

Bruce R. McCord,¹ Ph.D. and Frederic W. Whitehurst,² Ph.D.

The Analysis and Characterization of TNT Using Liquid Chromatography with Photodiode Array Detection

REFERENCE: McCord, B. R. and Whitehurst, F. W., "Analysis and Characterization of TNT Using Liquid Chromatography with Photodiode Array Detection," *Journal of Forensic Sciences*, JFSCA, Vol. 37, No. 6, November 1992, pp. 1574–1584.

ABSTRACT: The forensic characterization of various samples of TNT is described. The analysis technique consists of extraction followed by normal phase liquid chromatography with photodiode array detection. The samples are examined by enhancing the minor components and impurities left over from manufacture, and using these components as points of comparison. Initial attempts to order the data using spectral searching and pattern recognition techniques are described.

KEYWORDS: forensic science, TNT, explosives, forensic, liquid chromatography, diode array detection

When explosive materials such as trinitrotoluene (TNT) are found in a criminal investigation, important questions are immediately asked of the analyst, such as where the material came from and whether it ties in with some previous incident. As is the case with many explosives, the final product which results from the manufacture of TNT is a highly purified substance. The reason for this fact is simple, lives can depend on the ability to precisely determine the nature and explosive force of the blast. Specifications for finished TNT state maximum impurity levels of no more than 0.5 percent for 2,4 dinitrotoluene (DNT) and trinitrobenzene and 0.3 percent or less for most other impurities [1]. Actual values are of course much less [2]. While the high level of purity of TNT makes good sense from an applications point of view, it makes the search for ways to distinguish between different TNT samples quite difficult.

A number of different methods for extracting, concentrating, and analyzing the impurities and manufacturing by-products present in TNT have been developed [3,4,5]. Typically, a recrystallization is performed on the TNT, and the waste water, with its higher concentration of impurities is analyzed. In one procedure, the pure TNT crystals

Received for publication 6 January 1992; revised manuscript received 28 February 1992; accepted for publication 28 February 1992.

Presented in part at the 42nd Annual Meeting of the American Academy of Forensic Sciences, Cincinnati, OH, 1990.

This is publication number 91-02 of the Laboratory Division of the Federal Bureau of Investigation. Names of commercial manufacturers are provided for identification only and inclusion does not imply endorsement by the Federal Bureau of Investigation.

¹Research Chemist, Forensic Science Research Unit, FBI Laboratory, FBI Academy, Quantico, VA.

²Special Agent, Materials Analysis Unit, FBI Laboratory, 10th Street and Pennsylvania Avenue, Washington, DC.

are grown slowly on a copper wire which is removed prior to analysis of the supernatant material [3]. In a second procedure the impurities present in a sample are concentrated by placing an excess amount of TNT in a solvent in which it has low solubility. If the impurities present are more soluble in the solution than the bulk TNT they will be selectively concentrated [5].

Once the TNT samples are prepared, various methods can be used to analyze the material. Procedures used include mass spectrometry, gas chromatography, nuclear magnetic resonance, and liquid chromatography [2-9].

While the goal in all these studies has been to examine ways to define the sets of impurities present in TNT, little work has been done in the analysis of collected samples in order to determine points of comparison and analysis. The goal for this project has been to define a reproducible procedure by which selected TNT samples can be analyzed and the analytical data stored for later retrieval. Once this data has been compiled, unknown samples collected at a later date can be analyzed to see if they are similar to previously examined TNT samples.

For the analyses described in this paper, an extract of the samples was performed using a modification of the procedure described by Basch and Kraus [5]. The samples were next analyzed by liquid chromatography. Liquid chromatography was chosen as a method over gas chromatography for two main reasons. Firstly, due to the large amount of TNT present in the sample even after preconcentration of the impurities, it was believed that the large capacity of the packed liquid chromatographic column would allow larger sample injections and thus greater sensitivity for impurities. Secondly, the TNT molecule and its related compounds show good UV absorbance, and the use of diode array detection was expected to enable impurities from one sample to the next to be easily tracked. There is also an advantage to using HPLC due to the possible chance that some of these compounds may be subject to thermal decomposition in the GC, although for the most part, this does not seem to be a significant problem [2].

