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ARTIFACTS AND LOSSES IN THE SAMPLING OF CHLORINATED WATERS BY XAD ADSORPTION

by
Albert M. Cheh, Ph.D.

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FINAL REPORT

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Abstract

Chlorination of natural waters generates mutagens that most likely are electrophiles. These electrophiles are often recovered for testing and analysis by adsorption to XAD resins. It was found that the production of artifacts stemming from the action of free chlorine on XAD-4 resin could be suppressed at least ten fold by converting the free chlorine to chloramine. Kinetic studies indicate that free chlorine is consumed at least ten times as rapidly by XAD-4 as is chloramine.

Sampling losses during XAD recovery of electrophiles and mutagens were also examined. Mutagenic activity bound to resins generally decreased over a period of several days, but some increases were seen. Electrophiles labeled by 4-nitrothiophenol generally decreased concurrently, but sometimes new species were seen.

Organics concentrates in ethanol did not appear to lose mutagenic activity as rapidly as concentrates in DMSO. Simultaneous electrophile assays, however, indicated up to 50% loss of some electrophiles present in ethanol concentrates.

Artifacts

Attention has focused on electrophiles present in chlorinated water because of their possible mutagenicity/carcinogenicity.[1] XAD adsorption methods are commonly used to recover and concentrate organics from water. [2] When chlorine is present in the water, however, it may react with the XAD resin to produce spurious compounds, including electrophiles.[3] This project assayed the production of electrophilic compounds from XAD-4 resin, as a function of chlorine concentration and species, and investigated the kinetics of reaction between XAD-4 resin and chlorine as a function of chlorine species, chlorine concentration, temperature and pH. The goal of this aspect of the project was to define the conditions resulting in significant production of artifacts, as well as define ways to avoid this problem.

Sampling Losses

Columns of XAD resin used to sample waters in the field may be shipped to a central laboratory for processing. This unavoidable delay before processing could result in the loss of unstable or reactive compounds such as electrophiles. Previous experiments had shown that when identical batches of tap water were passed through XAD, processed to recover organics, and assayed in parallel for mutagens, good reproducibility was seen. Day to day reproducibility was less certain, however, so a chemical assay for electrophiles, complementing mutagenesis assays, was used to detect losses over a period of days.

Storage Losses

Toxicological evaluation of hazardous materials may require extended dosing over days to weeks. Thus, even after water samples are brought to the laboratory and are concentrated, they may

be kept in cold storage for long periods of time while the toxicological study progresses. Losses of unstable or reactive materials during storage could lead to an undesirable underestimate of toxicological hazards. The final aspect of this project was to examine losses of electrophiles in water concentrates upon storage at different temperatures, using either ethanol or dimethyl sulfoxide (DMSO) as the solvent.

Methodology

General

XAD Resins: XAD-4 resin was prepared for use by repeated washing with 1N HC1, water, 1N NaOH and water until all visible color was removed. This was followed by Soxhlet extraction using water, acetone, methylene chloride and methanol. This procedure is more extensive than the usual one.[2]

Concentration of Water Organics: Following methods described previously. [3,4] Twenty liter volumes of finished drinking water, taken from a distribution system tap, were passed through 100 ml bed volumes of cleaned XAD-4 at an approximate flow rate of one liter/hr. The resin was then washed with distilled water to displace any material trapped but not adsorbed. The adsorbed organics were eluted with acetone followed methylene chloride. The aqueous phase was discarded, while the organic solvent was evaporated in a rotary evaporator to yield a dry residue. This limited the study to nonvolatiles. The residual organics were redissolved in a volume of ethanol or DMSO equal to 1/10,000 the original volume of water, yielding the water concentrate solution. All concentrate volumes are expressed in terms of the original volume of water. Thus, 50 μl of concentrate corresponds to 0.5 liter of original water.

Mutagen Bioassay: (Ames Assay) The Ames assay was used as a bioassay for mutagenic electrophiles. Testing was performed following the usual protocol[5]) except that the bacteria were grown in 1 to 2 diluted Oxoid Broth No. 2 instead of Difco. Testing used strains TA100 and TA100-FR1 because they

gave the maximum response to drinking water mutagens. S-9 was omitted because it only suppreses the mutagenic response.

Particular attention was paid to including positive controls for comparison of day to day responses. Styrene oxide was used as the principal positive control, because epoxides and alkyl halides are among the mutagenic electrophiles likely to be found in drinking water. Plating without added mutagen or water sample constituted the negative control. Ethanol or DMSO up to 50 µl per plate had little effect on the response. Since the volume of water concentrate added was kept below 50 µl, solvent controls were not needed. Slopes of the dose responses were calculated by least squares fits. In general, the guidelines proposed by an expert group [6] were followed.

Chemical Assay for Electrophiles – (NTP Labeling/HPLC Separation and Detection)

Electrophiles were detected in a chemical assay based on derivatization with 4-nitrothiophenol (NTP) (4):112.5 µl of water concentrate was mixed with 37.5µl 0.05 M aqueous potassium phosphate buffer, pH 7.4 and either 0, 2.5×10^{-4} M, 5×10^{-4}M, 10^{-3} M or 2 x 10^{-3}M NTP. Reactions proceeded 60 mins at room temperature. 50 µl DMSO was then added to solubilize the reaction components. High performance liquid chromatography (with a Spectra Physics 8100 instrument) was used to separate the derivatized electrophiles. These were detected by their absorbance at 345nm, using an SP8400 variable wavelength detector. Chromatograms were plotted using a Hewlett Packard 3390A reporting integrator. The methanol-water gradient used with water samples was changed from the previously used one to the following: Time 0, 40% methanol; time 2 mins, 40% methanol; time 30 min, 100% methanol; time 36 min, 100% methanol, and stop run. Chromatography was done on a 0.46 x 25 cm reverse phase C-8 column with a flow rate of 2 ml/min. The oven temperature was 35° C.

ARTIFACTS

Reaction between chlorine and XAD-4 Resin

25 ml packed volume of resin was placed in 900 ml of temperature equilibrated, chlorine-demand-free water (tap water purified by reverse osmosis and then passed through a Millipore Milli-Q system) together with 2mM buffer (phosphate for pH 6-7; borate for pH 8-9). Reagent grade sodium hypochlorite and water were added to produce one liter volumes containing the free chlorine concentrations indicated below.

Monochloramine was produced by adding a slight excess of ammonium chloride to 900 ml volumes of temperature equilibrated, chlorine-demand-free water containing buffer and hypochlorite. Resin was added after the free chlorine was converted to monochloramine.

The solutions were gently stirred (4 site synchronous magnetic stirrer) at a rate just enough to completely suspend the resin. Simultaneous blanks omitted resin. The rate of reaction was followed by monitoring the disappearance of chlorine or monochloramine with the DPD colorimetric method.[7] ^{k}observed values were computed by $^{k}_{obs} = 0.693 \div tl/2$, where tl/2 = half life of chlorine.

Artifact production

20 liter volumes of chlorine-demand-free water containing various concentrations of free chlorine or monochloramine buffered to pH 7.4 with 5mM phosphate were passed through XAD columns as described above. The columns were processed, and assayed for mutagens and electrophiles as described above.

Three identical samples of tap water were passed in parallel through identical columns of XAD-4 under the conditions described above. One column was processed immediately, a second 24 hours later and the third 72 hours later. These were labeled day 1, day 2, and day 4. To detect losses over time, bioassays and chemical assays for electrophiles in each of the three water concentrates were conducted as described above. This procedure was repeated weekly over several months to generate sufficient sample for testing storage losses and to see if there was any pattern of sampling loss that was consistent despite changes in the tap water.

Storage Losses

Water concentrates in DMSO or ethanol were placed in tightly sealed 13 x 100 tubes with Teflon lined screwcaps. After varying periods of storage at -80°C, -20^0 C, or 4^0 C, they were reassayed for mutagens and electrophiles by the Ames and chemical assays following the procedures described above.

Results and Discussion

Artifacts

Mutagens

Table I lists the mutagenesis (Ames) assay results showing the generation of mutagens by chlorination of XAD-4 resin. The slopes of the dose responses are given in the fourth column. Day to day variation in the bioassay was anticipated, thus in each run a styrene oxide dose response was included. Each of the slopes of the dose responses due to chlorination artifacts could then be normalized by dividing by the styrene oxide dose response slope (in revertants per μg SO) for that run. These normalized values are given in the rightmost column of Table I. Figure 1 is a plot of the observed slopes and their normalized values from Table 1. The lines drawn on Fig. 1 are least squares fits of the points. As can be seen, free chlorine gives rise to somewhat more than ten times

TABLE I

Artifacts

Mutagen production due to reaction between chlorine and XAD-4

Run #	Chlorine Species	ppm chlorine (as Cl_2)	Ames assay slope of dose response (revertants/ liter)	Styrene oxide response (revertants/μg)	Normalized Ames assay slope
1	free chlorine	0.5 1.0 2.0	39 38 188	0.84 0.84 0.84	46 45 224
2	free chlorine	0.5 1.0 2.0	64 108 217	0.38 0.38 0.38	169 284 572
3	free chlorine	0 0.5 1.0 2.0	-21 65 141 246	0.58 0.58 0.58 0.58	(-21) 113 245 428
4	monochloramine	2	19	0.68	79
5	monochloramine	4 8 12	52 63 90	0.33 0.33 0.33	161 195 279
6	monochloramine	0 2 4	-15 0 30	0.46 0.46 0.46	(-15) 0 65
7	monochloramine	4 8 12	60 86 72	0.49 0.49 0.49	123 176 148

amount of mutagenic artifacts as does monochloramine, per ppm of chlorine. It should be noted that the individual Ames plate values obtained with chloramine often were not much greater than background, so there is less confidence that the slopes reflect true dose responses. Therefore, the estimate of ten times more mutagenic artifacts could easily be more or less than that.

Electrophiles

Figures 2-6 illustrate the detection by NTP adduct formation of electrophilic artifacts due to chlorination of the resin. Fig. 2 shows a series of reagent blanks, run in the standard gradient. As has been noted, the major contaminant in NTP is the disulfide (8) which in these HPLC runs emerges at about 20 mins. The next major peak from NTP is unionized NTP-SH, appearing at 6 mins. Very small peaks are also seen at 18, 24, and 25 mins as well.

Fig. 3 shows a sample generated by passing purified water without chlorine through the XAD-4 resin. Substantial peaks are seen at 21 and 29 minutes. Curiously, these are not seen in many of the chlorinated water samples. Solid residue was seen after rotary evaporation to remove resin desorption solvent. This could be due to resin throw, residual organic matter in the purified water or residuals from the solvents. Resin throw is probably the most likely.

With the addition of NTP, however, little increase is seen beyond the peaks observed in the NTP blank* indicating the absence of electrophiles. By contrast, NTP products from the 2.0 ppm free chlorine sample of run #3 of Table 1 are shown in Fig. 4a. The peaks at 20 and 21 mins in the absence of NTP are more sizable than those in Fig. 3, thus an impact of chlorine is observed. With the addition of NTP, the region between 10 and 18 min in particular rises above the baseline obtained for sample minus NTP.

* The large peak seen at 20 mins is NTP disulfide from the NTP.

Figure 1a. Observed values (,slopes of dose responses) for mutagens produced by chlorination of XAD-4 resin. (•) -free chlorine (o) -chloramine.

1b. Observed valued normalized for the response of styrene oxide (positive control). (•) -free chlorine (o) -chloramine.

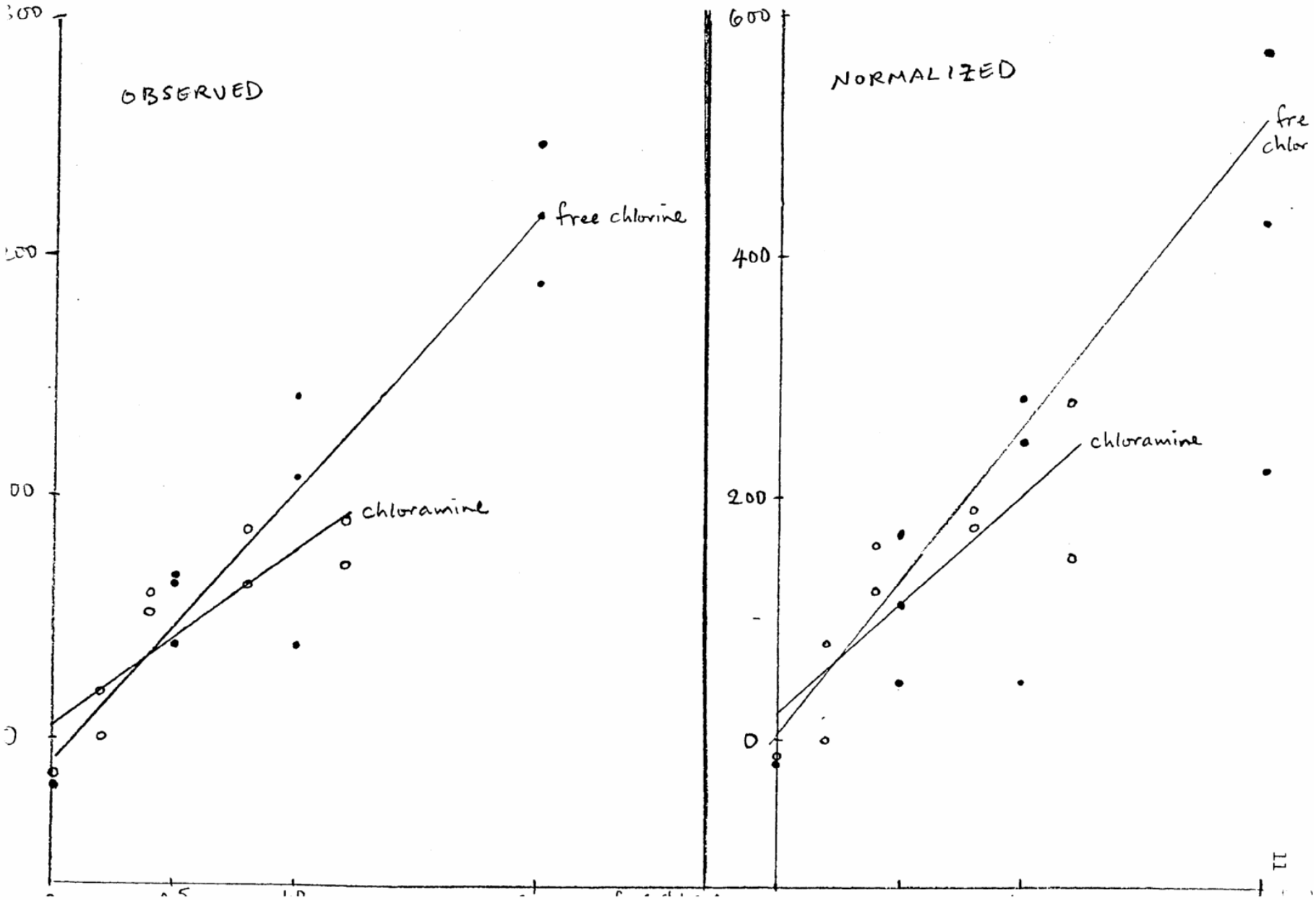

OBSERVED
free chlorine
chloramine
NORMALIZED
600
400
200
0
free chlor
chloramine

Figure 2. Reagent blanks

2a. (top) Ethanol 112.5µl; 0.05 M potassium phosphate, pH 7.4, 37.5 µl; DMSO 50 µl.

2b. (middle) same plus 5×10^{-4} M NTP

2c. (bottom) same plus 2×10^{-3} M NTP

REAGENT BLANKS

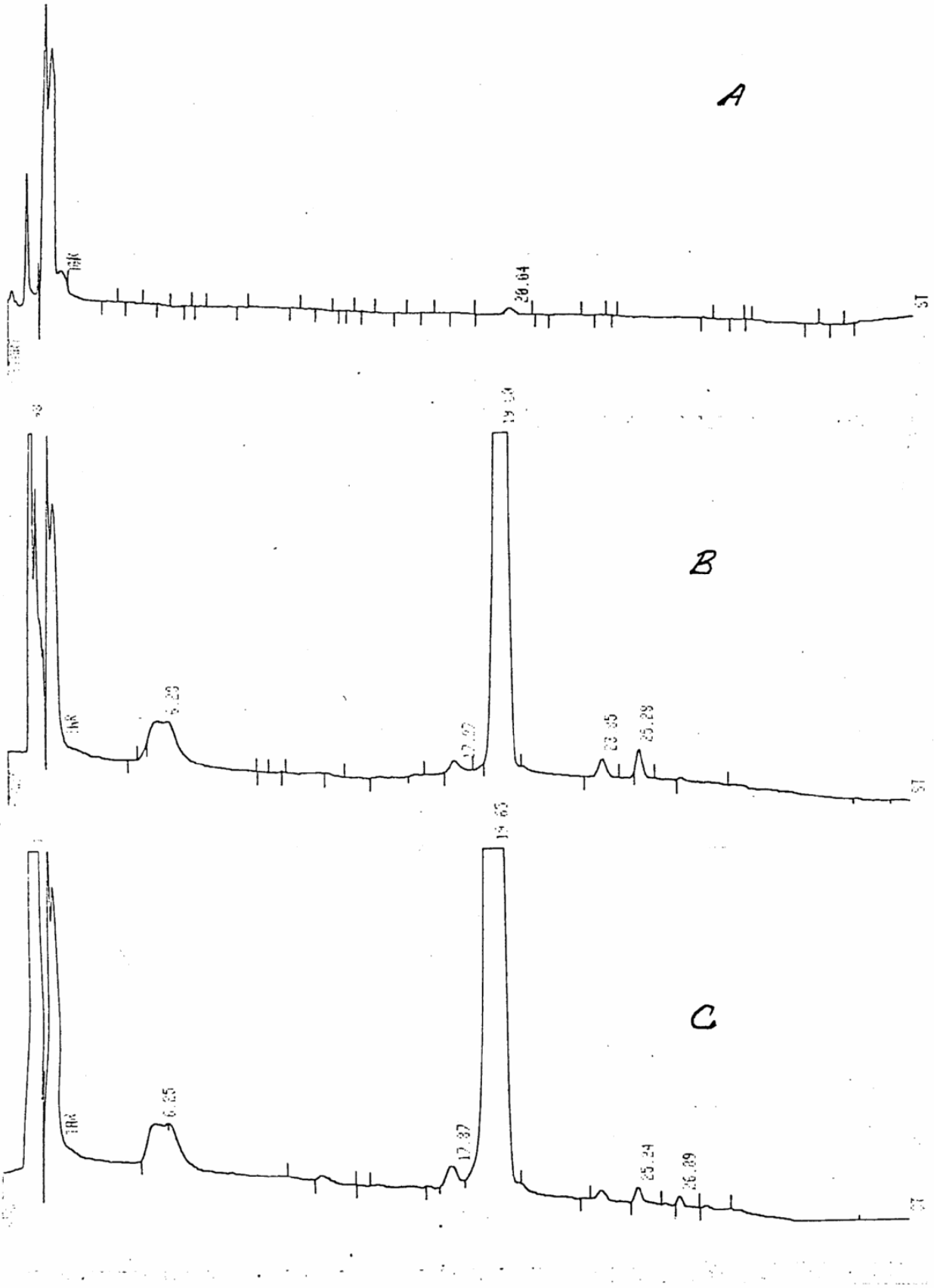


Figure 3. 0 ppm chlorine – artifacts sample

3a. (top) no added NTP.

