

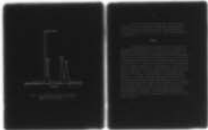
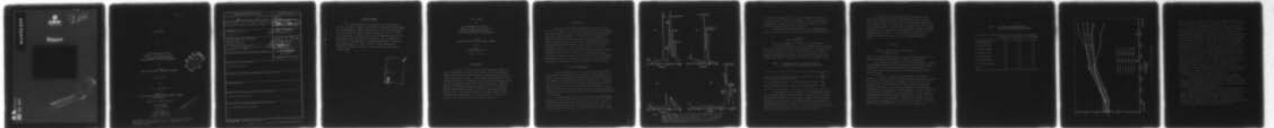
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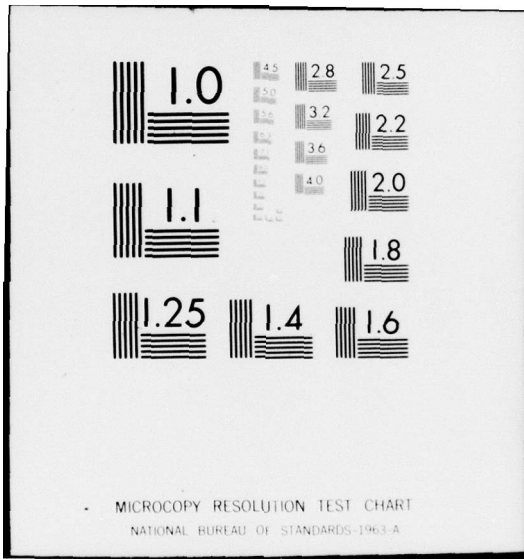
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FINAL REPORT

on

THE DETERMINATION OF TETRYL  
AND 2,3-, 2,4-, 2,5-, 2,6-, 3,4-  
AND 3,5- DINITROTOLUENE USING  
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

to

U.S. ARMY  
MEDICAL RESEARCH AND DEVELOPMENT COMMAND

July 27, 1977

by

T. B. Stanford, Jr.

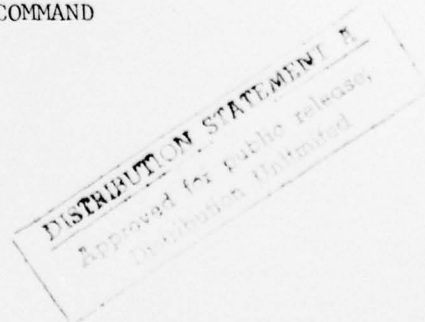
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EXECUTIVE SUMMARY

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This report summarizes work conducted under an extension of the title contract to examine the feasibility of applying High Performance Liquid Chromatographic (HPLC) techniques to the low level determination of tetryl and the six isomeric dinitrotoluenes (2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-DNT). A method suitable for the determination of tetryl in the presence of HMX, RDX and TNT is described. Also, a separation of the five of the six DNT isomers is described and evidence is presented which suggests that with proper control of column efficiency and selectivity a complete separation of all six of these isomers may be accomplished.  
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FINAL REPORT

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THE DETERMINATION OF TETRYL  
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to

U.S. ARMY  
MEDICAL RESEARCH AND DEVELOPMENT COMMAND

from

BATTELLE  
Columbus Laboratories

January 31, 1977

INTRODUCTION

As a part of the work conducted under Contract No. DAMD-17-74-C-4123, a methodology based on high-performance liquid chromatographic (HPLC) techniques was developed suitable for detecting as low as 50 ppb of HMX, RDX, and TNT in water. The development and applications of this method are described in a summary report for the subject contract entitled: "Holston Army Ammunition Plant (HAAP) Pilot Plant Bioassay: Chemical Analysis of Selected Water Samples" submitted to the U.S. Army Medical Research and Development Command by Battelle's Columbus Laboratories, August 26, 1976. As this method proved to be rather unique in terms of its sensitivity, selectivity, and ease of operation, it would be of interest to extend its application in munitions analysis.