Experimental Procedure

Materials and Methods

Preparation of Samples—TNT samples obtained from the FBI's explosive reference files were extracted in order to concentrate the impurities present. The following procedure was used: 100 mg of TNT was placed in a small test tube and 2 mL of heptane (Burdick and Jackson) was added. The mixture was then allowed to reflux for 5 min in a Temp Block Module Heater (Lab Line Instruments Inc.) set at 110°C. During this time the TNT was observed to melt and the test tube was occasionally shaken. Next the mixture was allowed to cool to room temperature, and the supernatant liquid was decanted into a second test tube. This solution was then blown dry using a light stream of dry nitrogen, and then diluted with 3 mL of mobile phase.

Liquid Chromatography—The liquid chromatograph used was a Waters 600-MS pump attached to a 990 photodiode array detector and a 710B Wisp auto sampler, (Millipore Corporation, Waters Chromatography Division). The 990 detection system uses an NEC IBM compatible computer for data storage and retrieval, and includes UV spectral library search routines. The mobile phase used was 80/20 heptane/methylene chloride by volume (Burdick and Jackson), and was prepared by mixing 265.1 g of methylene chloride with 547.2 g of heptane. The flow rate used was 2.0 mL/min., and the column was a Supelco LC-SI 15 cm, 3 micron column, (Separation Technologies). Sample detection was carried out using the photodiode array detector with a wavelength range of 224 to 350 nm, and a nominal detection wavelength of 235 nm.

Data Reduction—The data obtained from the liquid chromatograph consisted of chromatograms and associated UV spectra. After obtaining the chromatograms, peaks were identified using the library search routines of the diode array detector, and using their retention times relative to the main TNT peak. These results were then placed in a file for later retrieval and analysis. This data analysis was carried out using pattern recognition software present in the Systat software package (SYSTAT Inc.).

Results and Discussion

The initial goal of this study was to develop a procedure to compare captured samples of TNT in order to determine if certain samples originated from the same source. Such a goal requires that several important protocols be established. First of all, a highly reproducible method is needed in order to assure that samples measured months apart can be related to each other. There must be adequate resolution between the impurities present, many of them such as 2,4 DNT and 2,6 DNT, differing only by the position of attachment of the nitro group. Proper understanding of the modes of decomposition of TNT is also important in order to relate two samples which may be separated in time. The effects of light and long-term storage on the decomposition of TNT should be understood in order to better relate two samples with very similar characteristics.

Method Development

The initial phase of the work involved development of an analysis method for TNT. The extraction procedure developed by Basch and Kraus was selected mainly because it was felt that a hexane extraction would be more controllable and reproducible than the procedure developed by G. Kriegel, which involved recrystallization onto a copper wire [3,5]. The method used a known amount of TNT and heated it for a specified period of time in hexane, then cooling the sample and decanting the supernatant for analysis. For this work, the procedure was altered slightly by using heptane as the solvent, for it was observed that certain samples had a brownish coating on the outside as a result of photodecomposition. The boiling point of heptane was hot enough to melt the samples while that of hexane was not. Thus it was felt that a more homogeneous sampling was done if the extraction was carried out on liquid rather than crystalline TNT. The net effect of the extraction was a 6-fold improvement in the relative concentration of 2,4 DNT to 2,4,6 TNT. The relative concentration of the nonsymmetric TNTs increased as well (Fig. 1).

Tests were done in order to determine reproducibility of the extraction by analyzing 10 different individual extracts of a TNT canister of military explosive. The results showed good reproducibility with a relative standard deviation of 7 percent for the area of the 2,4 DNT peak.

Development of a suitable procedure for analysis of the extracted TNT impurities proved to be a more difficult problem. Initial trials with reverse phase HPLC failed to give adequate separation of the TNT isomers, so a normal phase separation was tried. Previous work by Sanders showed only a few solvent mixtures to be suitable for this analysis [9]. Tests in this laboratory of eluents consisting of heptane and methylene chloride mixtures with a Supelco 3 micron silica column showed that an 80/20 mixture of heptane with methylene chloride was near the optimum polarity for the analysis. This eluent gave good separation but the UV cutoff for methylene chloride was a relatively high 235 nm (Fisher), making identification by UV spectra difficult.