3b. (middle) 5×10^{-4} M NTP added.

3c. (bottom) 2×10^{-3} M NTP added.

0ppm Cl2

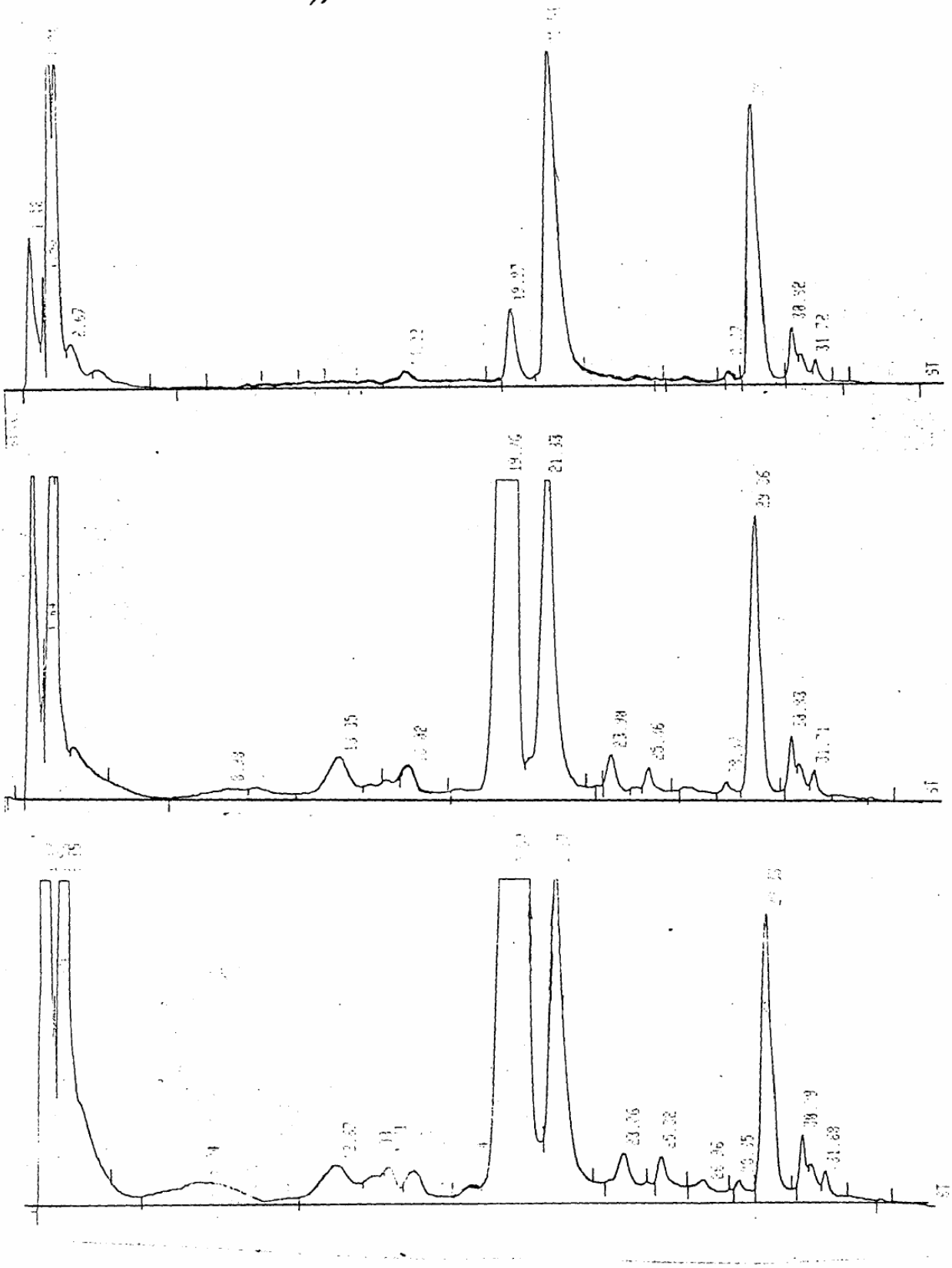

19.93
38.52
31.72
19.46
21.33
39.36
23.98
25.46
38.83
31.71
23.36
25.32
25.36
25.35
39.19
31.68

Figure 4. Artifacts due to the action of free chlorine on XAD-4.

4a. 2.0 ppm free chlorine

(top) no added NTP.

(middle) 5×10^{-4}M NTP added.

(bottom) 2×10^{-3}M NTP added.

4b. 1.0 ppm free chlorine.

(top) no added NTP.

(middle) 5×10^{-4}M NTP added.

(bottom) 2×10^{-3}M NTP added.

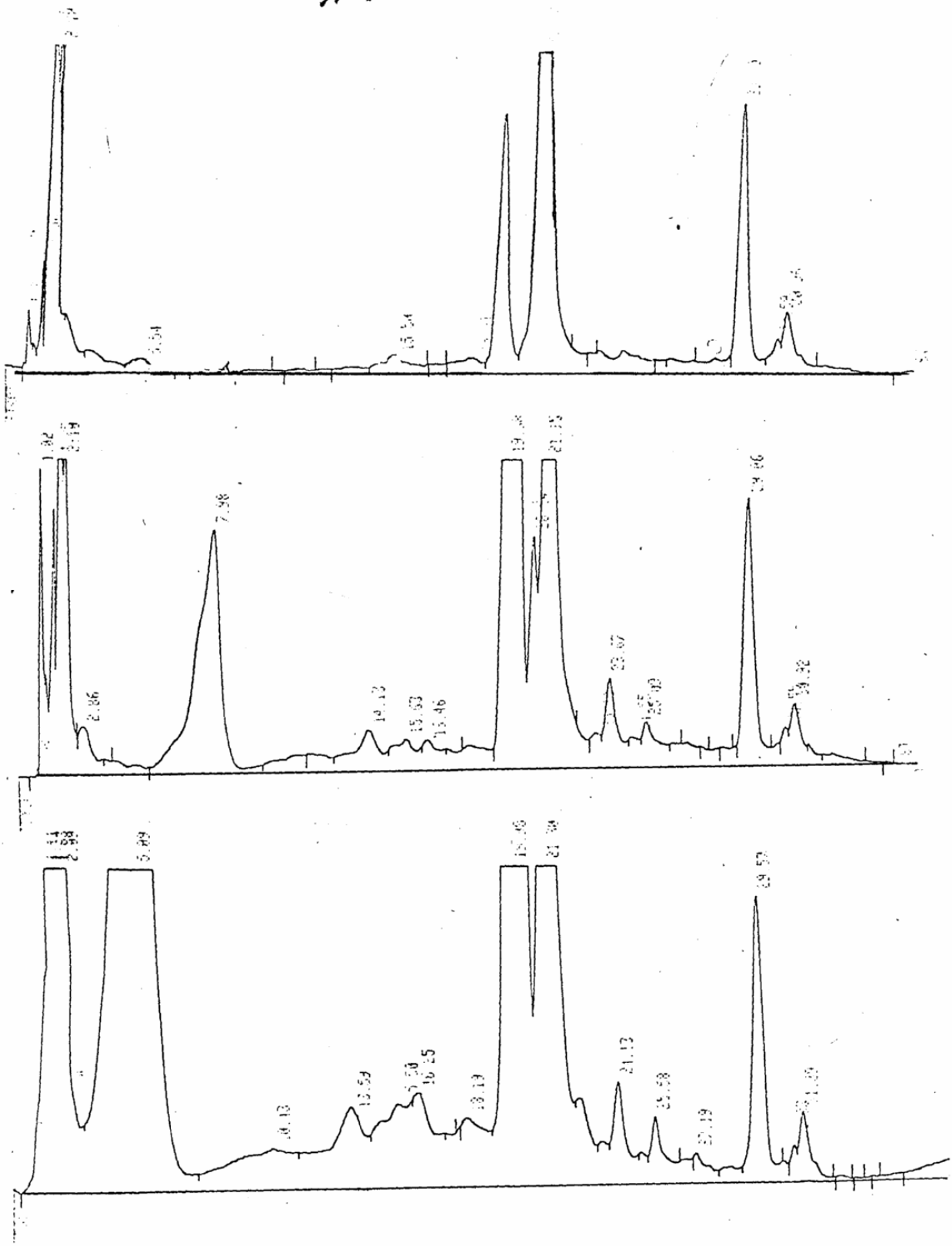
4c. 0.5 ppm free chlorine

(top) no added NTP.

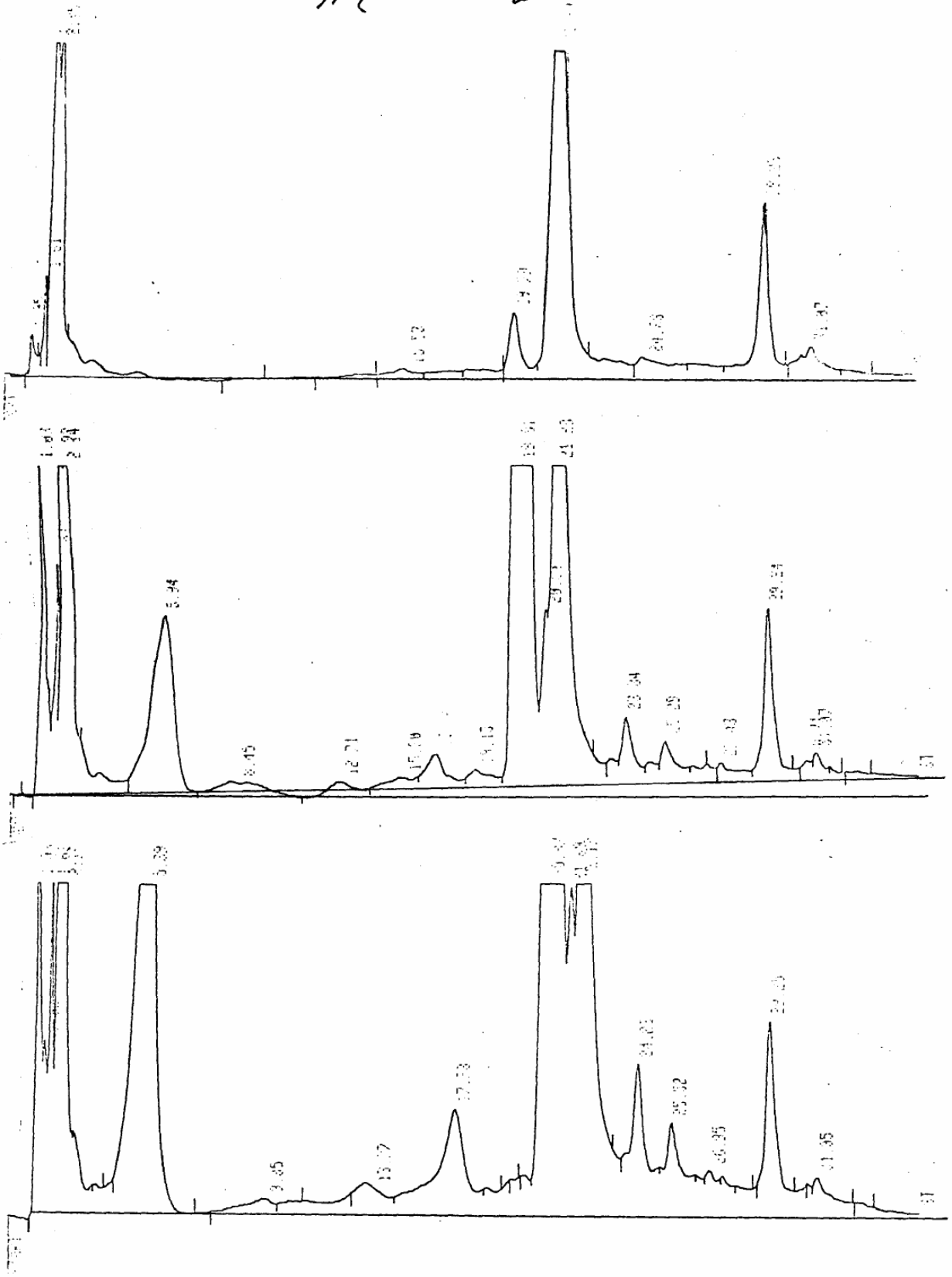
(middle) 5×10^{-4}M NTP added.

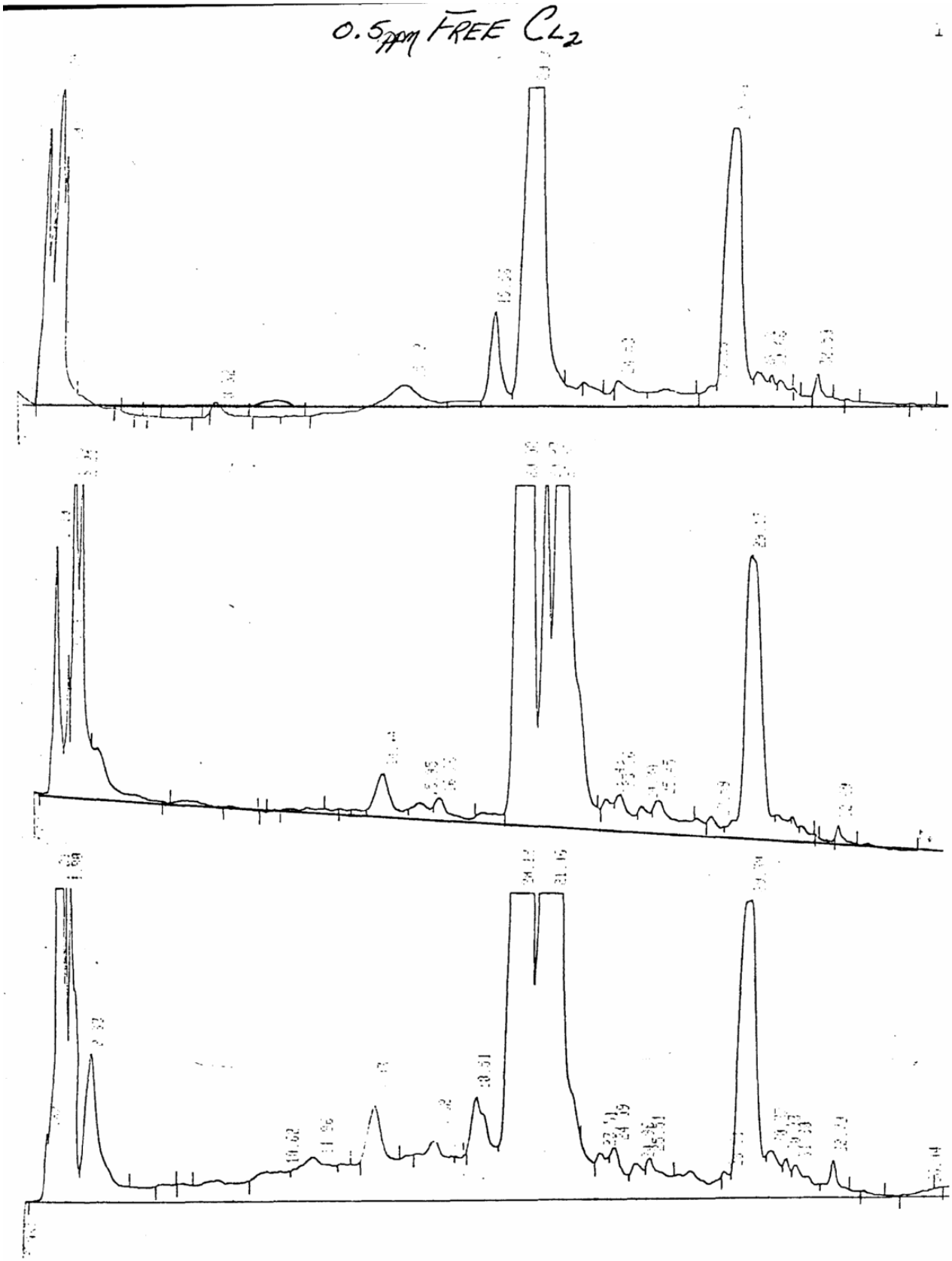
(bottom) 2×10^{-3}M NTP added.

2.0ppm FREE Cl_2



1.0ppm FREE Cl_2




0.5ppm FREE Cl_2

The 1.0 ppm, sample of run #3 of Table 1 was also reacted with NTP, with the results shown in Fig. 4b. There is some increase over the baseline, but substantially less than with the 2.0 ppm sample.

The 0.5 sample of run #3 of Table 1 is shown in Fig. 4c. The curves are not readily super imposable upon each other because of baseline drift. The 0.5 ppm sample of run #2 shows increases above background less than those of the corresponding 1.0 ppm sample, although for the entire series of run #2, the HPLC detector was not always stable. (chromatograms not shown).

The impact of monochloramine is shown in Fig. 5a-c. In figure 5a the 4 ppm NH_2C1 sample of run #7 shows increases somewhat smaller than those observed with l ppm free chlorine (Fig. 4b). In both the 8 and 12 ppm NH_2C1 samples (Fig. 5b and 5c) there are increases similar to those seen with l ppm free chlorine (Fig. 4b). In Figs. 5b and 5c the highest NTP concentrates have a large response between 21 and 29 mins which is believed to be due to a detector malfunction rather than represent actual NTP adducts.

Reaction between chlorine and XAD-4 resin.

Table II is a summary of the rate constant observed k_{obs} for the reaction between free chlorine and XAD-4 resin. As is expected, there is a consistent increase in reaction rate with increasing temperature. There is also a drop in reaction rate as the pH rises from 6 to 8. With monochloramine, reaction times of several hours showed little reaction, while free chlorine concentrations showed significant reactions at 1/10 the amount of time allowed for monochloramine reaction under the same conditions of pH and temperature. Thus in these kinetic runs monochloramine appears to be less than 10% as reactive as is free chlorine

Table II

Reaction Between Free Chlorine and XAD-4 resin-

K_{obs} values in min $^{-1}$

Run #	Temperature	pH (values in parentheses)						
1	0º			(7.1)		(7.7)		
				0.017		0.0055		
2	0º	(5.9)		(7.0)		(7.7)		
		0.016		0.011		(0)		
3	0º	(5.8)		(7.0)				
		0.017		0.010				
4	15º			(7.0)		(7.7)		
				0.033		0.014		
5	15º			(6.9)	(7.5)			(9.0)
				0.034	0.015			0
6	15º			(7.0)			(8.0)	(9.0)
				0.029			0.006	0
7	25º			(7.0)			(8.0)	
				0.050			0.007	
8	25º			(7.0)		(7.8)		
				0.047		0.009		
9	25º			(7.1)		(8.0)		
				0.039		~0		
10	25º	(5.8)		(7.1)		(8.0)		
		0.063		0.046		~0		
11	25º	(6.0)	(6.5)	(7.1)				
		0.060	0.051	0.040				

Figure 5. Artifacts due to the action of monochloramine on XAD-4.