### Experimental

All analyses were conducted using 25 cm x 4.6 cm x 1/4 in., partial 10-ODS columns. A Varian 8500 Gradient Elution High Performance Liquid Chromatography System was used consisting of two solvent delivery pumps and a solvent programmer. By filling one of the pumps with 40 percent  $\text{CH}_3\text{OH}/\text{H}_2\text{O}$  and the other pump with 40 percent  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ , all ternary mobile phase compositions between 40 percent  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  and 40 percent  $\text{CH}_3\text{OH}/\text{H}_2\text{O}$  may be obtained by proper selection of the solvent programmer controls (i.e., a mobile phase composed of 20 percent  $\text{CH}_3\text{OH}/20$  percent  $\text{CH}_3\text{CN}/60$  percent  $\text{H}_2\text{O}$  would result by pumping 50 percent from Pump A and 50 percent from Pump B). All runs were performed isocratically, with uv detection at 230 nm using a du Pont 837 variable wavelength uv detector. All solvents used in this study were distilled-in-glass grade obtained from Burdick and Jackson, Muskegan, Michigan.

### Results and Discussion

An initial examination of a mixture of the six isomeric DNTs and TNT under the analytical conditions employed in the HAAP study and using a 50 percent  $\text{MeOH}/\text{H}_2\text{O}$  mobile phase revealed that although TNT was well separated, the DNTs eluted as a poorly resolved group. Subsequent examination with a 40 percent methanol/water mobile phase still did not achieve an adequate separation of these individual compounds. These results indicated that lower percentages of methanol/water mobile phases would merely extend analysis time without affecting an adequate separation of these isomers.

Acetonitrile/ $\text{H}_2\text{O}$  mixtures were also examined as mobile phases. A reversal of selectivity was observed (see Figure 1) when a 40 percent acetonitrile/water mobile phase was used, as the relative retention values of TNT and the DNTs were reversed as compared with their order of elution with methanol/water. TNT now eluted after the DNTs. However,