Examination of polarities of other solvent mixtures showed that only two other solvents were likely to produce similar results with better UV cutoffs, tetrahydrofuran, THF, and

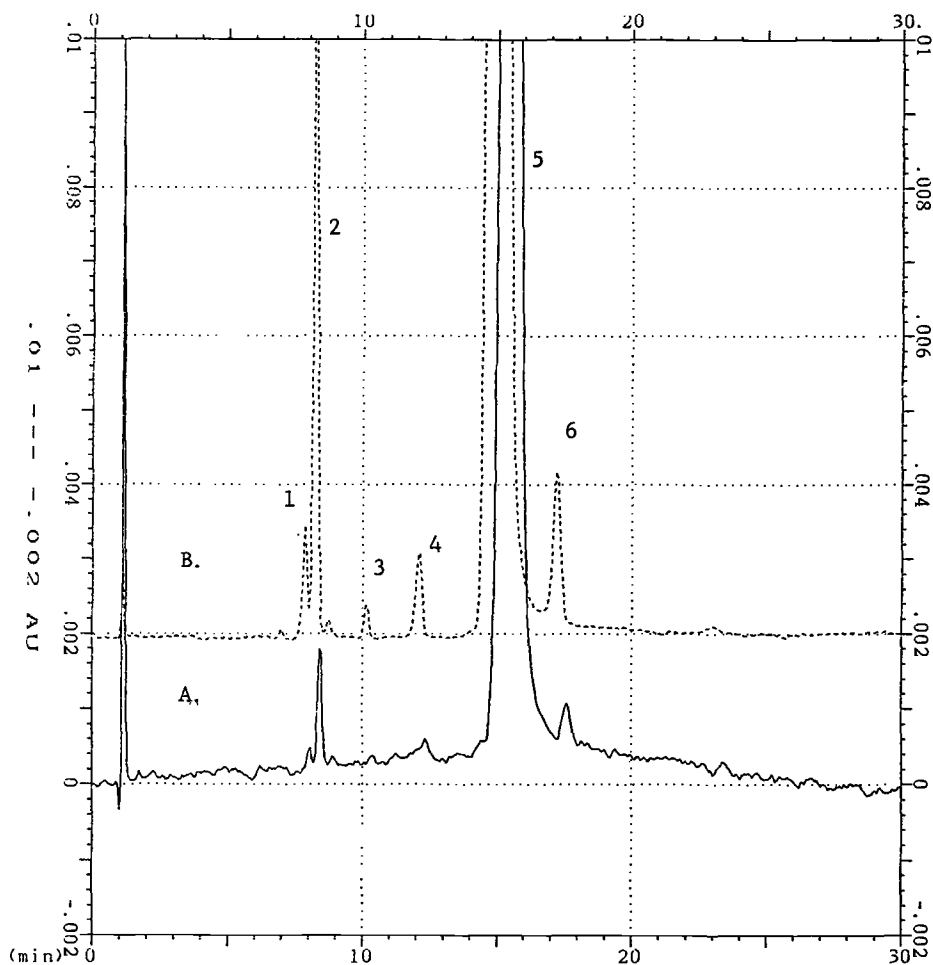


FIG. 1—Chromatogram of TNT; (a) prior to extract, (b) extract. Peak identification: (1) unk4, (2) 2,4 DNT, (3) unk1, (4) 2,3,4 TNT, (5) 2,4,6 TNT, (6) 2,4,5 TNT.

methyl *t*-butyl ether, MTBE [10]. Since MTBE had a strong and noxious odor, analyses using mixtures of THF and heptane were attempted. The result was an optimum mixture of 1.5% THF to 98.5% heptane. Unfortunately, while the solvent absorbance cutoff was better with this compound, the separation was not as good or as ordered as that with the methylene chloride/heptane mixture. In the methylene chloride/heptane mixture the broad TNT peak was well separated from the other peaks, and the toluene isomers eluted by degree of nitration. This was not the case with the THF/heptane eluent, so the method picked for routine analysis used the methylene chloride/heptane eluent.

Another problem observed with this procedure was a gradual loss of resolution and increase in retention time of peaks in a particular column as time went on. In an attempt to increase the stability of the analysis, amino and cyano columns were tried, however, resolution of the isomeric forms of TNT once again suffered. Cleaning the column by flushing it with 50 to 100 mL of pure methylene chloride restored the column, and the problem appears to be due to certain polar materials hanging up on the column.