5a. 4 ppm NH_2C1.

(top) no added NTP.

(middle) 5×10^{-4}M NTP added.

(bottom) 2×10^{-3}M NTP added.

5b. 8 ppm NH_2C1.

(top) no added NTP.

(middle) 5×10^{-4}M NTP added.

(bottom) 2×10^{-3}M NTP added.

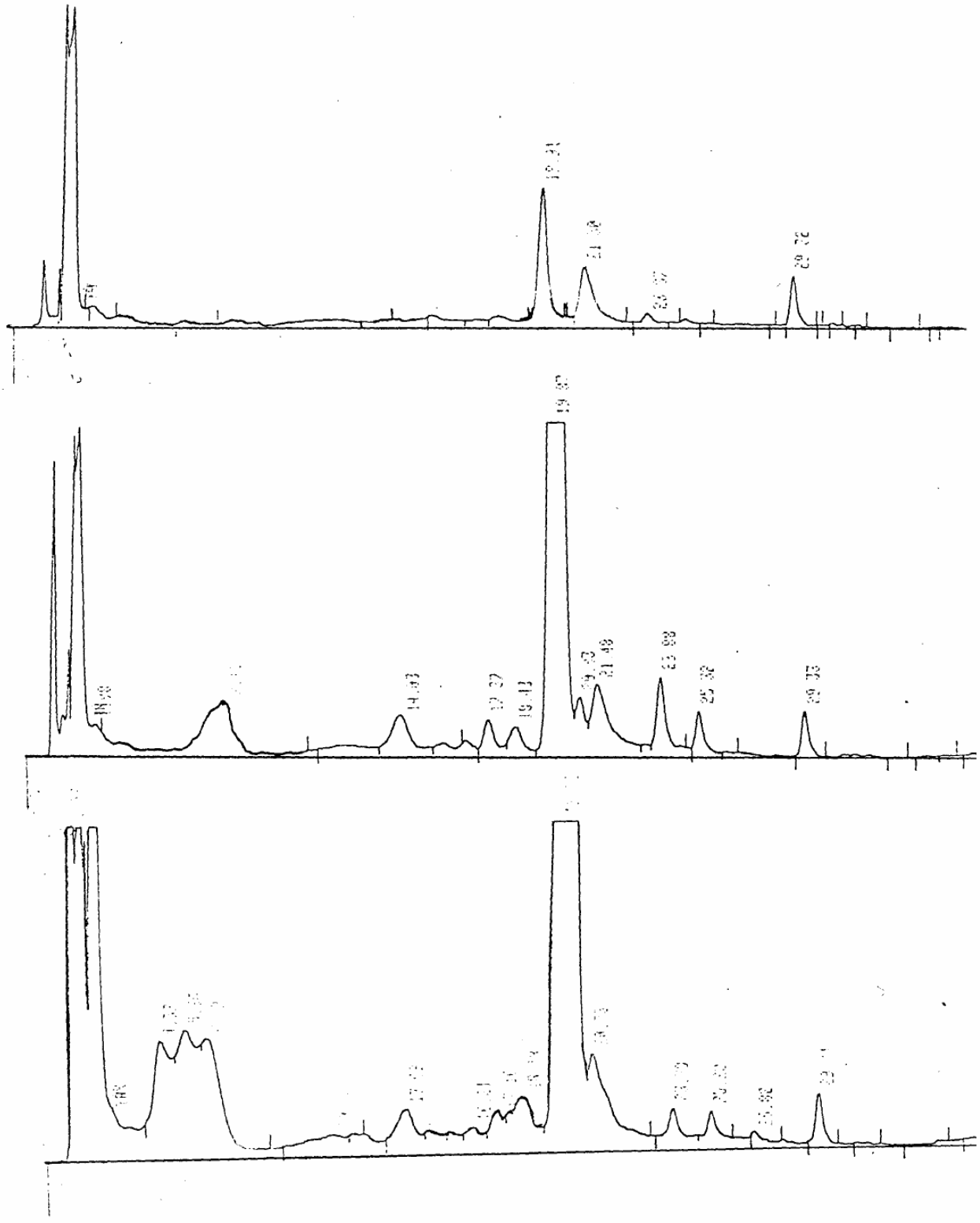
5c. 12 ppm NH_2C1.

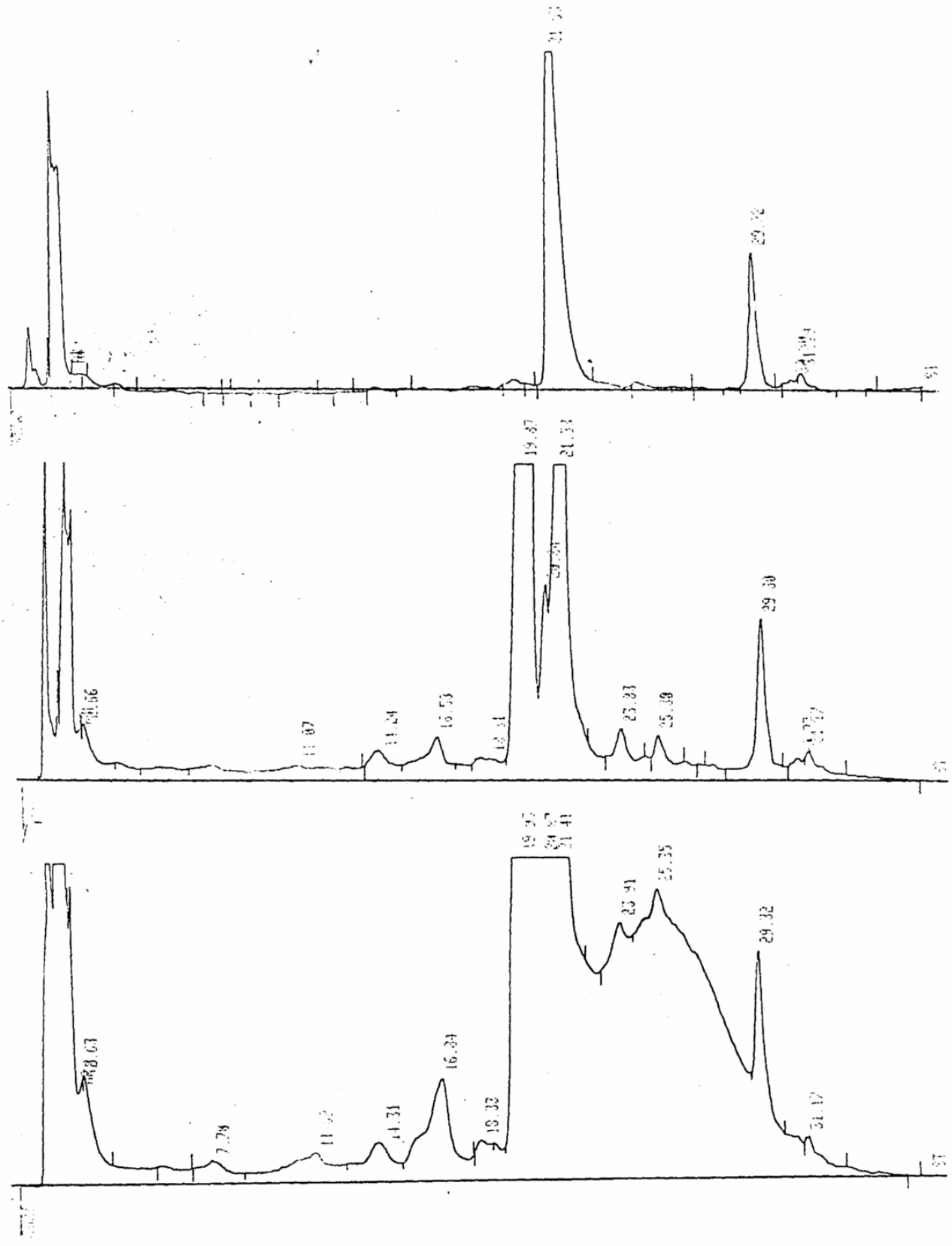
(top) no added NTP.

(middle) 5×10^{-4}M NTP added.

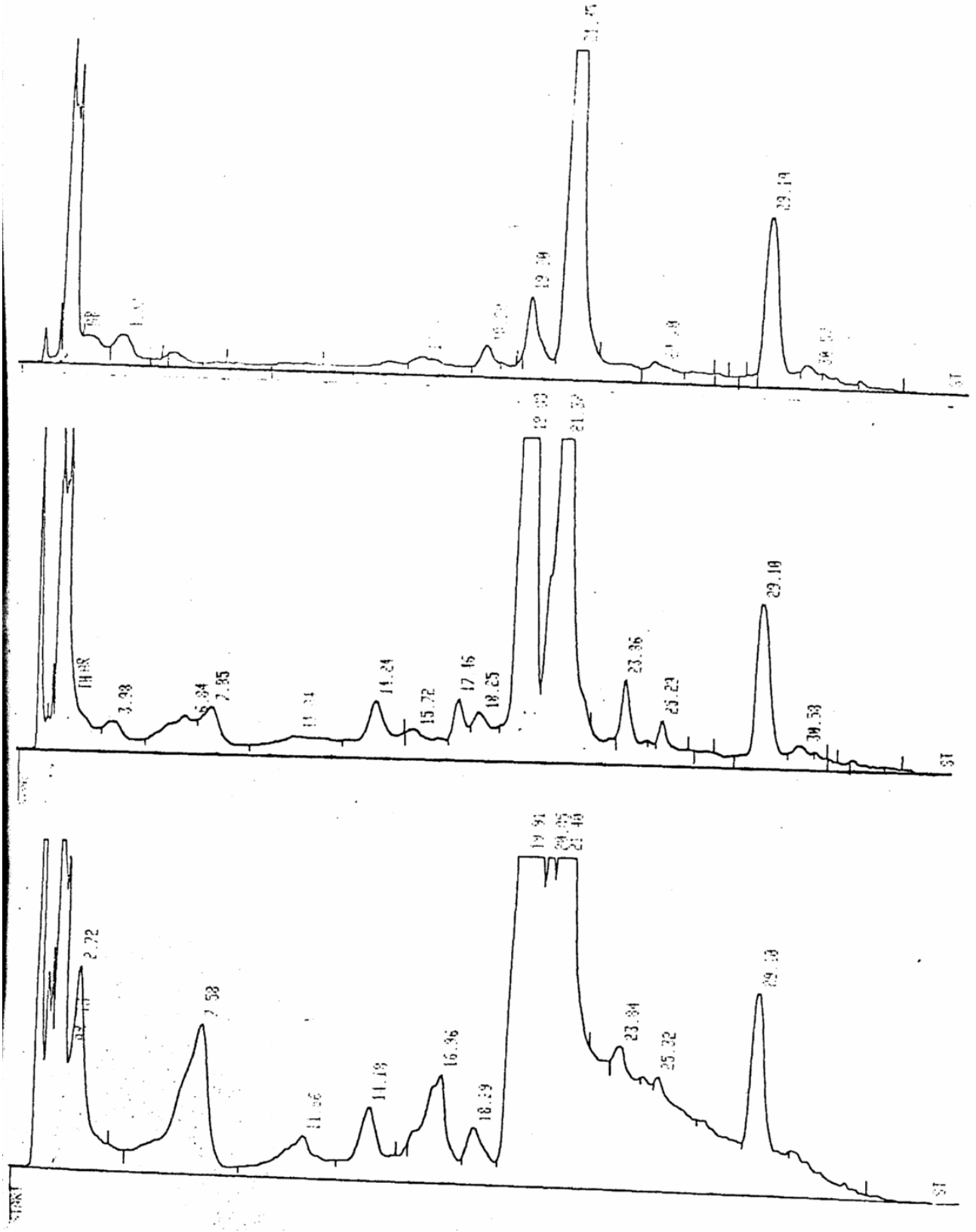
(bottom) 2×10^{-3}M NTP added.

4pp-nl NH2Cl




21.33
29.76
19.87
21.32
29.30
16.53
29.32
16.34

12 ppm NH_2Cl


12.39
21.45
23.19
3.93
6.84
7.95
11.24
15.72
17.16
18.25
12.93
21.52
23.36
25.23
29.10
30.58
2.72
7.58
11.46
11.89
16.36
18.29
19.91
23.84
25.32
29.59

The nature of the most reactive chlorine species in free chlorine is of some interest. The bulk of reaction would be expected to come from HOC1. Based on pk_as for $HOCl \leftrightarrow H^+ + OCl^-$ of 7.8 to 7.5 for temperatures from 0 to 25°C, [9] a decrease in pH from 7.0 to ≤ 6.0 would result in less than a 50% increase in [HOC1]. Since the increase in rate constants at OºC, as the pH drops from 7 to 5.8-5.9, is greater than 50%, there could be another species, dependent on H^+, which is kinetically more rapid than HOC1. Likewise, as one raises the pH, there is a more rapid decrease in reaction rate than is expected on the basis of HOC1→less reactive $OC1^-$. The impact of pH on the other reactant, namely resin, is not known, nor is it definite that another species formed at low pH, such as H_20C1^+ or $C1_2$ is involved in the reaction. Therefore, suffice it to say that a simple assumption of HOC1 being reactive, and $OC1^-$ being unreactive, combined with calculations of [HOCl] and [$OC1^-$] vs pH (from the pk_a and the Henderson-Hasselbach equation) does not explain the magnitude of increase in k_{obs} as the pH drops from 8 to 6**.**

Sampling Losses

Table III illustrates the changes in mutagen levels that were observed when identical water samples were processed immediately after passage through XAD resin (day 1), one day later (day 2) or three days later (day 4). The expected outcomes presumably would be to see losses with time, although it is possible that large polymeric electrophiles might break down to smaller, more reactive or mutagenic ones, much as the production of THMs has been observed to continue in water samples. However, no obvious pattern of day to day changes was observed either before or after normalization. At the bottom of Table III, all the runs of day 1 or day 2 or of day 4, before or after normalization, were averaged (not including run #2 where day 2 was lost) to see if there were any overall trends not seen in the individual runs. No clear pattern is seen either before or after normalization.

The slopes for styrene oxide positive control responses were also averaged for all 14 dose responses. The mean value of 0.446 revertants/μg had a standard deviation of 0.162 revertants/μg which is 36% of the mean. Thus, the day to day variations were substantial. The historical value tabulated

TABLE III

Sampling Losses

Run #	XAD processing day	Mutagen content-Ames assay slope (revertants/liter)	Styrene oxide response (revertants/μg)	Normalized Ames assay slope
1	1	669	0.422	1656
	2	1067	0.675	1580
	4	1152	0.564	2043
2	1	370	0.303	1221
	2			
	4	309	0.633	488
3	1	481	0.339	1419
	2	680	0.379	1794
	4	299	0.206	1451
4	1	702	0.410	1712
	2	641	0.575	1115
	4	426	0.323	1319
5	1	442	0.228	1939
	2	678	0.487	1392
	4	926	0.700	1323
		observed slopes	normalized	
Avg. of	d1	581	1682	
	d2	767	1470	
	d4	700	1534	

before this project for styrene oxide dose responses in this lab was (mean ± standard deviation) 0.84 ± 0.23 revertants/μg for 60 dose responses. Thus, the set of responses described here are significantly lower than the historical average, and show greater variation.

The pattern for electrophile adducts with NTP was slightly more consistent. In run #4 of the sampling loss series (Fig. 6a-c) a complex chromatogram for the day 1 sample without NTP becomes simpler on successive days. In addition, the curves with NTP do not rise as high above the zero NTP baseline. There is a striking reduction between day 1 and later days in the peaks indicated by the arrows. Thus losses seem to occur, which is in keeping with the mutagenesis assay response (Table III).

The chromatograms for run #5 (Fig 7a-c) show a sample gaining peaks on subsequent days. However, the new peaks are much like the ones seen in the Oppm $C1_2$ artifacts sample (Fig. 3). The overall increase over background, though, appears to rise, while the 24 and 25 min peaks are about the same on day 1 and 4, but lower on day 2. This somewhat parallels the observed mutagen content but not well. One problem with the day 4 sample is another instrument malfunction.

The chromatograms of run #3 could not be compared readily because of detector malfunctioning resulting in varying baselines. However, NTP peaks at 24 and 25 mins, which are very large on day 1 fall to very small sizes on day 2 and further on day 4 just like they do in Figs. 6a-c. (run #3 not shown).

Figure 6. Sampling losses – run # 4 of Table III.

6a. Water sample processed on day 1.

(top) no added NTP.

(middle) 5 x 10^{-4}M NTP added.

(bottom) 2 x 10^{-3}M NTP added.

6b. Water sample processed on day 2.

(top) no added NTP.

(middle) 5 x 10^{-4}M NTP added.

(bottom) 2 x 10^{-3}M NTP added.

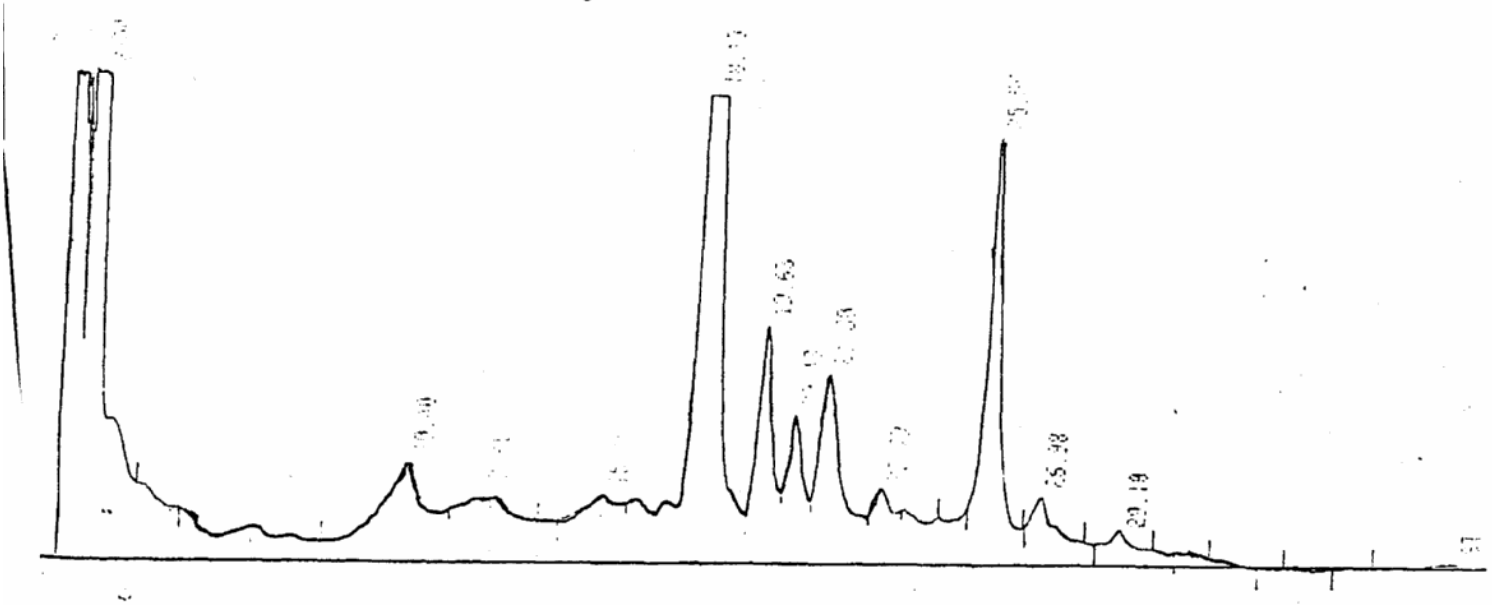
6c. Water sample processed on day 4.