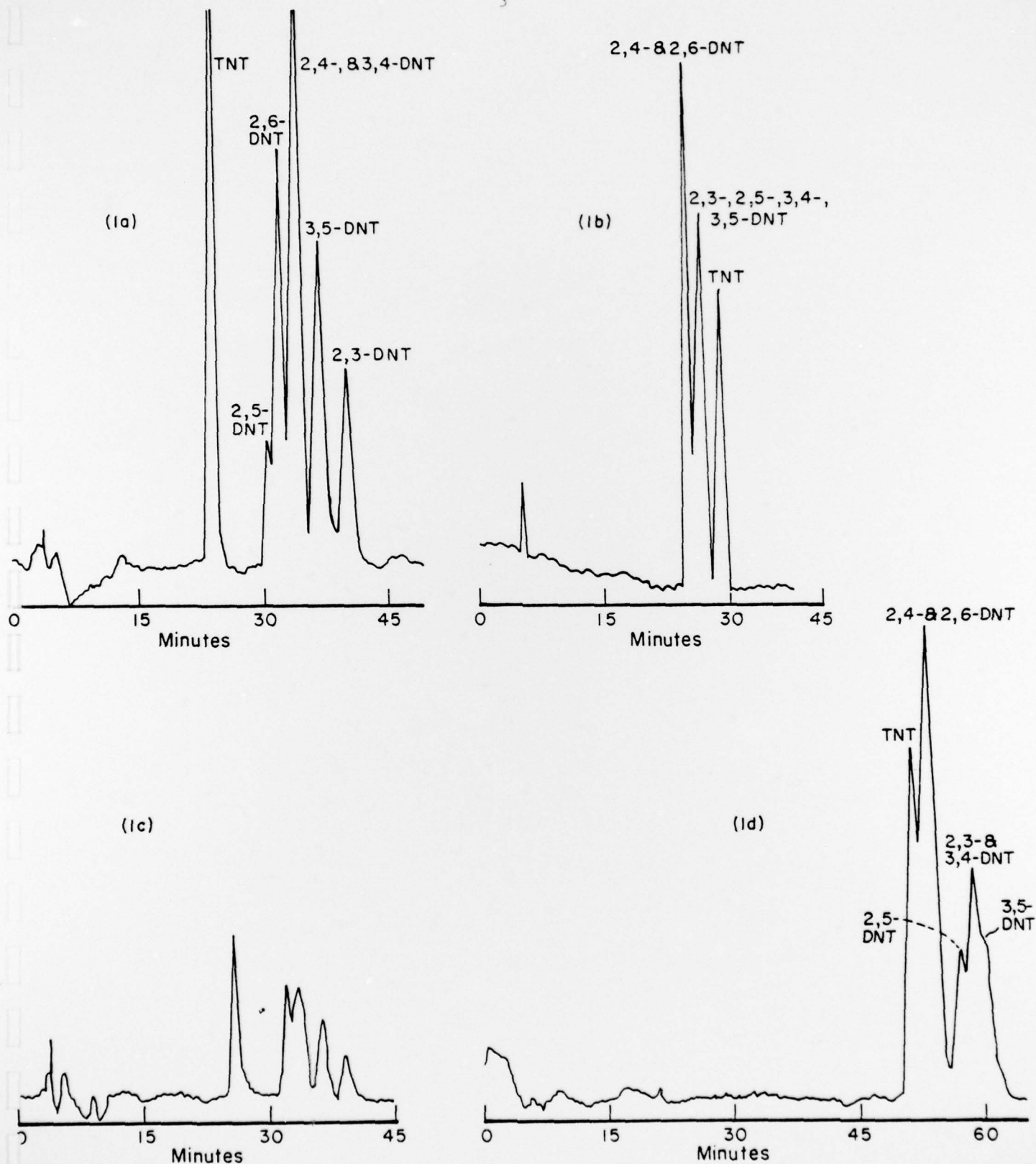


FIGURE 1. HPLC CHROMATOGRAMS OF THE SIX DNTs AND TNT OBTAINED WITH (1a) A 40%  $\text{CH}_3\text{OH}$  MOBILE PHASE AT 100 ml/hr FLOW,  $N_{\text{eff}} = 7327$ , (1b) A 40%  $\text{CH}_3\text{CN}$  MOBILE PHASE AT 100 ml/hr FLOW,  $N_{\text{eff}} = 7937$ , (1c) A 5%  $\text{CH}_3\text{CN}/35\%$   $\text{CH}_3\text{OH}$  MOBILE PHASE AT 100 ml/hr FLOW,  $N_{\text{eff}} = 7569$ , and (1d) A 17%  $\text{CH}_3\text{CN}/23\%$   $\text{CH}_3\text{OH}$  MOBILE PHASE AT 60 ml/hr FLOW,  $N_{\text{eff}} = 10,462$

the latter were resolved into two groups and the desired individual separation of these isomers was not achieved. Again, lower percentages of acetonitrile in water merely resulted in an extended analysis time, without effecting individual isomer separation.

A measure of the separating efficiency of a chromatographic system is given by the number of effective theoretical plates defined as

$$N_{\text{eff}} = 16 \left( \frac{t_R - t_0}{W} \right)^2$$

Where  $W$  = the bandwidth at baseline for a selected peak.

In an effort to increase  $N_{\text{eff}}$  for our chromatographic system and hence, the separation efficiency, the column length was increased to 50 cm, the flow rate reduced to 60 ml/hr, and dead volume in the system was reduced to minimize peak broadening. These modifications increase the analysis time; however, the resultant effect on  $N_{\text{eff}}$  is shown in Table 1.

TABLE 1. OPTIMIZATION OF  $N_{\text{eff}}$ , BASED ON TNT PEAK  
OBTAINED WITH 40%  $\text{CH}_3\text{OH}/60\%$   $\text{H}_2\text{O}$  MOBILE PHASE

	$N_{\text{eff}}$
One $\mu\text{C}_{18}$ column; flow rate, 100 ml/hr	4940
Two $\mu\text{C}_{18}$ columns; flow rate, 100 ml/hr; reduced dead volume	6704
Two $\mu\text{C}_{18}$ columns; flow rate, 60 ml/hr; reduced dead volume	7885

This optimization of efficiency for our chromatographic system gave little improvement in the separation of the DNTs using a 40 percent acetonitrile/60 percent water mobile phase. However, an almost complete resolution of each DNT isomer was obtained with 40 percent methanol/60 percent water (see Figure 1). However, 2,4- and 3,4-DNT are not resolved and the analysis time was increased to 1 hour 15 minutes using a 60 ml/hour flow, and to almost 45 minutes with a 100 ml/hour flow rate.