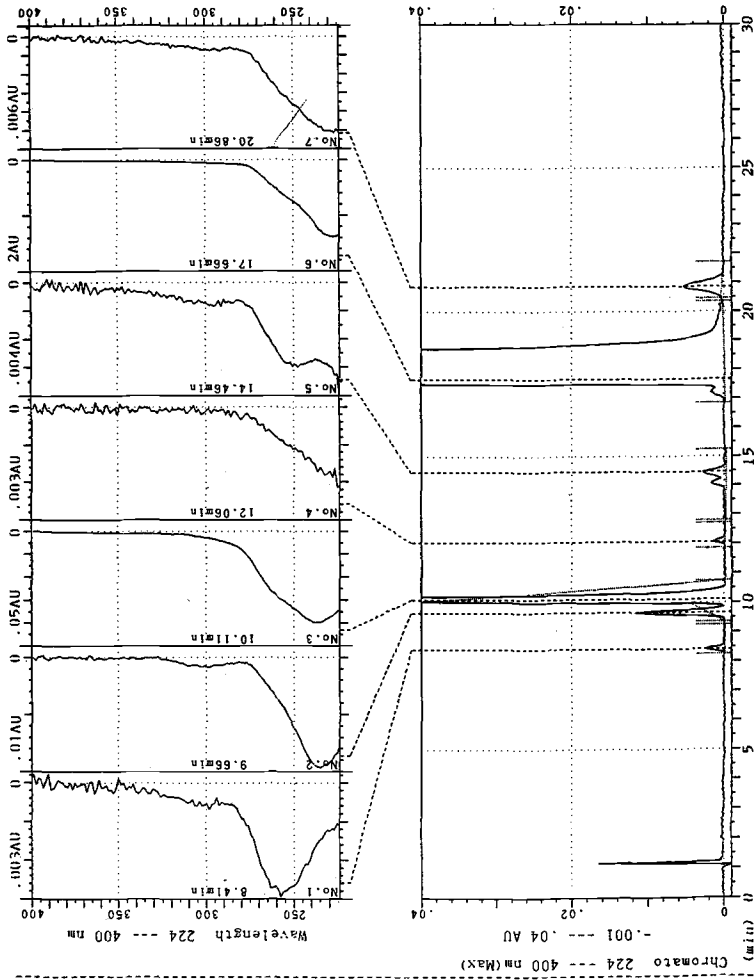


FIG. 2—Chromatogram of a TNT sample showing the UV spectrum of each of the major peaks. Peak identification: (1) unk, (2) unk4, (3) 2,4 DNT, (4) unk2, (5) unk 5, (6) 2,4,6 TNT, (7) 2,4,5 TNT.

Diode Array Detection

The problem of peak identification was made more difficult given the shifting retention times on the silica column. These problems were solved by referencing all peaks to a relative retention time based on 2,4,6 TNT, and using the peak matching and library capabilities of the Waters 990 diode array detector. Spectra were recorded for each chromatographic peak and stored for later comparison with a spectral library. Figure 2 shows a chromatograph with the related spectra obtained from each peak. Typically, a chromatographic peak's UV spectra would be recalled from disk and matched with spectra stored in a library of all known and unknown peaks. Peaks whose retention time and UV spectra did not match any known standard were stored as library unknowns. Thus as each TNT sample was analyzed, a data base was built up of known and unknown compounds. Table 1 gives a list of the compounds identified, their relative retention times, and their UV absorbance maxima. In preparing the library of unknown compounds, it was particularly important to do background subtraction at the shoulders of the peaks to ensure that the UV spectra obtained contained no contribution from the solvent, neighboring peaks, or column bleed. This was especially relevant to peaks present at very low levels.

Sample Stability

A series of tests were carried out to assess the effects of storage and sunlight on the TNT samples and to determine how such effects might alter the quality of the analysis. It should be noted that TNT, a hard crystalline material, is typically cast into blocks or poured into forms which are generally well protected from sunlight; however, samples taken from these larger blocks of material have been observed to turn brownish with time and exposure to light. To see how this discoloration affects the analysis, four 50 mg samples of a military grade TNT were placed on a window sill and exposed to sunlight for various periods of time. As can be seen in Fig. 3, very little effect was seen, even though this particular sample was exposed to sunlight for a period of 4 months.