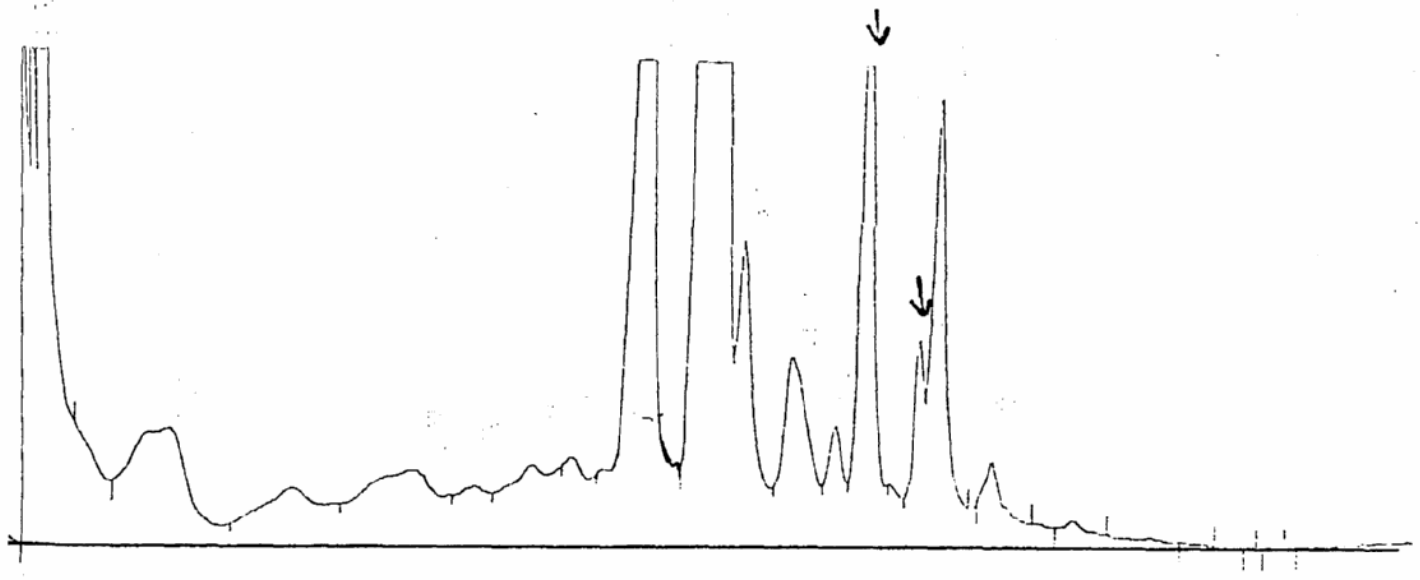
(top) no added NTP.

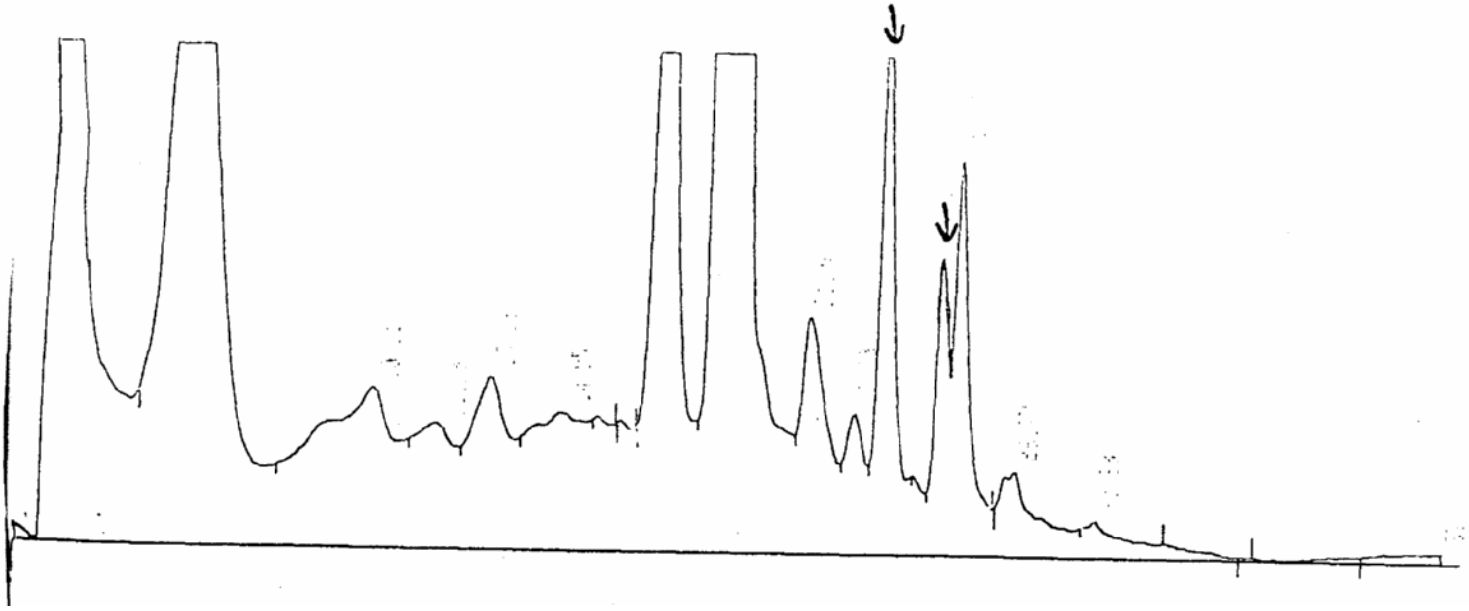
(middle) 5 x 10^{-4}M NTP added.

(bottom) 2 x 10^{-3}M NTP added.