Because acetonitrile had demonstrated this rather unusual selectivity for the DNTs and TNT, it was anticipated that a ternary mobile phase composed of methanol, acetonitrile, and water might achieve the selectivity necessary for separation of the DNT isomers within a much shorter analysis time. Retention data were obtained for each of the DNT isomers and TNT in several mixtures of methanol, acetonitrile, and water and are presented in Table 2. The retention data given in Table 2 describes these results in terms of the net retention parameter  $k'$ , where:

$$k' = \frac{t_R - t_o}{t_o}$$

with  $t_R$  = retention time of each compound

$t_o$  = nonsorbed time.

Thus,  $k'$  is a dimensionless measure of the retention characteristics of a compound regardless of mobile phase flow rate or column length.

By examining the dependence of  $k'$  for each DNT isomer as a function of mobile phase composition, one may determine the optimum mobile phase composition for the best possible separation of these materials. A plot of  $k'$  versus mobile phase composition for each DNT isomer and TNT is shown in Figure 2.

For a  $N_{eff}$  of ~ 8000 plates, a difference of 5 percent or greater in  $k'$  values is required to achieve generally acceptable resolutions ( $R_s = 1$ ) of two closely eluting components. From the data in Figure 2, it appears that such a separation for the DNT isomers may be possible within the range 10/30 to 5/35 percent  $CH_3CN/CH_3OH$ . The chromatogram obtained with a 5 percent  $CH_3CN/35$  percent  $CH_3OH$  mobile phase (see Figure 1c) shows significant broadening of the peak containing 2,4- and 3,4-DNT as compared with the 40 percent  $CH_3OH$  chromatogram, but 2,5- and 2,6-DNT are now unresolved. It may be possible to accomplish the required resolution using a lower total percentage of organic solvent in water (i.e., 5 percent  $CH_3CN/25$  percent  $CH_3OH/70$  percent  $H_2O$ ), or with an increased  $N_{eff}$ .

TABLE 2.  $k'$  VALUES OF SELECTED MUNITIONS  
USING SEVERAL DIFFERENT MOBILE PHASES

	50% MeOH/H <sub>2</sub> O	40% MeOH/H <sub>2</sub> O	40% CH <sub>3</sub> CN/H <sub>2</sub> O
2,3-Dinitrotoluene	6.63	11.56	5.94
2,4-Dinitrotoluene	6.00	9.79	5.55
2,5-Dinitrotoluene	5.44	8.32	6.41
2,6-Dinitrotoluene	5.50	8.78	6.51
3,4-Dinitrotoluene	5.63	9.58	6.40
3-5-Dinitrotoluene	6.38	10.39	6.44
2,4,6-Trinitrotoluene	3.22	6.50	6.97
Tetryl	2.78	6.11	7.37