The effects of storage were further checked by allowing the sample to sit at 50° for an extended period of time. The result was a gradual loss of the more volatile impurities in the sample.

The fact that the conditions of storage of TNT (or of any other chemical composition) can have an effect on its chromatographic profile is not necessarily a liability from a forensic standpoint, particularly if the question is one of determining whether two samples

TABLE 1—Identity, absorbance maxima, and retention times of the impurities found in the TNT samples.

Peak	Retention Time (min)	Relative Retention	UV Maxima (nm)
1. 2,4 DNT	7.66	0.57	238
2. 2,4,5 TNT	15.96	1.18	226.8
3. 2,3,4 TNT	11.81	0.87	229.6
4. unk 2	11.31	0.84	250.6, 297
5. unk 1	8.38	0.62	231
6. unk 4	7.20	0.53	235.2, 297
7. unk 3	21.21	1.57	—
8. unk 5	8.41	0.62	228.2
9. 2,6 DNT	4.26	0.31	239.4
10. unk 7	5.91	0.43	231
11. 2,4,6 TNT	13.51	1.00	228.2

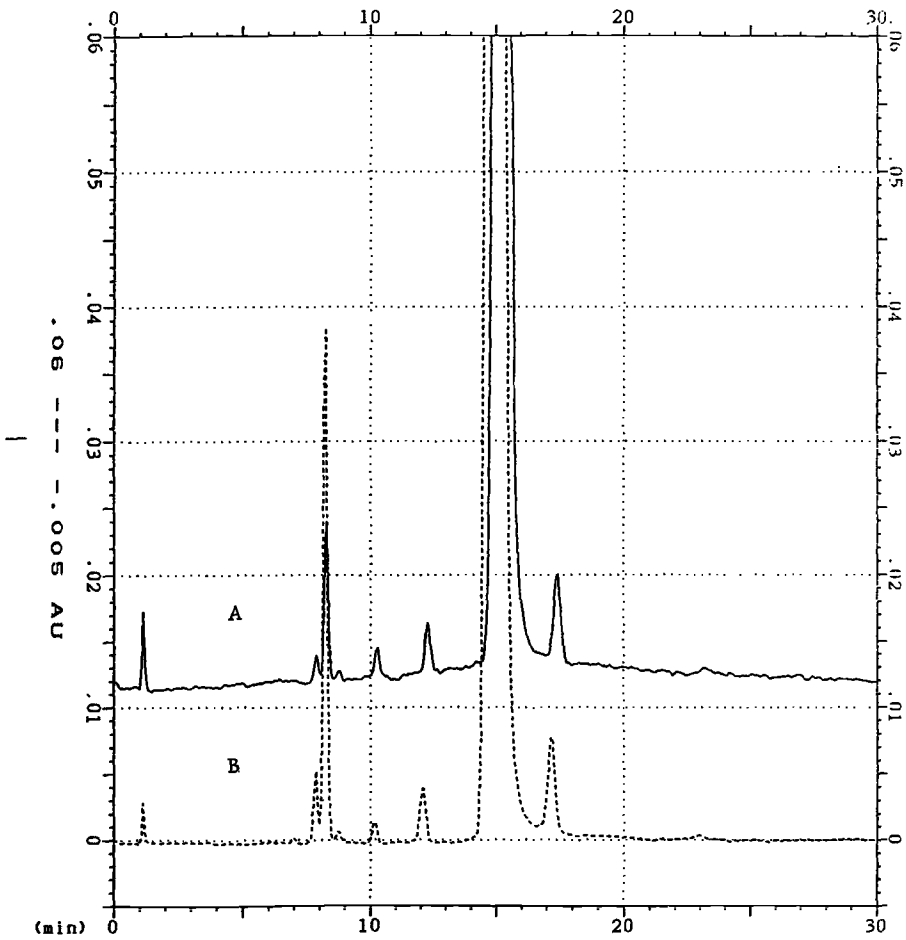


FIG. 3—Chromatogram of TNT; (a) before, and (b) after, exposure to sunlight for 4 months.

are of the same origin. If two bombs are captured containing material from the same terrorist cache, then the TNT inside the bombs will have experienced the same conditions from the time of manufacture up until the point at which the material was separated and placed in the bomb. Thus similarity between the TNT used in the two explosive devices can be used as an indication of common origin. Furthermore, since a variety of differing manufacturing processes are used to manufacture this material, the presence of certain impurities may help indicate origin as well [11].