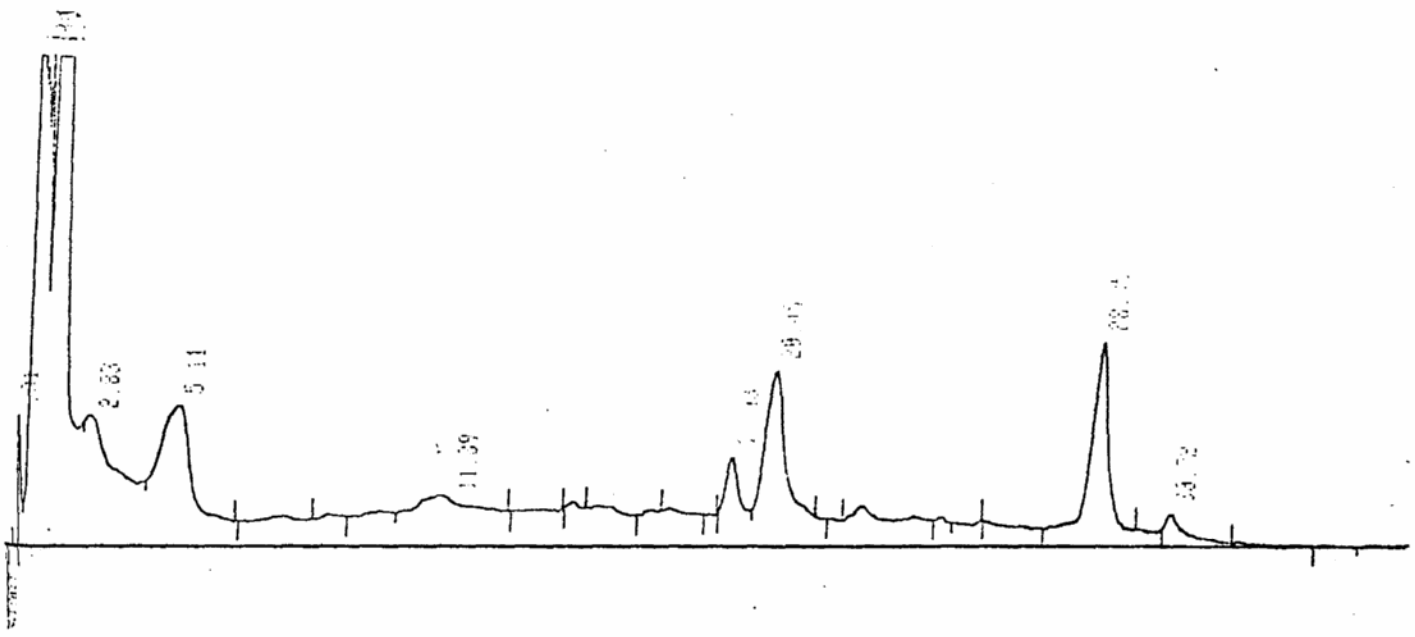
Day 1

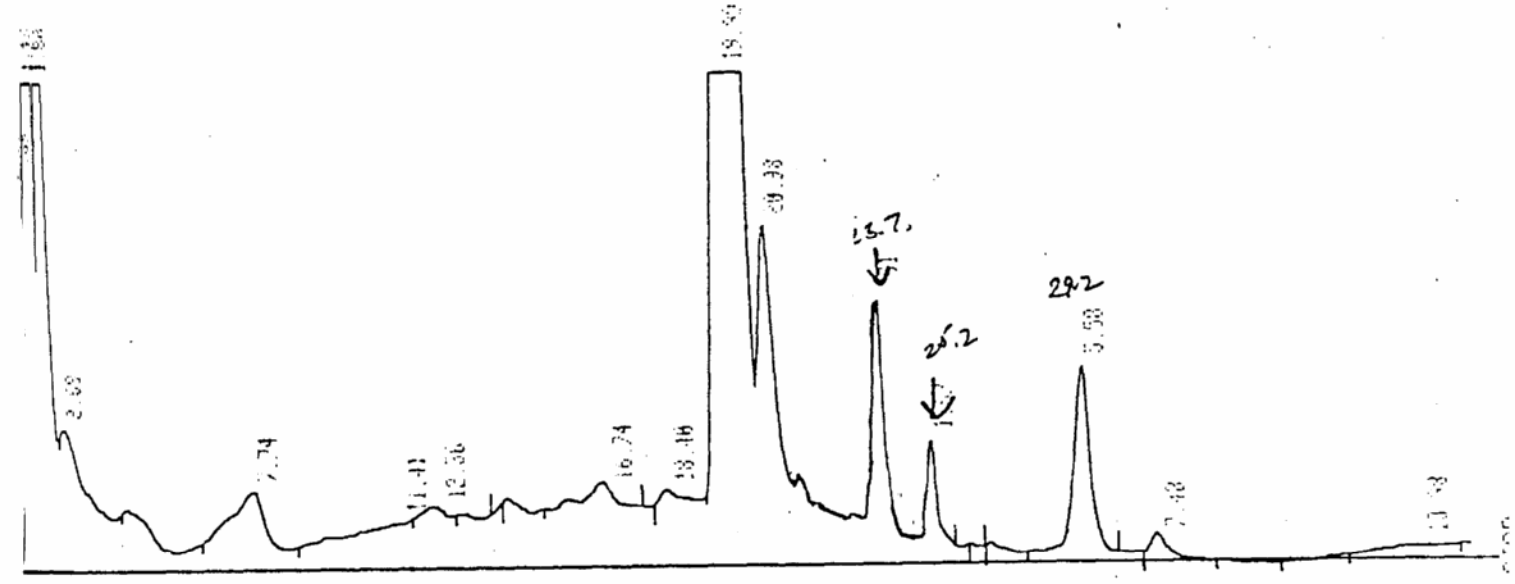


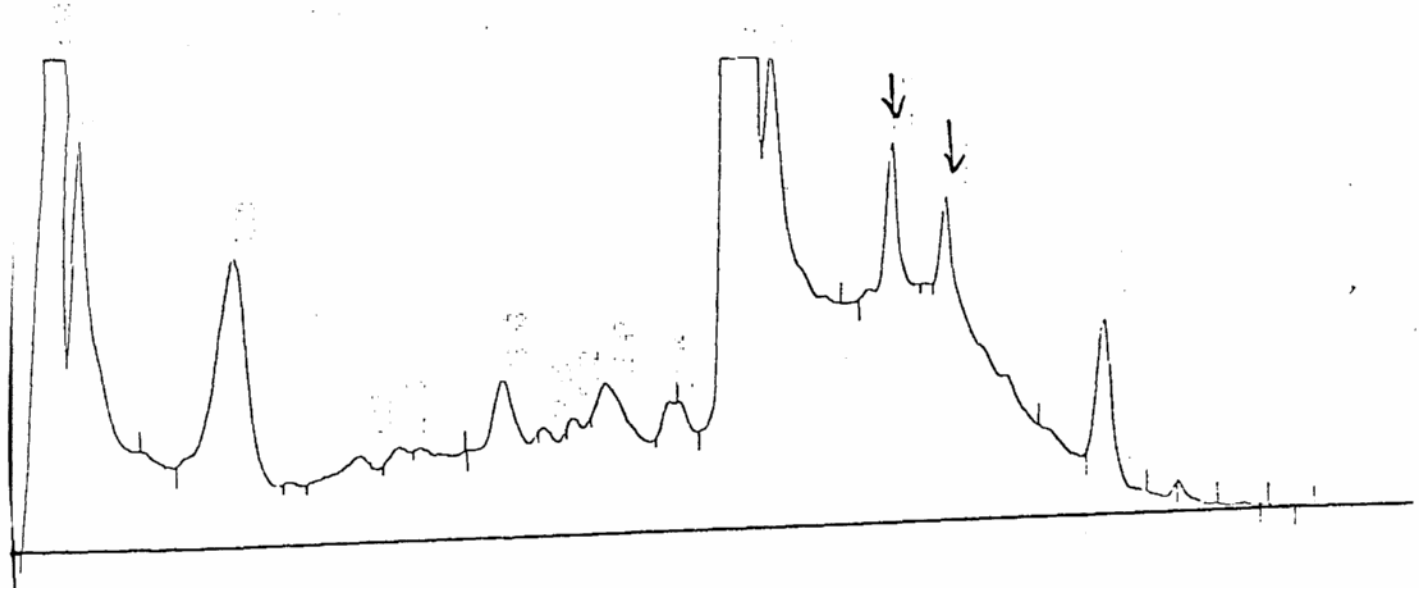




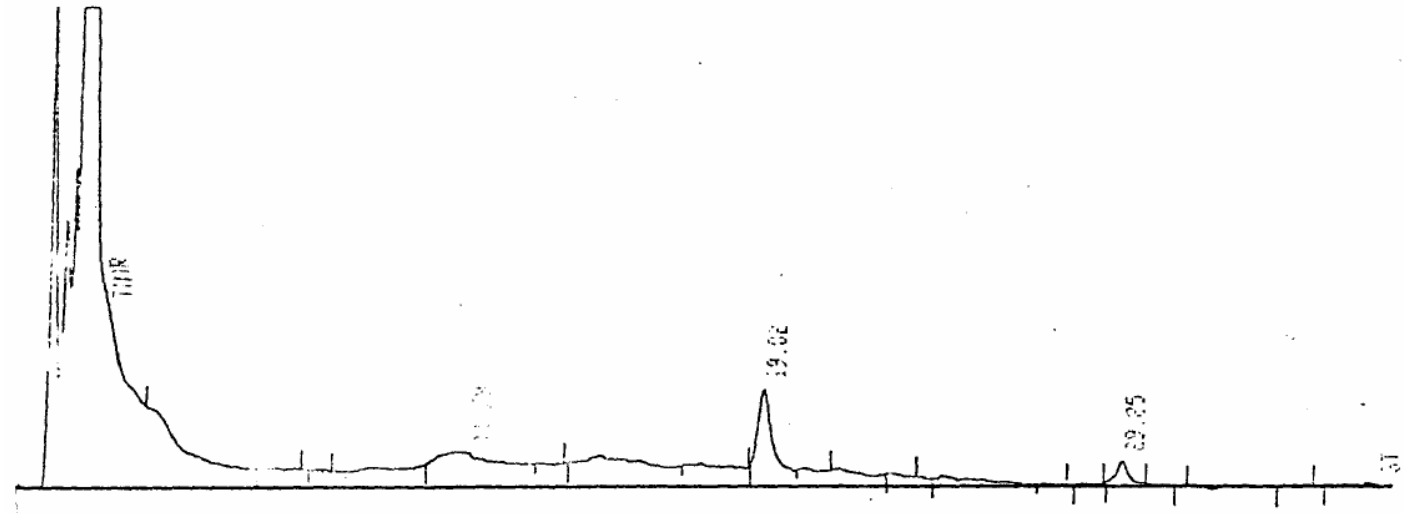
Day 2

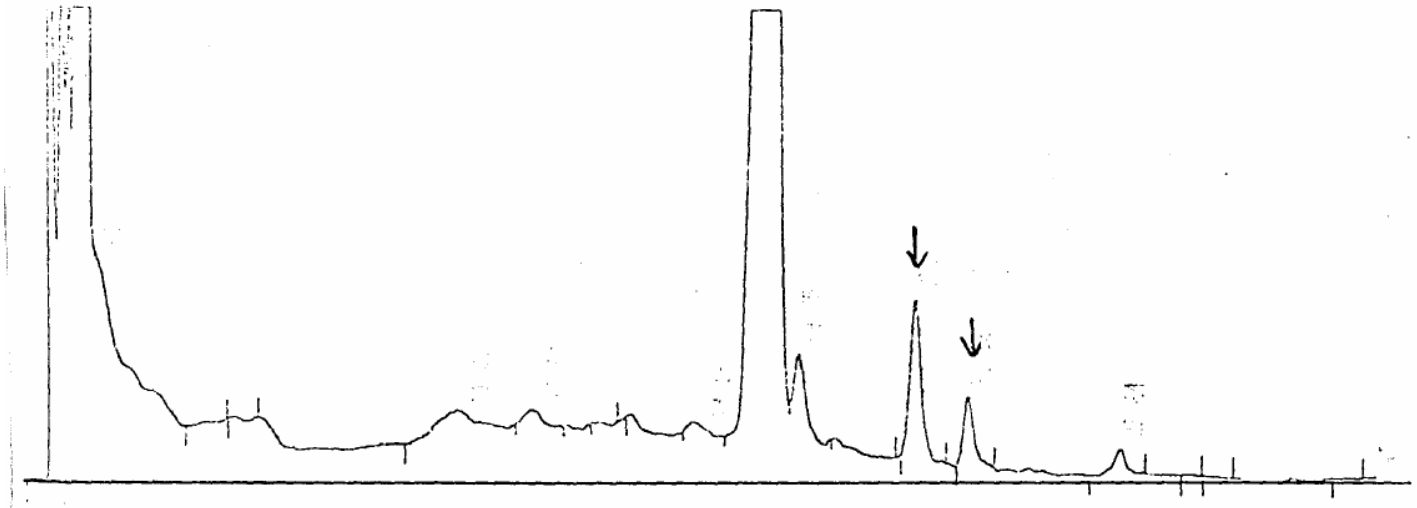






Day 4





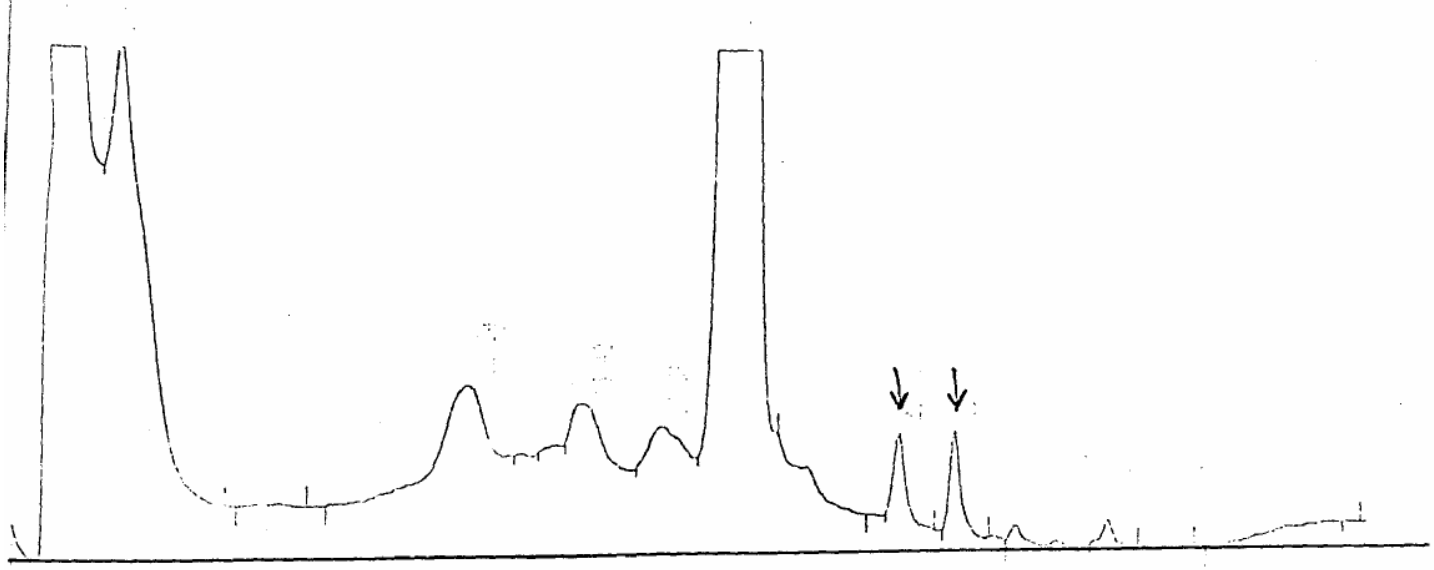


Figure 7. Sampling losses - run #5 of Table III.

7a. Water sample processed on day 1.

(top) no added NTP.

(middle) 5 x 10^{-4}M NTP added.

(bottom) 2 x 10^{-3}M NTP added.

7b. Water sample processed on day 2.

(top) no added NTP.

(middle) 5 x 10^{-4}M NTP added.

(bottom) 2 x 10^{-3}M NTP added.

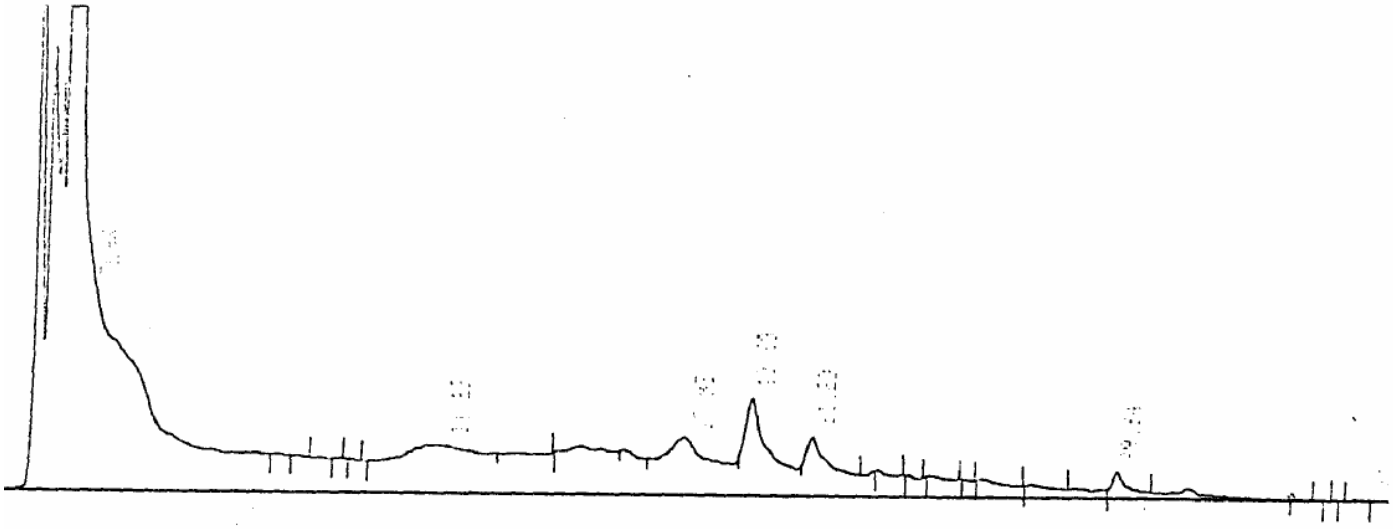
7c. Water sample processed on day 4.

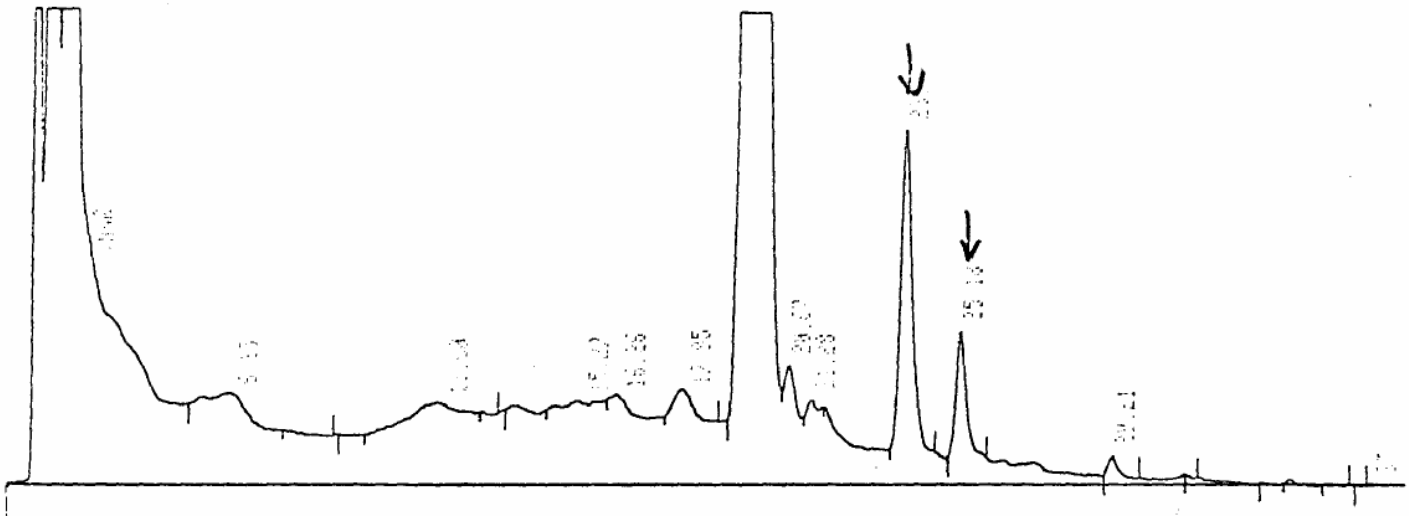
(top) no added NTP.

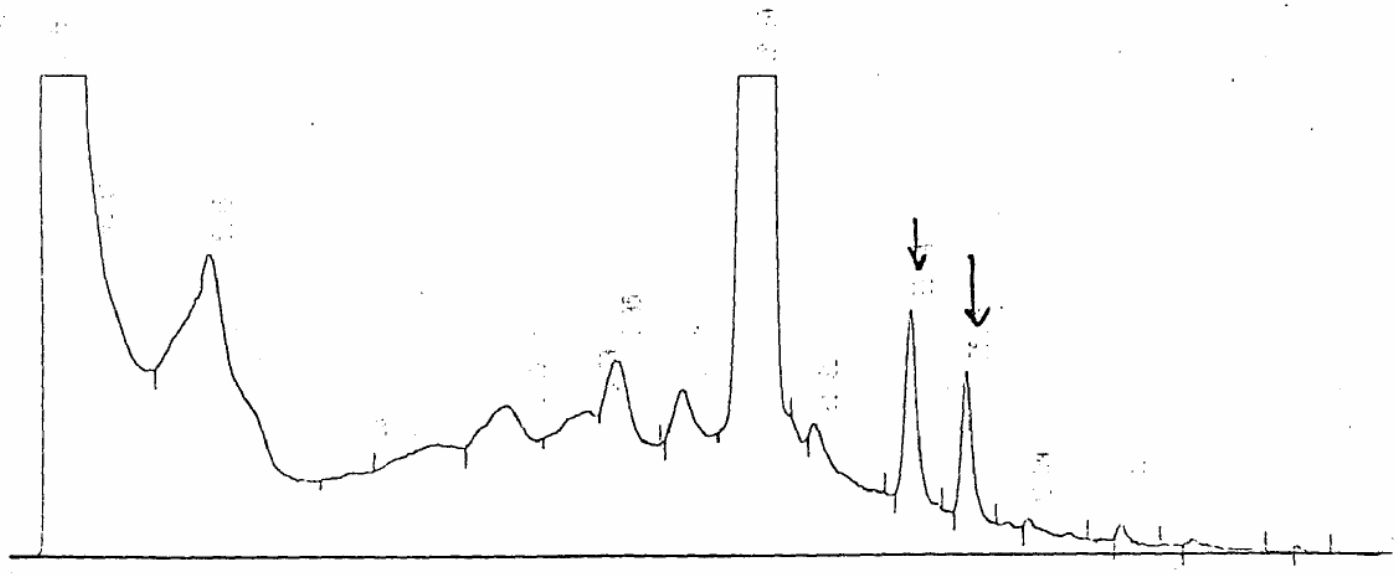
(middle) 5 x 10^{-4}M NTP added.

(bottom) 2 x 10^{-3}M NTP added.

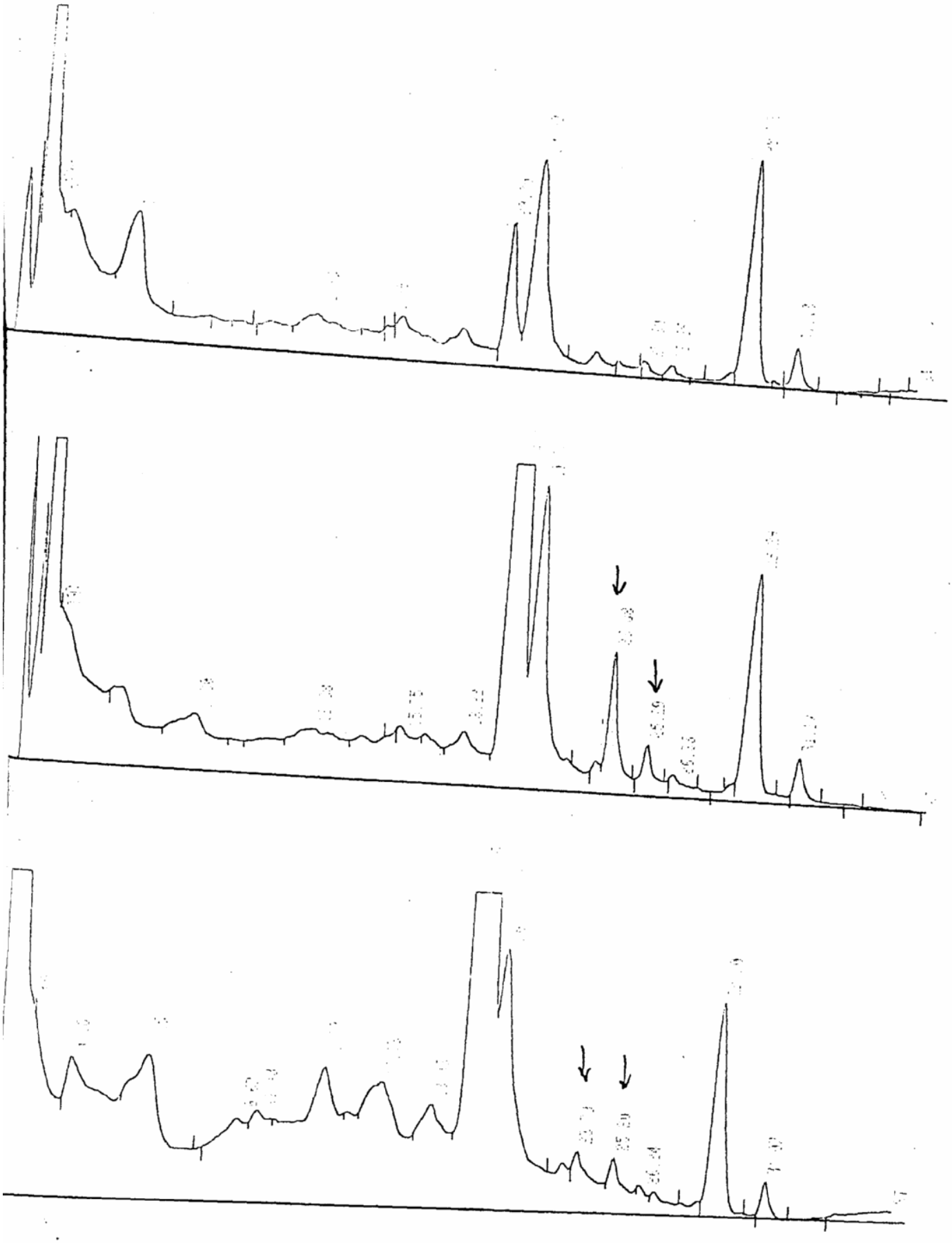
Day 1



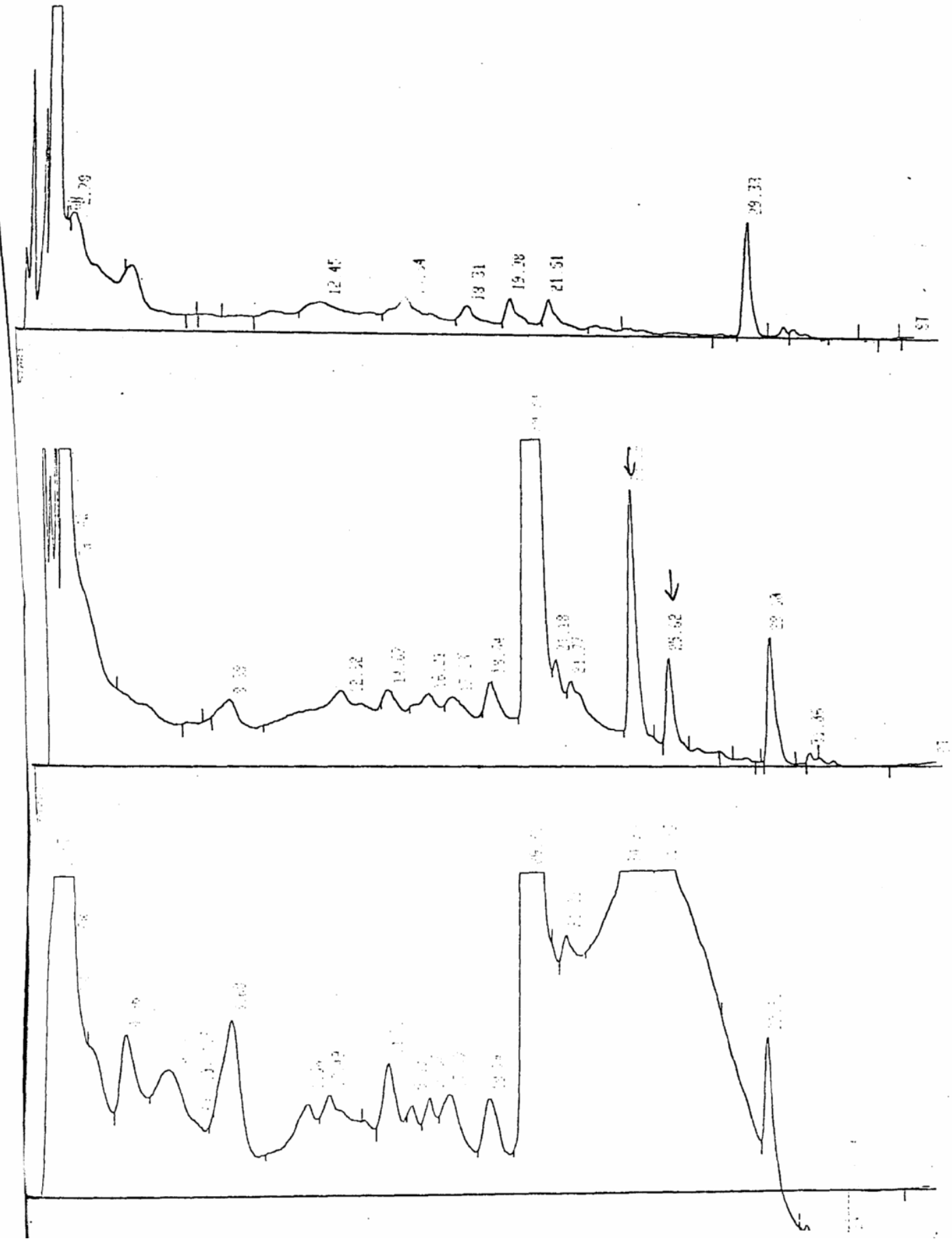




Day 2



Day 4



Storage Losses

Table IV is a list of results showing changes in mutagen content in stored water concentrates. Samples stored in ethanol appeared to retain mutagenic activity or actually show increases in activity over time. Samples stored in DMSO showed consistent decreases in mutagen content, although the normalized results are highly scattered. The ethanol samples stayed liquid, but DMSO (m.p.18) freezes out from a water concentrate, sometimes leaving a more concentrated liquid portion, thus the physical conditions of storage are different in the two solvents.

