo- pentene	61142-07-2		X			X
72	1-Ethenyl-3-methylenecyclo- pentene	61142-07-2		X			X
82	1,3,5-Cyclooctatriene	1871-52-9			X		
118	1,3,5-Cyclooctatriene	1871-52-9			X		
125	Propylbenzene	103-65-1		X			
132	1-Ethyl-3-methylbenzene	620-14-4	X				X
141	1,3,5-Trimethylbenzene	108-67-8	X				X
149	(1-Methylethyl)benzene	98-82-8	X				
167	1,3,5-Trimethylbenzene	108-67-8	X				X
192	1,3,5-Trimethylbenzene	108-67-8	X				X

OKS-1-2 cont.

286

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
206	1-Ethenyl-2-methylbenzene	611-15-4		X			
218	1,2-Diethylbenzene	0-00-0		X			
225	1,4-Diethylbenzene	105-05-5			X		
252	1-Ethyl-2,3-dimethylbenzene	933-98-2		X			
285	2-Ethyl-1,4-dimethylbenzene	1758-88-9		X			
310	(1-Methylpropyl)benzene	2039-93-2		X			
971	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5		X			X
1299	Diisooctyl ester 1,2-benzene dicarboxylate	27554-26-3		X			X

OKS-1-3

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
815	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5	X				X
1064	Diisooctyl ester 1,2-benzene dicarboxylate	27554-26-3	X				X

OKS-2-1

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
14	1,3,5-Trimethylbenzene	108-67-8		X			X
873	Pyrene	129-00-0			X		
885	2,3,5-Trimethylphenanthrene	3674-73-5		X			
898	2,3,5-Trimethylphenanthrene	3674-73-5		X			
932	1-Methyl-7-(1-methylethyl)- phenanthrene	483-65-8			X		
945	1-Methylpyrene	2381-21-7			X		
958	1-Methylpyrene	2381-21-7			X		
987	1,2,3,5-Tetrachloro-4- methoxybenzene	938-22-7			X		
1020	1,1':2',1"-Terphenyl	84-15-1			X		

OKS-2-1 cont.

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Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
1026	5,6-Diphenylbicyclo[3.1.0]-hex-2-ene	56143-24-9			X		
1131	5-Methylchrysene	3697-24-3			X		
1185	7,12-Dimethylbenz[a]-anthracene	57-97-6			X		

OKS-2-2

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
963	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5		X			X
971	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5	X				X
1147	Bis(4-methylpentyl)-1,2-benzenedicarboxylic acid	146-50-9		X			X
1158	Bis(4-methylpentyl)-1,2-benzenedicarboxylic acid	146-50-9			X		X
1172	Bis(4-methylpentyl)-1,2-benzenedicarboxylic acid	146-50-9		X			X
1182	Bis(4-methylpentyl)-1,2-benzenedicarboxylic acid	146-50-9		X			X
1191	Bis(4-methylpentyl)-1,2-benzenedicarboxylic acid	146-50-9		X			X
1201	Bis(4-methylpentyl)-1,2-benzenedicarboxylic acid	146-50-9		X			X
1293	Isodecyl octyl ester 1,2-benzenedicarboxylate	1330-96-7		X			X
1304	Isodecyl octyl ester 1,2-benzenedicarboxylate	1330-96-7		X			X
1391	Diisononyl ester 1,2-benzenedicarboxylate	28553-12-0		X			X
1460	Diisononyl ester 1,2-benzenedicarboxylate	28553-12-0		X			X
1497	Diisononyl ester 1,2-benzenedicarboxylate	28553-12-0			X		X

OKS-2-3

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Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
95	1[2-(Dimethylamino)-1,1-dimethylethoxy]-2,2-dimethylpyrrolidine	14123-51-4			X		
126	1,1-Dichloro-2,2-difluoroethane	471-43-2			X		
211	1,1-Dichloro-2,2-difluoroethane	471-43-2		X			
218	1,1-Dichloro-2,2-difluoroethane	471-43-2			X		