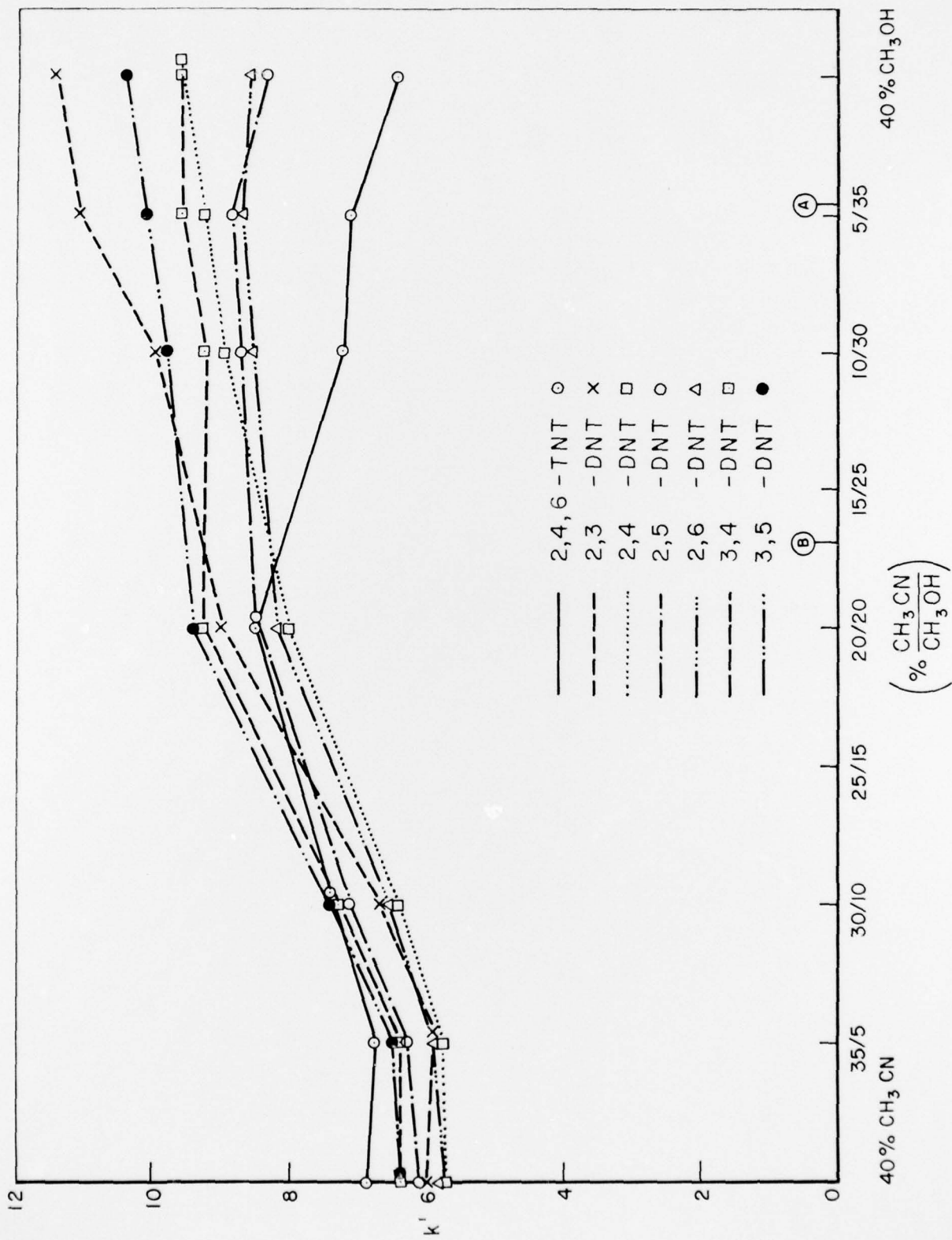


FIGURE 2. PLOT OF  $k'$  VS MOBILE PHASE COMPOSITION

However, the rapid separation of DNT and the six isomeric DNTs seems best accomplished using 40 percent methanol/60 percent water with  $N_{\text{eff}} = 7880$ .

However, if a determination of all six isomers is not sought, by proper selection of mobile phase composition, a satisfactory separation of the isomers of interest may be possible. A very rapid preliminary separation of these materials is achieved with a 25 cm column, a 40 percent acetonitrile/60 percent water mobile phase, and a flow rate of 100 ml/hr. Under these conditions, TNT is well separated and the six isomeric DNTs are resolved into two groups in about 12 minutes. The first of these groups elutes with a  $t_R = 9.7$  minutes and contains 2,3- and 2,4-DNT. The second fraction elutes with a  $t_R = 10.3$  minutes and contains 2,5-, 2,6-, 3,4-, and 3,5-DNT. Although the maximum resolution of each DNT isomer is not obtained, these results achieve a preliminary separation of these materials into two groups with a total analysis time of less than 15 minutes. Furthermore, the 2,3-, 2,5-, and 3,4-DNTs are significantly more toxic than the other three isomers. If only these three DNTs are to be quantitated, Figure 2 indicates that a mobile phase of 17 percent acetonitrile/23 percent methanol/60 percent water will effect the resolution of 2,5-DNT as a single peak while 2,3- and 2,4-DNT will elute together but separated from the other isomers (see Figure 1d).

Retention data for tetryl was obtained and is presented in Table 1. This munition elutes very near TNT, but a separation of TNT and tetryl may be achieved using a 50 percent methanol water mobile phase. An example of this separation is shown in Figure 3 in which RDX, TNT, and tetryl are separated in less than 8 minutes.

Minimum detectable amounts for the DNTs, TNT, and tetryl all ranged between 5 and 25 ng in this study. This level of sensitivity will permit a lower limit of detection of between 50 and 250 ppb (for the direct injection of a 100  $\mu$ l water sample). Of course these values are dependent upon a visible response in the LC chromatogram of these materials and will vary with peak shape and hence operating conditions.