Fig. 8-12 show the changes in electrophile pattern occuring upon storage of water concentrates. Fig. 8a-d shows a sample stored in ethanol at -80°C. Changes are seen upon storage. The 14 day stored sample has substantially smaller 24 and 25 min. peaks than the fresh sample, and new peaks appear earlier in the chromatogram. The 7 day old sample chromatogram, however, is virtually super imposable upon a fresh one. From Table IV it may be noted that in this run (la), the 14 day value for mutagen content was higher on an observed basis, but lower after normalization.

Fig. 9 shows a sample stored in ethanol at -20°C. No decrease is seen in the NTP reaction profile after 14 days of storage. Here, the mutagen count (Table IV, run 2).

In Fig. 10, it can be seen that a sample stored at $+4^0$C in ethanol had losses in 7 days in peaks at 22, 24 and 25 mins (arrows) despite the apparent rise in mutagen count (Table IV, run 3a).

With samples stored in DMSO (Fig. 11, -80°C and Fig. 12, -20°C) there is no clear decrease in 24-25 min peaks, since these were not present in those water samples. Instead, the only hint of a change is a possible shifting of some of the peaks before disulfide to shorter retention times in the -80°C sample and in the -20°C sample.

TABLE IV

Storage Losses

Run #	Solvent Storage	Storage Temperature (ºC)	Days in Storage	Mutagen Content (revertants per liter) A	Styrene oxide response (revertants per µg) B	Normalized mutagen content (A+B)
1	Ethanol	-80	0	699	0.422	1656
			7	492	0.303	1624
			15	594	0.339	1752
1a	Ethanol	-80	0	442	0.228	1939
			7	469	0.165	2842
			14	884	0.555	1593
2	Ethanol	-20	0	481	0.339	1419
			7	501	0.410	1222
			14	666	0.228	2921
3	Ethanol	+4	0	370	0.303	1221
			8	619	0.339	1826
			15	657	0.410	1602
3a	Ethanol	+4	0	702	0.410	1712
			7	819	0.228	3592
4	DMSO	-80	0	697	0.165	4224
			7	681	0.555	1227
			14	508	0.18	2822
5	DMSO	-20	0	512	0.165	3103
			7	430	0.555	775
			14	343	0.18	1905

Figure 8. Sample storage losses. Ethanol, -80º C.

8a. fresh sample, no added NTP.

8b. fresh sample, 5×10^{-4}M NTP added.

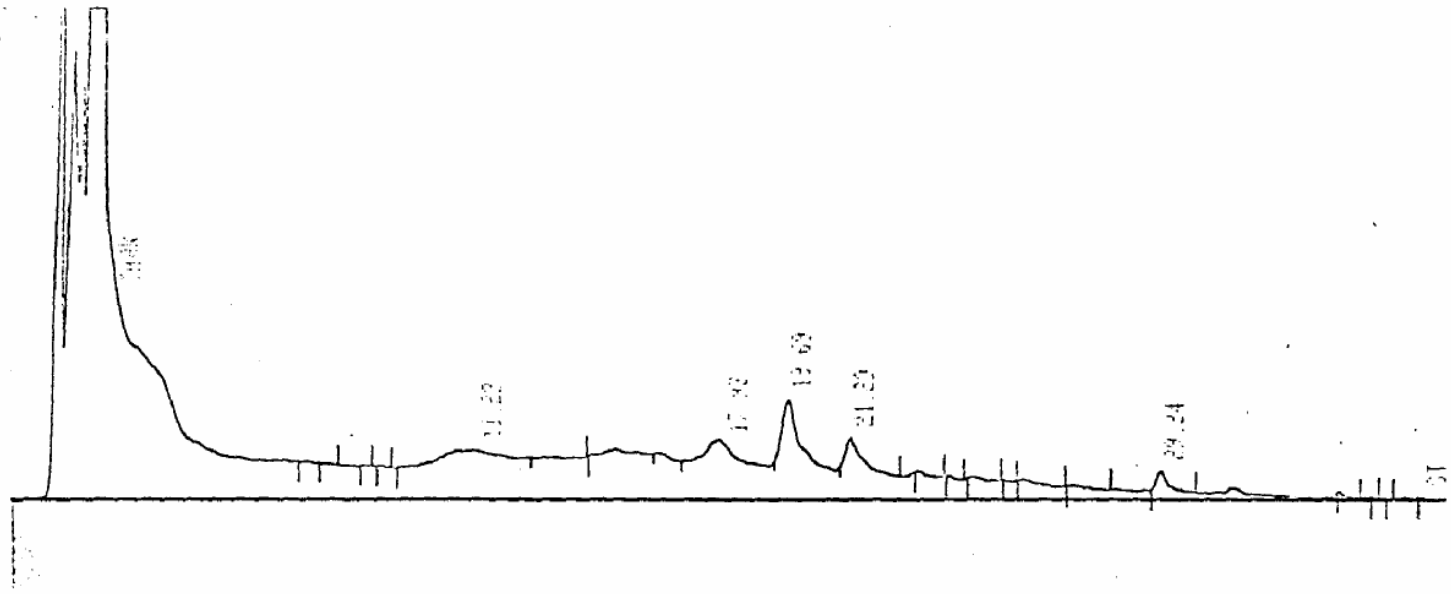
8c. after storage for 7 days at -80° C, 5×10^{-4}M NTP added.

8d. after storage for 14 days at -80° C, 5×10^{-4}M NTP added.

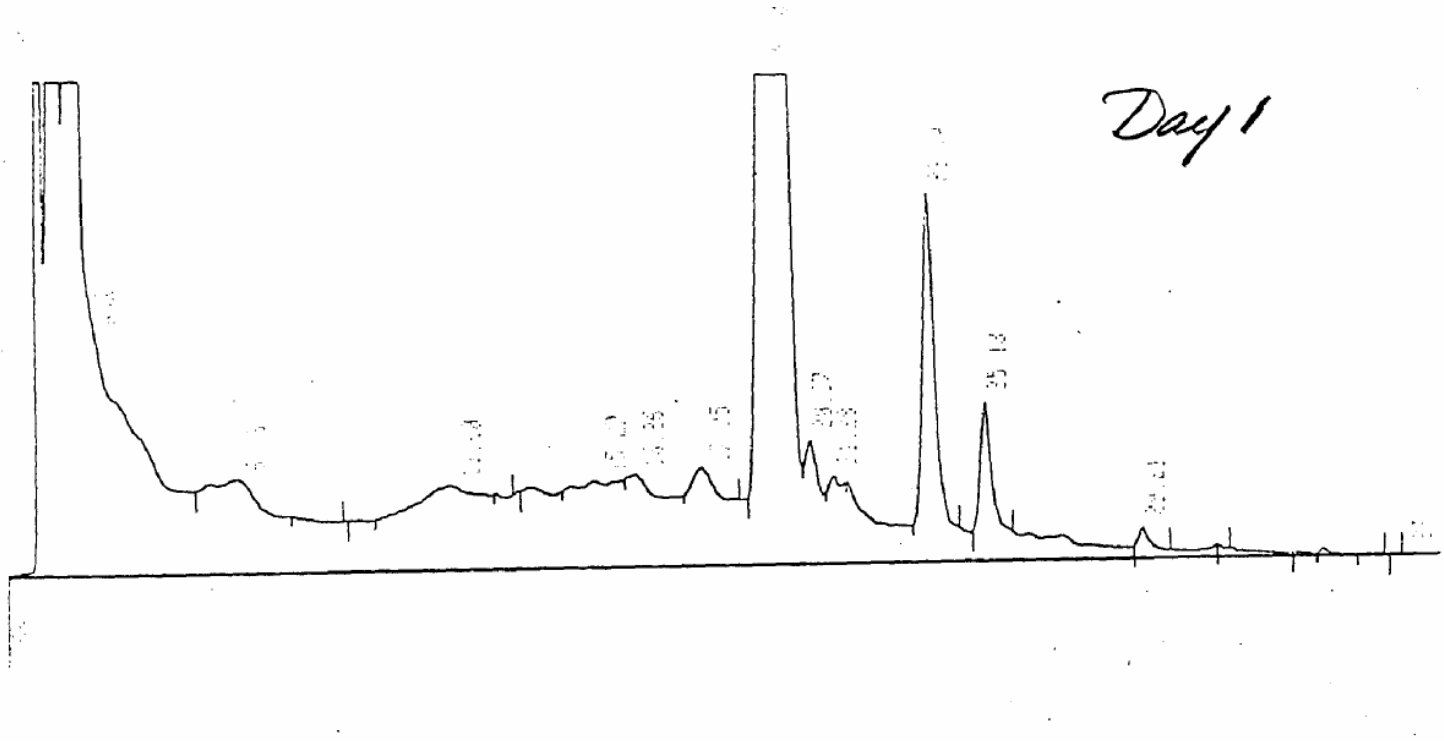
The chromatographic profile for no NTP did not change appreciably with time.

-80° Storage EtOH

A

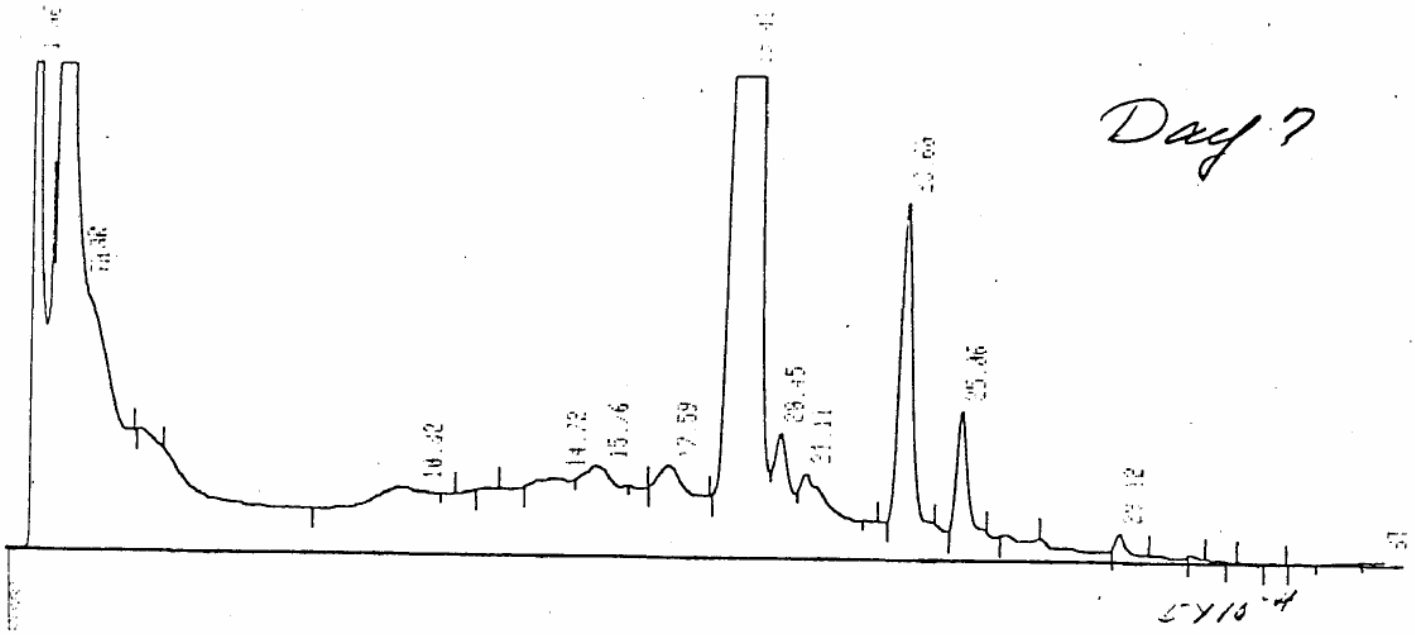


B



-80° Storage EtOH

C



D

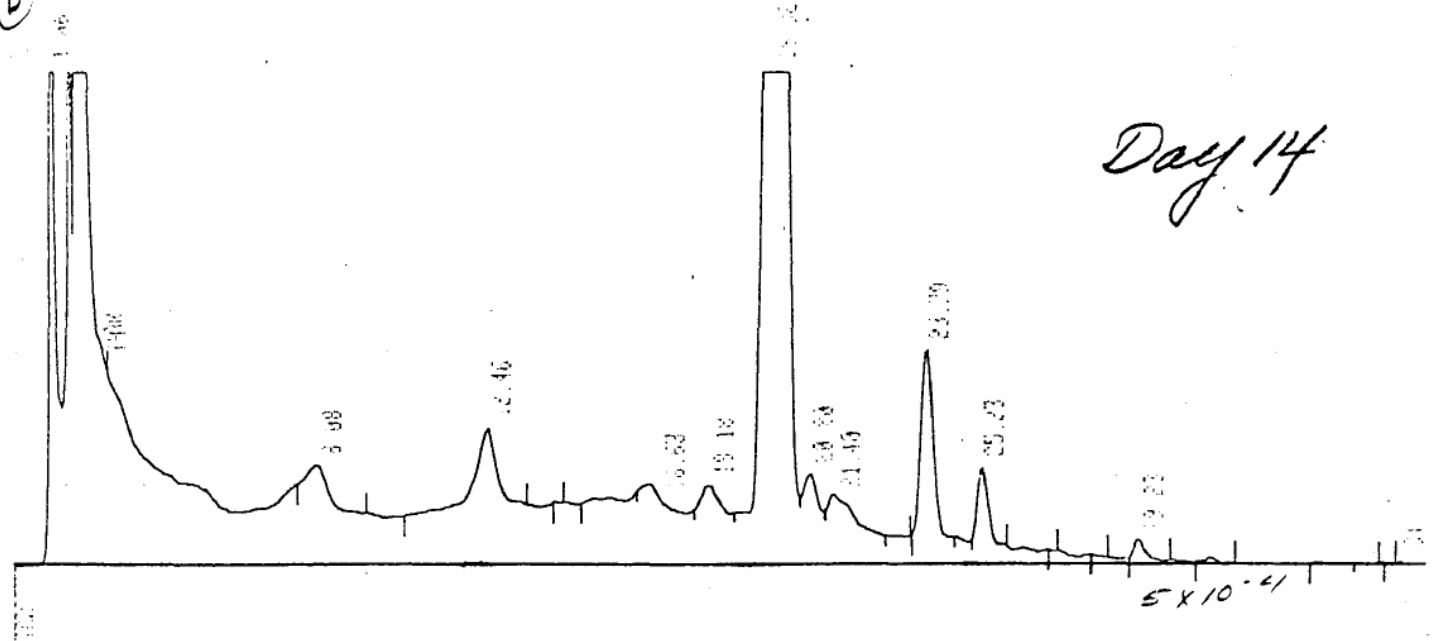


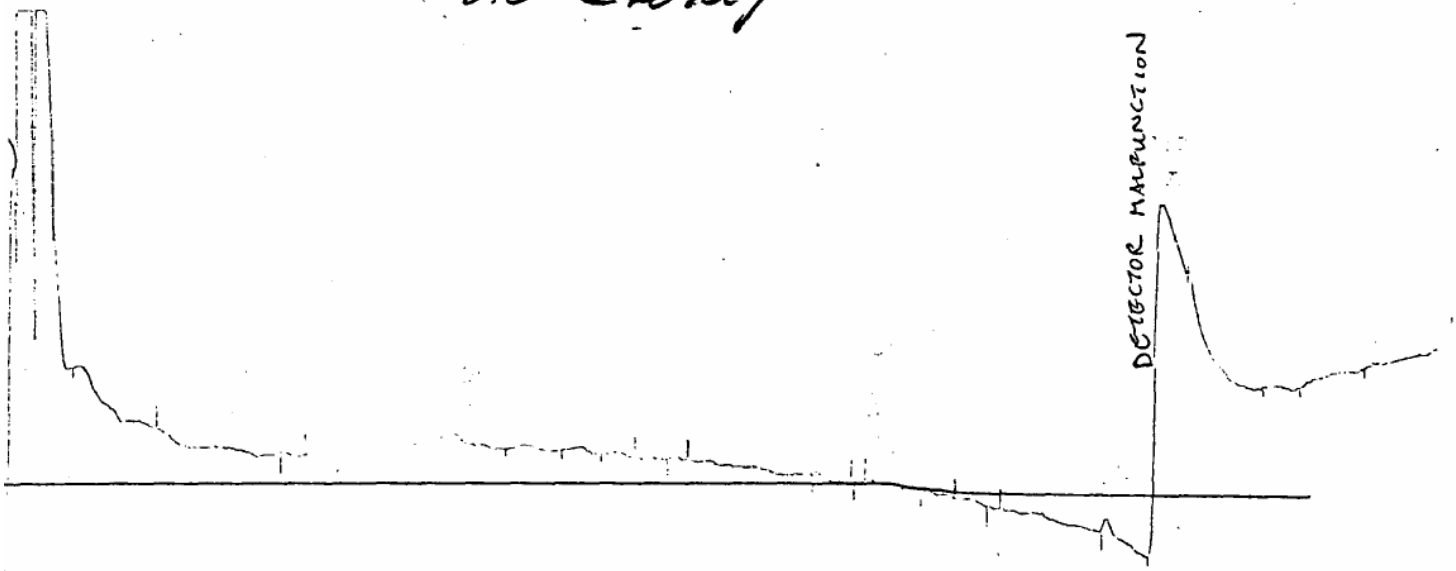
Figure 9. Sample storage losses. Ethanol, -20°C.