OKS-3-1

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
490	Diethyl phthalate	84-66-2		X			
659	Phenanthrene	85-01-8		X			
750	Butyloctyl ester-1,2-benzene dicarboxylate	84-78-6			X		X

OKS-3-2

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
53	1,3-Dimethylbenzene	108-38-3		X			
72	1,3-Dimethylbenzene	108-38-3		X			
132	(1-Methylethyl)benzene	98-82-8		X			
141	1,2,4-Trimethylbenzene	0-00-0		X			X
149	(1-Methylethyl)benzene	98-82-8		X			
165	1,3,5-Trimethylbenzene	108-67-8		X			X
192	1-Ethyl-3-methylbenzene	620-14-4			X		X
201	2,3,4-Trimethyl-1,4-pentadiene	72014-90-5		X			
282	2,3,4-Trimethyl-1,4-pentadiene	72014-90-5		X			
363	Naphthalene	91-20-3			X		

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
468	1-Methylnaphthalene	90-12-0			X		
972	Butyl-2-methylpropyl ester 1,2-benzenedicarboxylate	17851-53-5		X			X
1149	Bis(4-methylpentyl)-1,2- benzenedicarboxylic acid	146-50-9			X		X
1166	Bis(4-methylpentyl)-1,2- benzenedicarboxylic acid	146-50-9			X		X
1182	Decylhexyl ester 1,2- benzenedicarboxylate	25724-58-7			X		X
1200	Decylhexyl ester 1,2- benzenedicarboxylate	25724-58-7			X		X
1304	Bis(2-ethylhexyl) phthalate	117-81-7			X		
1362	Diisononyl ester 1,2- benzenedicarboxylate	28553-12-0			X		X
1367	Diisononyl ester 1,2- benzenedicarboxylate	28553-12-0			X		X
1402	Diisononyl ester 1,2- benzenedicarboxylate	28553-12-0			X		X
1595	Diisononyl ester 1,2- benzenedicarboxylate	28553-12-0			X		X
1675	Diisononyl ester 1,2- benzenedicarboxylate	28553-12-0			X		X

OKS-3-3

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor	Artifact
79	1,1,2-Trichloro-2-fluoro- ethane	359-28-4			X		
96	1[2-(Dimethylamino)-1,1- dimethylethoxy]2,2-di- methylpyrrolidine	14123-51-4			X		
1143	3-Nitro-1,2-benzene- dicarboxylic acid	603-11-2			X		X

Solvent Blanks

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Methylene Chloride

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor
56	Tetrachloroethene	127-18-4		X		
271	Trans-2-chlorocyclohexanol	6628-80-4		X		
312	1,2-Dichlorocyclohexane	1121-21-7		X		

Chloroform

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor
5	Tetrachloroethene	127-18-4			X	
299	1,2,3-Trichlorobenzene	87-61-6		X		
331	1,2,3-Trichlorobenzene	87-61-6			X	
1168	Diethyl ester hexanedioate	123-79-5				X
1246	Bis(2-ethylhexyl) phthalate	117-81-7		X		

Benzene

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor
11	1-Ethenyl-3-methylene-cyclopentane	61142-07-2			X	
59	1-Ethyl-3-methylbenzene	620-14-4			X	
64	1-Ethyl-3-methylbenzene	620-14-4			X	
71	3-Ethyl-2,5-dimethyl-hexane	0-00-0				X
82	1,3,5-Trimethylbenzene	108-67-8			X	
135	Cyclododecanol	1724-39-6			X	
148	2-Propyl-2-heptanal	34880-43-8			X	
181	Decahydro-2-methyl-naphthalene	2958-76-1		X		
195	9-Eicosyne	71889-38-2			X	

Solvent Blanks cont.

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Benzene cont.

Peak #	Library Match	Reference #	Exc	Good	Fair	Poor
243	1,2,3-Trichlorobenzene	87-61-6	X			
275	1,2,3-Trichlorobenzene	87-61-6	X			
309	2,6,10,13-Tetramethyl-pentadecane	17081-50-4				X
336	1-Dotriacontanol	6624-79-9				X
380	7-Cyclohexyl-7-cyclohexyl-tridecane	13151-92-3			X	

VITA 2

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