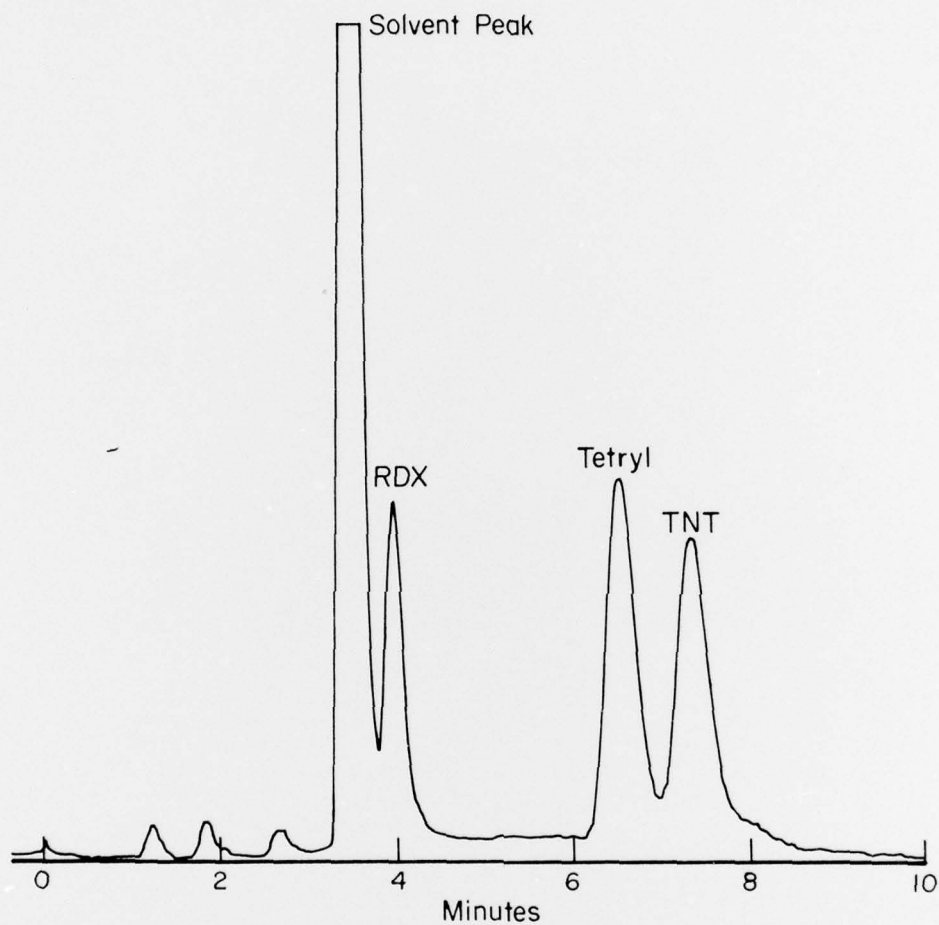


FIGURE 3. HPLC CHROMATOGRAM OF RDX, TNT, AND TETRYL OBTAINED WITH A 50% CH<sub>3</sub>OH/60% H<sub>2</sub>O MOBILE PHASE, 100 ml/hr FLOW RATE

In conclusion, present methodology based upon HPLC techniques has proven to be an effective means of separating and quantitating a variety of munitions in trace concentrations. Even such difficult to resolve mixtures as the six DNT isomers may be almost totally separated and quantitated.

#### SUMMARY

This report describes the separation of five of these six DNT isomers and judging from present data, we feel that all six isomers can eventually be separated once both the selectivity and efficiency of the HPLC conditions are optimized. We have shown strong evidence that solvents capable of specific interactions with these munitions (e.g., acetonitrile) can significantly affect their relative retention and hence the selectivity of their separation. In addition the efficiency of the analytical column can be increased, thus leading to a better separation. (Our laboratory is capable of packing highly efficient columns for specific projects involving difficult separations. This is accomplished by slurry packing smaller particle ( $>10 \mu$ ) columns. Column efficiencies of 20,000 plates are not unrealistic.) By the use of increased efficiency and selectivity (by incorporating different solvents in ternary mobile phase mixtures) there is a good chance that all six isomers could be separated. It is recommended that a thorough examination be made of the retention characteristics of these isomers with a variety of ternary mobile phase compositions and increased separating efficiencies. Possible candidate mobile phase compositions would be the possible ternary mixtures of acetonitrile, furfural, glycol, and water.