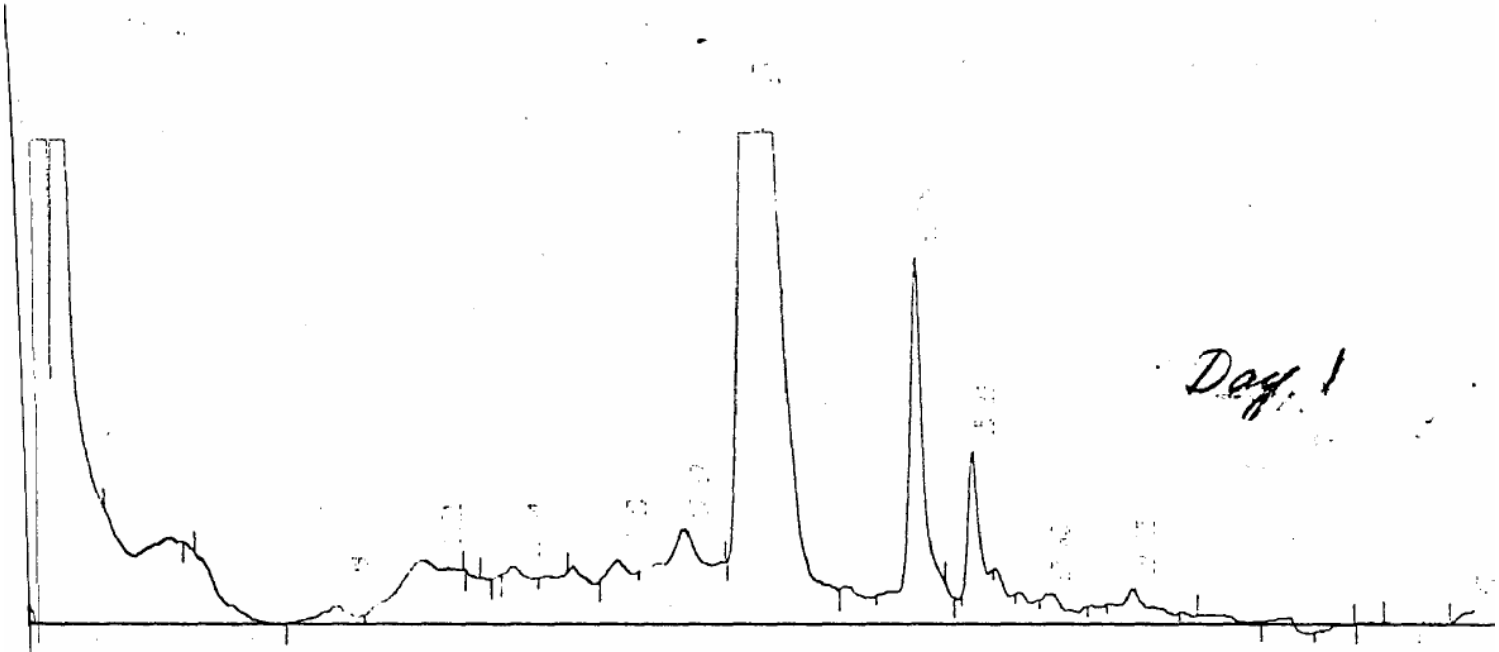
(top) fresh sample; no added NTP.

(middle) fresh sample, 5×10^{-4} M NTP added.

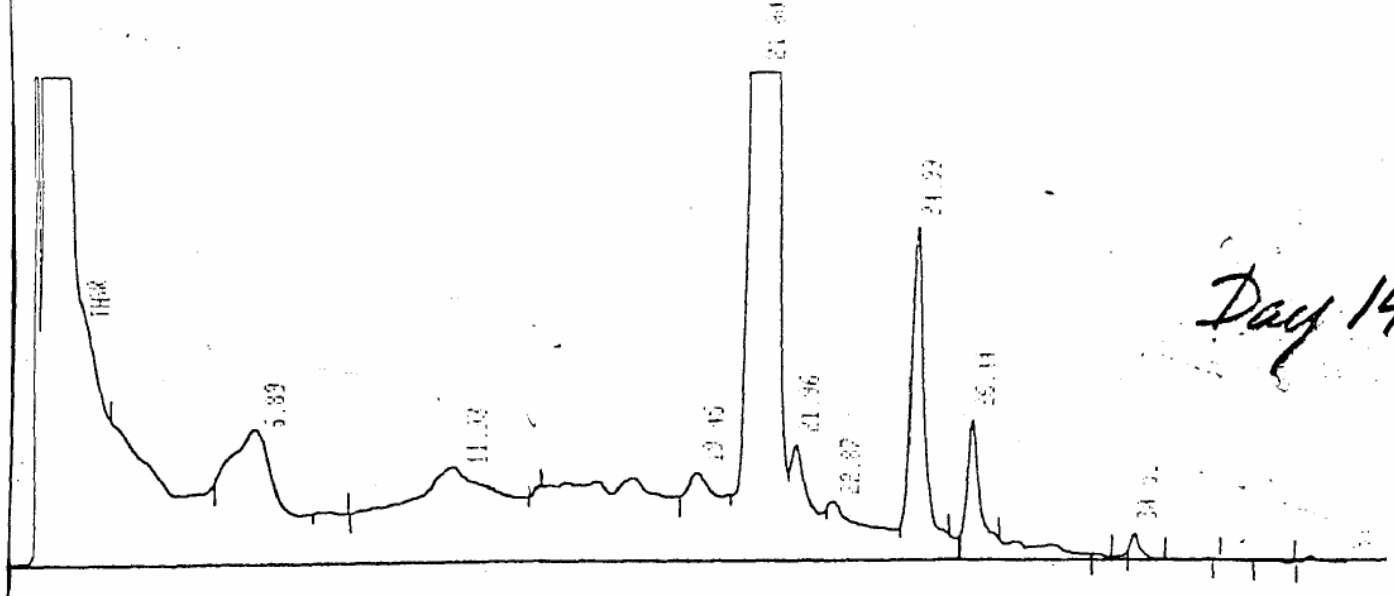
(bottom) after storage for 14 days, 5×10^{-4} M NTP added.

-20° Storage ETOH





Day 1



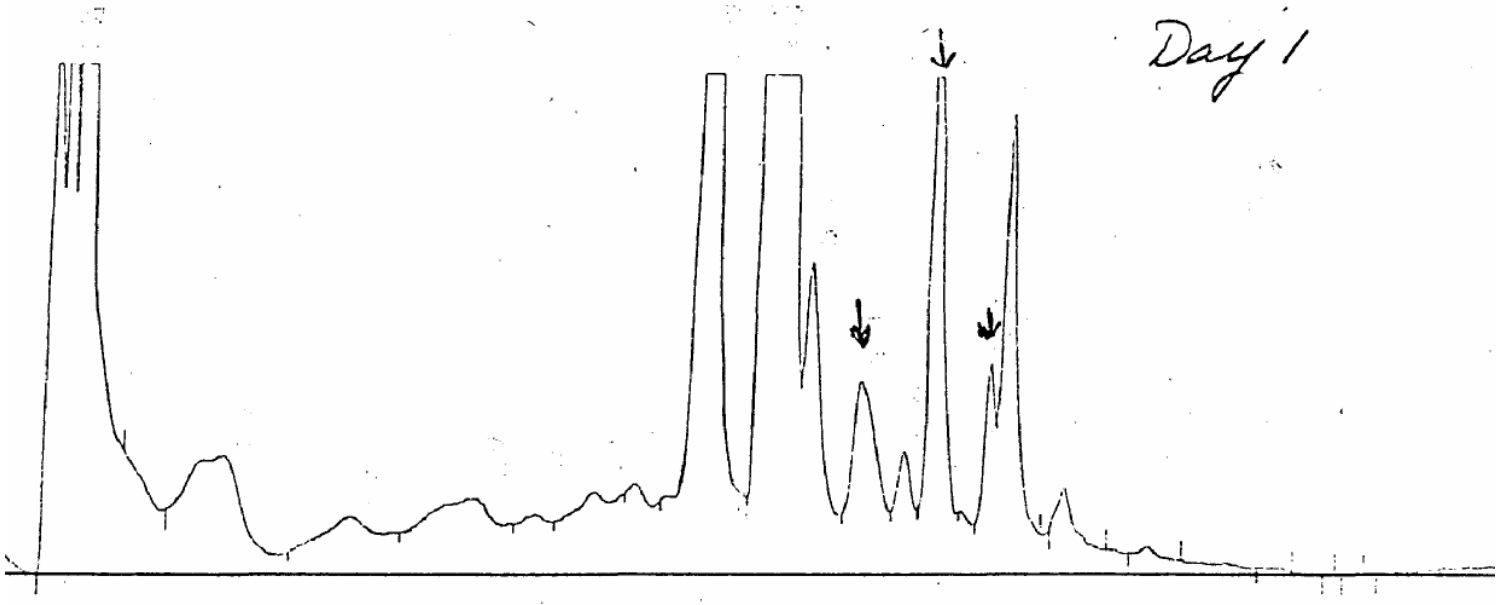
Day 14

Figure 10. Sample storage losses. Ethanol, $+4^0$C.

(top) fresh sample, 5 x 10^{-4}M NTP added.

(bottom) after storage for 7 days, 5 x 10^{-4}M NTP added.

4° Storage EtOH



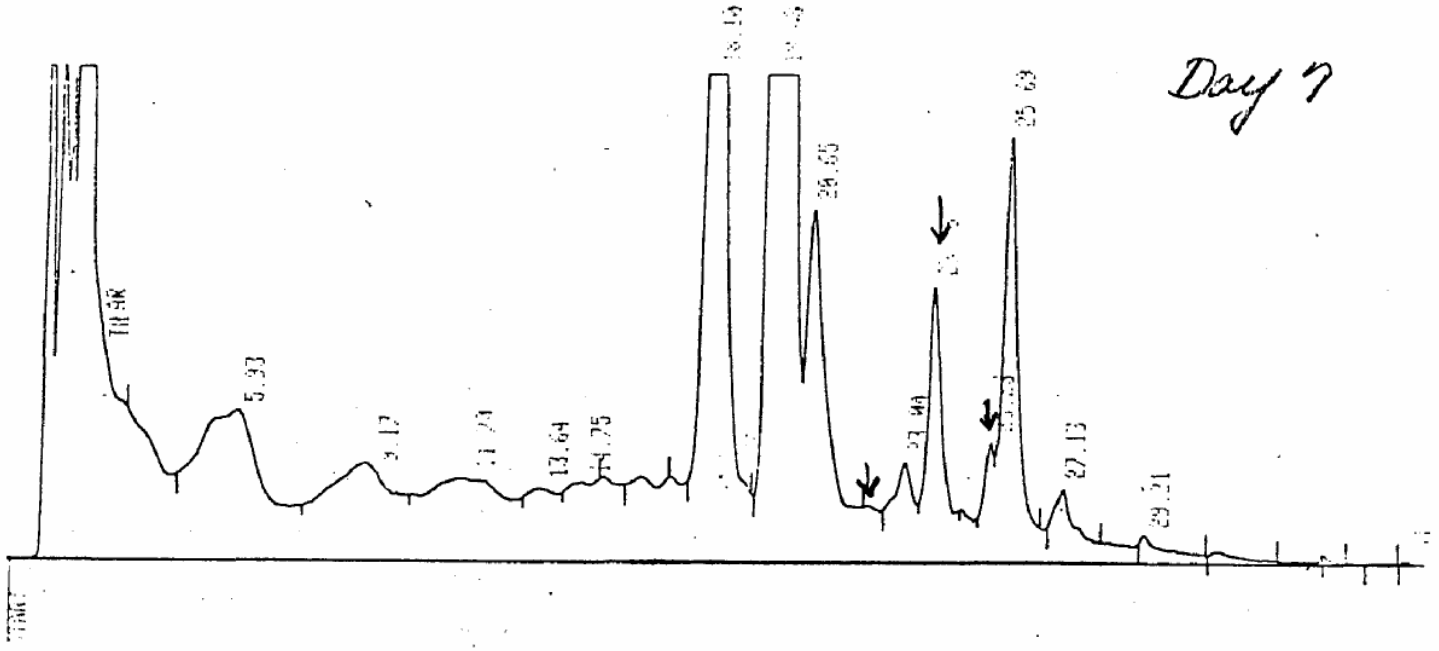


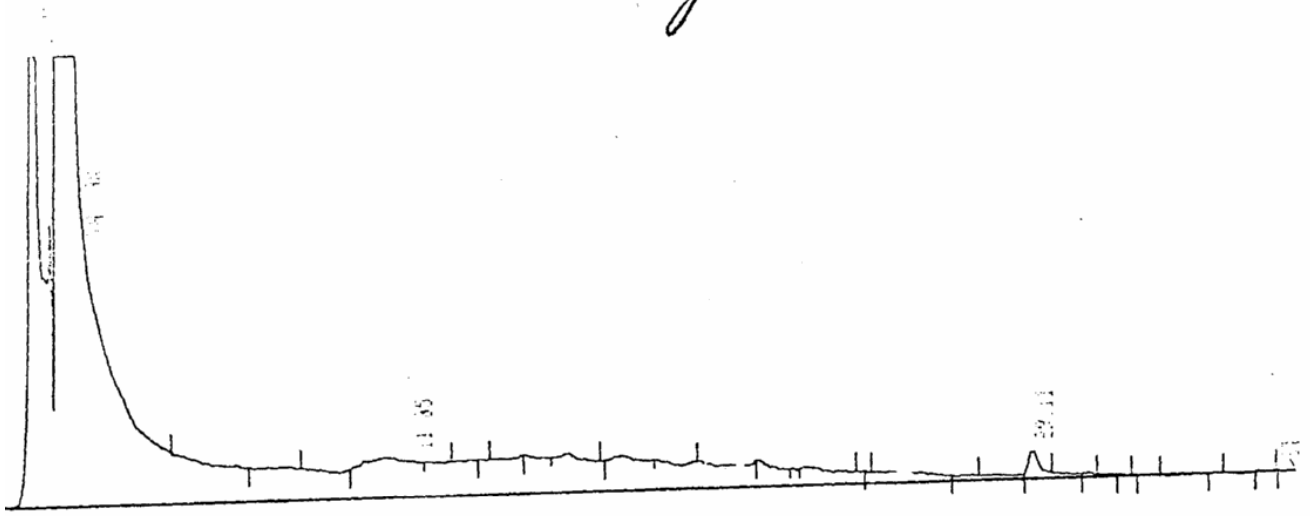
Figure 11. Sample storage losses. DMSQ, -80˚C,

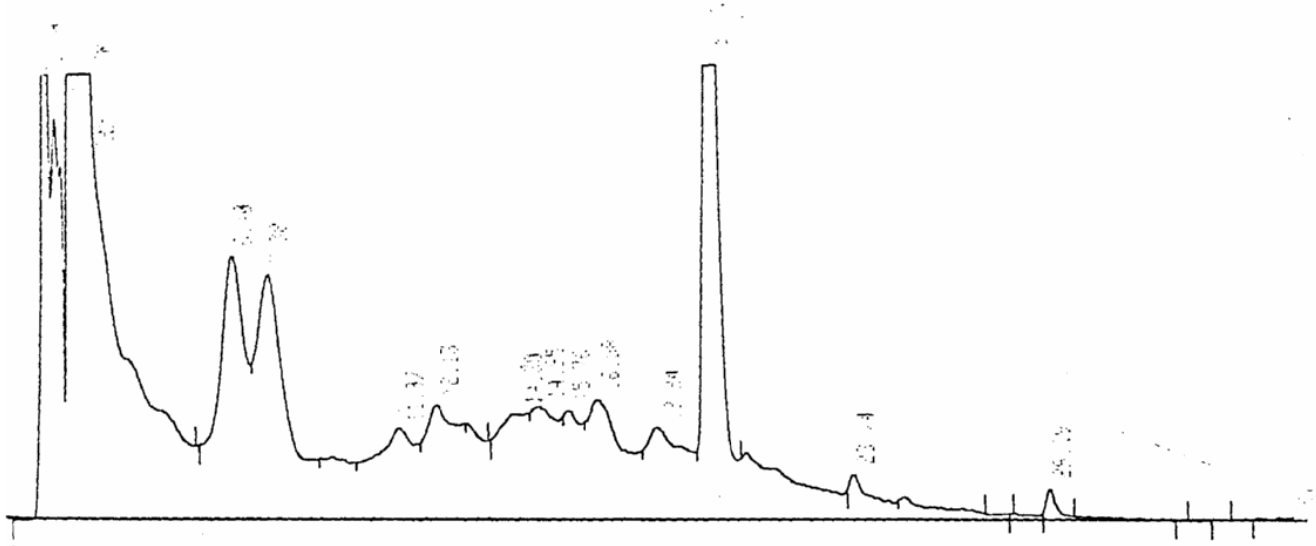
(top) fresh sample, no added NTP.

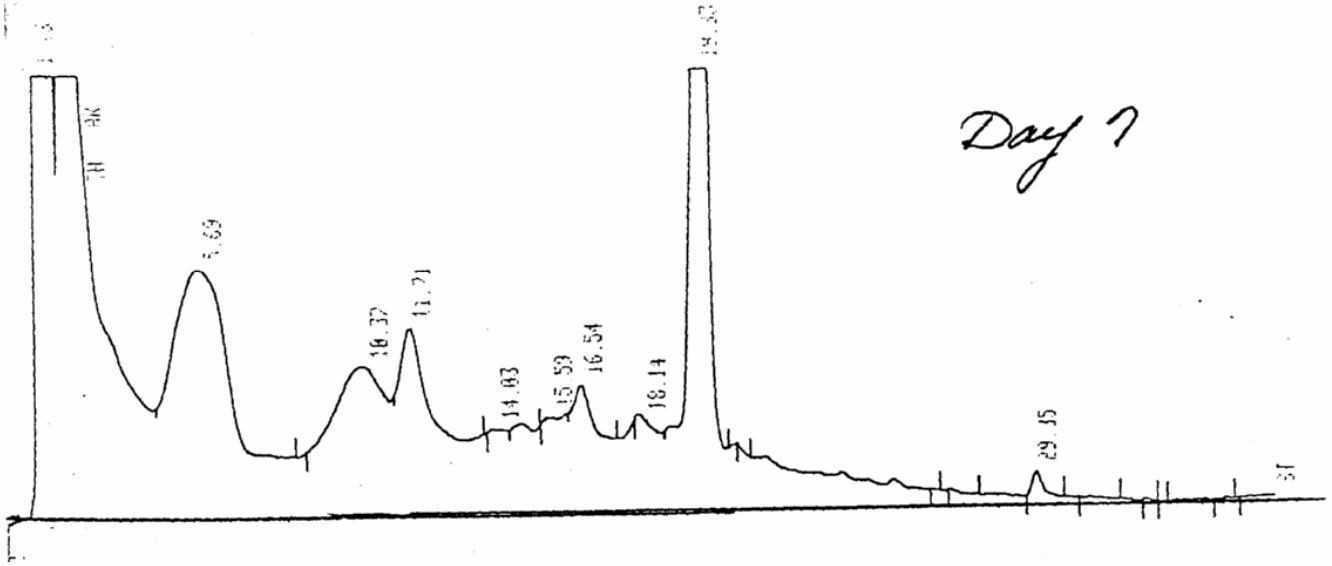
(middle) fresh sample, 5×10^{-4} M NTP added,

(bottom) after storage for 7 days, 5×10^{-4} M NTP added.