Chemometrics

Eighteen individual samples of TNT were collected from the FBI's explosive reference files. Figure 4 shows an example of 3 such samples. The figure reveals that the differences in the samples result from two main phenomena, peak ratios and the presence or absence of certain impurities. The resultant data, were placed in a file accessible to SYSTAT, a statistical software package capable of doing cluster analysis. In order to do the analysis, the raw data were first normalized by assigning the largest peak other than TNT the value of one, and dividing the area of the other peaks by that peak's area. This was done

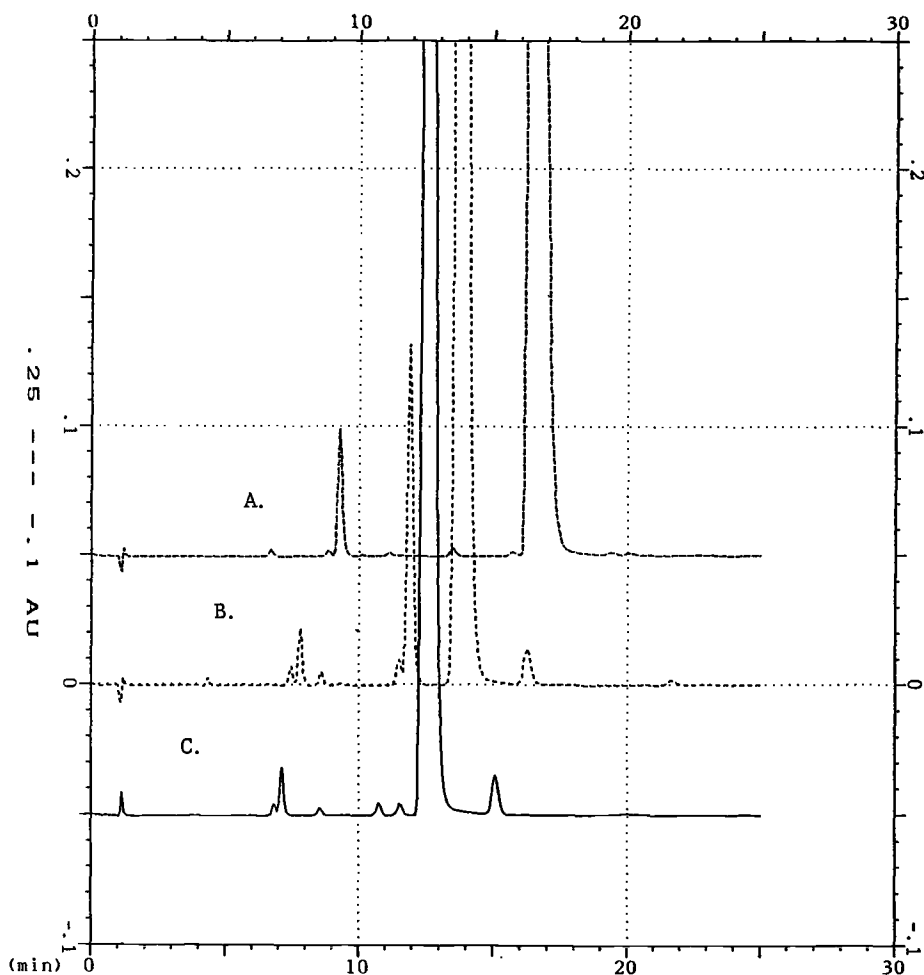


FIG. 4—Three different samples of TNT illustrating the variations present in the chromatographic profiles.

to eliminate any bias resulting from differences in sample weights prior to the heptane extraction. The data were also normalized or z-scored by compound detected. The sample with the largest percentage of a particular compound was assigned a 1, and all other samples were given fractional amounts with respect to that particular compound. This normalization was done to eliminate bias introduced as a result of peak area. Because the goal of cluster analysis is to sort samples according to differences between the samples, it is important to do these normalizations to ensure that small percent differences between large peaks do not overwhelm large differences between small peaks. Figure 5 shows the results of the cluster analysis. Individual samples are identified by letters. As can be seen, the closest matches are those between the replicate analyses.