-80° Storage DMSO







Day 7

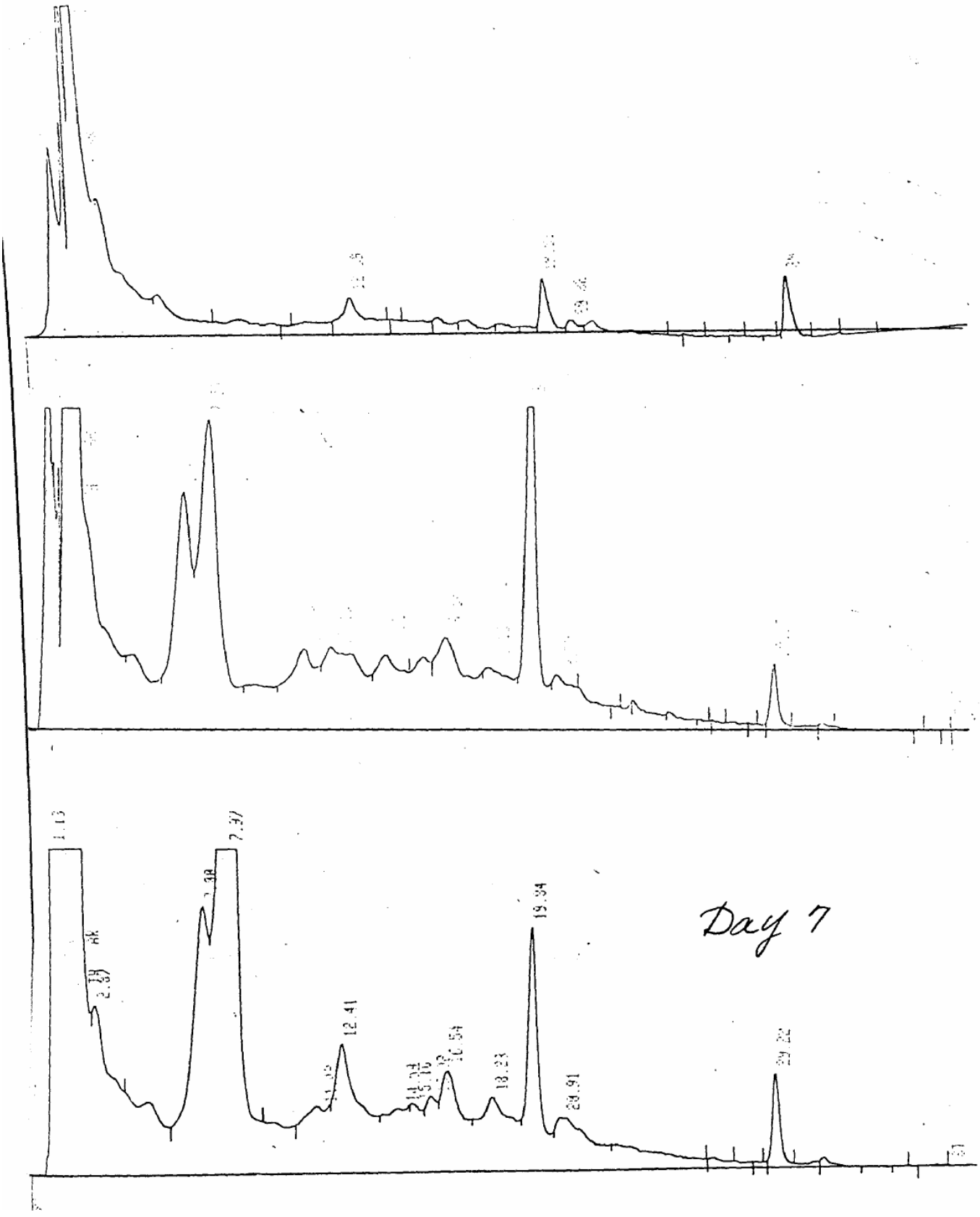
Figure 12. Sample storage losses. DMSO -20˚C.

(top) fresh sample, no added NTP.

(middle) fresh sample, 5×10^{-4}M NTP added.

(bottom) after storage for 7 days, 5×10^{-4}M NTP added.

-20° Storage DMSO



Titration of electrophiles with NTP.

In general, in this project, the water concentrates were reacted with 0, 2.5×10^{-4}M, 5×10^{-4}M. 10^{-3}M and 2×10^{-3}M NTP. Two samples were also monitored for simultaneous destruction of mutagens. These titrations done with TA 100-FRl are shown in Figs. 13 and 14. Past experience with various water samples (8) has shown a sharp reduction in mutagenic activity with 5×10^{-4}M NTP and a slower reduction in mutagenic activity as [NTP] is increased beyond that. Fig. 13 agrees with that pattern. Thus most of the figures presented in this report focus on ONTP, 5×10^{-4}M NTP and 2×10^{-3}M NTP. Fig. 14 shows a similar pattern of initial drop and leveling through 5×10^{-4}M NTP, but a significant continuation in the drop in mutagenic activity with 2×10^{-3}M NTP. The sample in Fig. 14 is a day 4 sample while Fig. 13 corresponds to a freshly prepared day 1 sample.

CONCLUSIONS

The most significant finding of this project is a demonstration that artifact production stemming from the action of chlorine of XAD resins can be suppressed 90% by converting free chlorine residuals to monochloramine. The simple addition of a slight stoichiometric excess of any ammonium salt will accomplish this. (With chlorine concentrations on the order of a few ppm and 1ppm$C1_2$= 1.4×10^{-5}M, there will be little impact on pH, regardless of the counter ion).

This procedure is best adapted to where large volume water samples are obtained and passed through resin beds under ambient conditions. Where a water supply is connected directly to a column, of course, it is not possible to convert the chlorine to chloramine. Then, the magnitude of artifact production by free chlorine might be predicted on the basis of the kinetic experiments reported in the artifacts section. The mutagenesis assays and electrophile assay in the artifacts section were performed after passing the various chlorine solutions through the XAD resin at ambient temperatures (about 25º C) and buffered to pH7.4. Based on the results of Table II, lower temperature or higher pH would be expected to yield lower

Figure 13. Titration of mutagenic activity with NTP (corresponds to chromatograms of Fig. 6a).

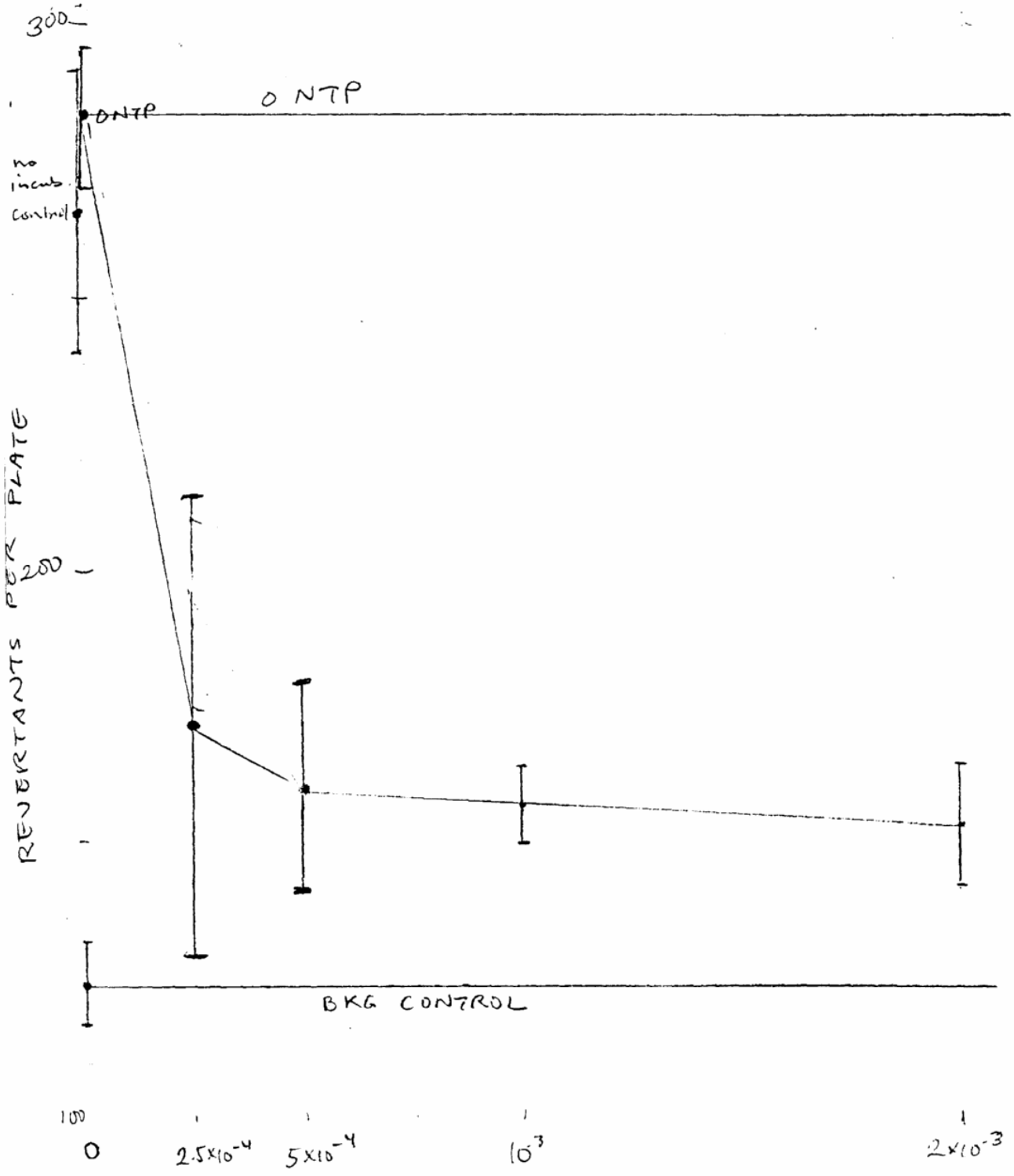
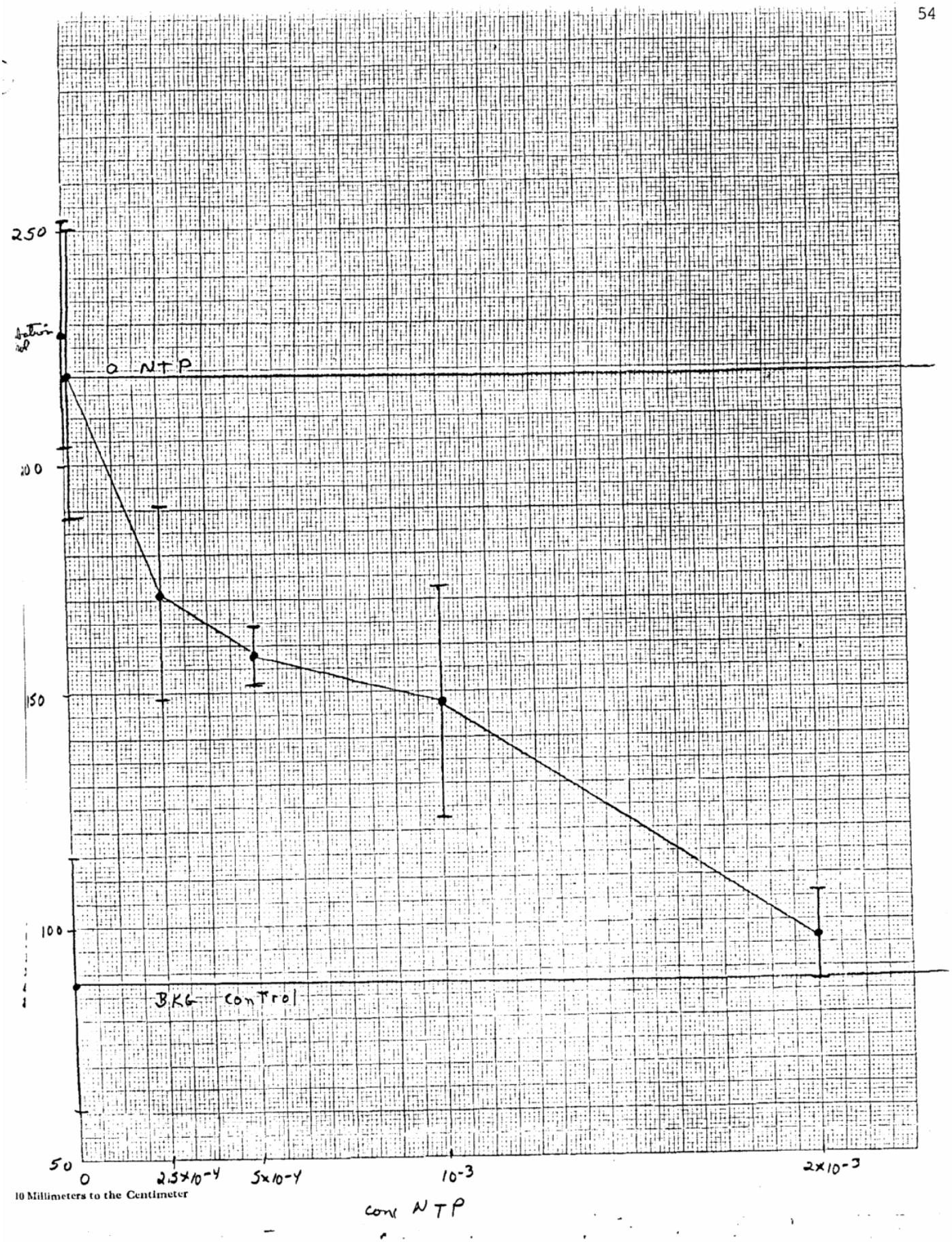

300
200
100
0 NTP
0 NTP
no incub. control
REVERTANTS PER PLATE
BKG CONTROL
0
2.5x10-4
5x10-4
10-3
2x10-3

Figure 14. Titration of mutagenic activity with NTP (corresponds to chromatograms of Fig. 7c).


250
200
150
100
50
0 NTP
BKG control
0
2.5×10⁻⁴
5×10⁻⁴
10⁻³
2×10⁻³
conc NTP
10 Millimeters to the Centimeter

levels of artifacts, while higher temperature or lower pH would increase the level, providing that the chlorine is not already completely consumed. In several of the above experiments testing artifact production by free chlorine, the resin effluent was collected, and almost no chlorine was found, indicating total consumption of free chlorine under those temperatures, pH and flow conditions (room temperature, pH 7.4, 1 liter/hr).Thus, the levels of artifacts observed in these experiments would likely represent an upper limit.

It should be mentioned that monochloramine concentrations were also monitored in several artifacts experiments. Here it was observed that contrary to the ≥ 90% consumption of free chlorine, only 10-40% of the monochloramine was consumed, consistent with the lowered reactivity seen in the kinetic runs, and with the lowered mutagen and electrophile production.

Sometimes XAD sampling is done after lowering the pH to 2, to increase the recovery of phenolic substances and carboxylic acids. The data reported here for pH 6-8 naturally would not apply there.

In the losses sections of the project certain problems prevented the drawing of firm conclusions. The mutagenesis assay showed greater variability in the positive control response than has been our experience in the past. In particular, the responses to the positive control (0.18 to 0.84 revertant/μg spanned a range wider than the one normally seen, besides being significantly lower than normal, suggesting a problem with the tester strains (and more so towards the end of the project). This wide variation in the positive control response is reflected in the wide variation in the experimental responses. A better behaving mutagenesis assay could provide more definitive results. The NTP assay for electrophiles was developed because a chemical procedure should be less subject to variations than a bioassay. Unfortunately, the late delivery of the HPLC meant that chromatograms for many of the earlier experiments do not exist. The fact that the detector lamp that was delivered was not reliable, and a replacement was slow to come by, meant still more samples were not subject to the NTP assay.

It is quite clear that a stable baseline is critical to the NTP measurements. In addition, although NTP enhances the detectability of electrophiles, there is still significant background absorbance in tap water samples. NTP adducts are superimposed upon this, making it more difficult to detect increases (artifacts) or decreases (losses). Methods to increase the signal (detection of electrophile adducts) and decrease the noise (background absorption) in this system are desirable. Thus, we are investigating a second generation of labeling nucleophiles beyond NTP.

Still, some interesting findings with NTP may be noted. Overall, the level of artifactual electrophiles produced by free chlorine is about IO× or greater than that produced by monochloramine, which agrees well with the findings using the bioassay, despite the variation in the latter. It should be noted that the styrene oxide positive control responses in the "artifacts" section (Table I) ranged from 0.33 to 0.84 (mean 0.54 SD 0.18) which is less variable than the responses seen in the other sections and is missing the extremely low (poor) responses. Thus the bioassay results in this section are among the more reliable ones.

Where NTP chromatograms were available, however, a pattern of storage change is observed. Thus it is seen that these samples can change whether bound to the resin or whether they are concentrates stored in the cold. In general there are losses, sometimes of individual peaks and sometimes in the overall mass of NTP adducts formed. It should be noted that where losses occur, this may be partially masked by the NTP reaction kinetics. Assuming that the NTP reactions do not go to completion (), where there are losses (fewer electrophiles), the NTP reactant is depleted less rapidly, and the higher concentrations of NTP remaining will react more rapidly with those electrophiles still present, partially offsetting the loss in electrophiles.

Better resolution of the NTP peaks would make it easier to detect changes in their size. Cochromatography artifacts are known to occur with water samples as some NTP disulfide co-

chromatographs with the 254 nm absorbance maximum of the water sample (8). Removal of the background non electrophilic material would reduce column loading and cochromatography artifacts; therefore, it would be extremely desirable if it could be accomplished. Elimination of the non electrophiles would improve resolution of electrophile adducts, and-as noted above, it would also improve the ability to detect changes in their concentration. It may be worthwhile to investigate separative methods in an attempt to separate electrophiles from the interfering background.

Declines over time in certain peaks were observed. In particular, the peaks at 24 and 25 mins are rather prominent, appearing in many chromatograms, and reflecting significant concentrations which are rather labile. Because of their prominence, these peaks may represent an appreciable portion of the overall electrophile content. It would be desirable to identify them.

References

1. See the series of volumes on "Water Chlorination: Environmental Impact and Health Effects." R.L. Jolley, ed.Ann Arbor Science Publishers, Ann Arbor, MI. 1975-1982.

2. Junk, G.A.; Richard, J.J.; Grieser, M.D.; Witiak, D.; Witiak, J.L.; Arguello, M.D.; Vick, R.; Svec, H.J.; Fritz, J.S. and Calder, G.V., J. Chromatogr. 99 745-762 (1974).

3. Cheh, A.M.; Skochdopole, J.; Koski, P. and Cole, L.,Science 207 90-92 (1980).

4. Cheh, A.M. and Carlson, R.E.,Anal Chem. 53 1001-1006 (1981).

5. Ames, B.N.; McCann, J. and Yamasaki, E.,Mutat. Res. 31 347-364 (1975).

6. DeSerres, F.J. and Shelby, M.D., Mutat. Res., 64 159-165 (1979).

7. American Public Health Association, American Water Works Association and the Water Pollution Control Federation "Standard Methods for the Examination of Water and Wastewater," 14th ed., 1975.

8. Cheh, A.M. and Carlson, R.E., in "Advances in the Identification and Analysis of Organic Pollutants in Water, Vol. l." L.H. Keith, ed. Ann Arbor Science, Ann Arbor, MI, 1982, pp:-457-465.

9. Morris, J.C., in "Water Chlorination: Environmental Impact and Health Effects, Vol.l." R.L. Jolley, ed. Ann Arbor Science, Ann Arbor, MI 1978 pp. 21-35.