Conclusions

The use of diode array analysis of these TNT samples in combination with retention time permits unique peak assignments of each of the impurities present in the chromato-

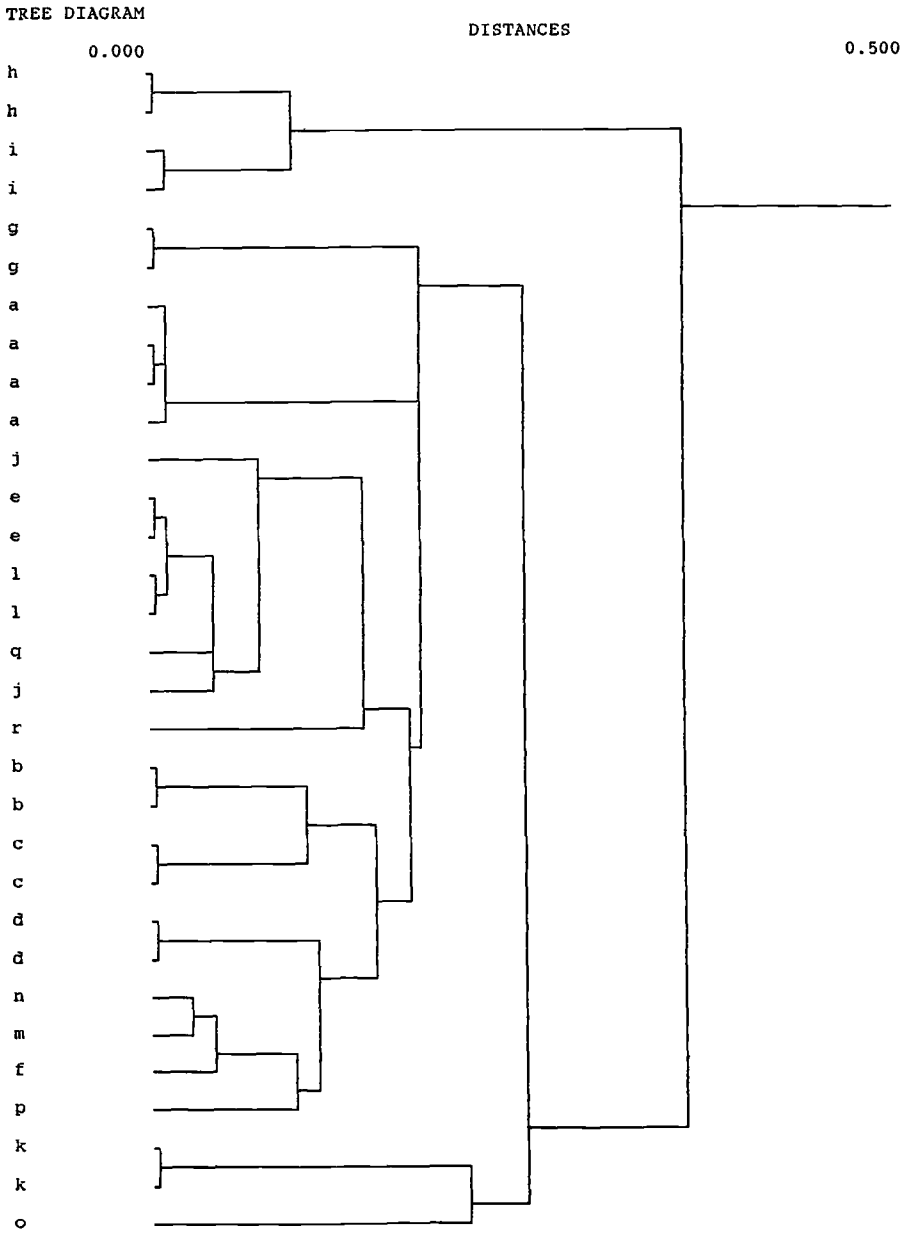


FIG. 5—Illustration of the results of a cluster analysis on TNTs from the FBI's explosive reference files. Individual samples are identified by letter.

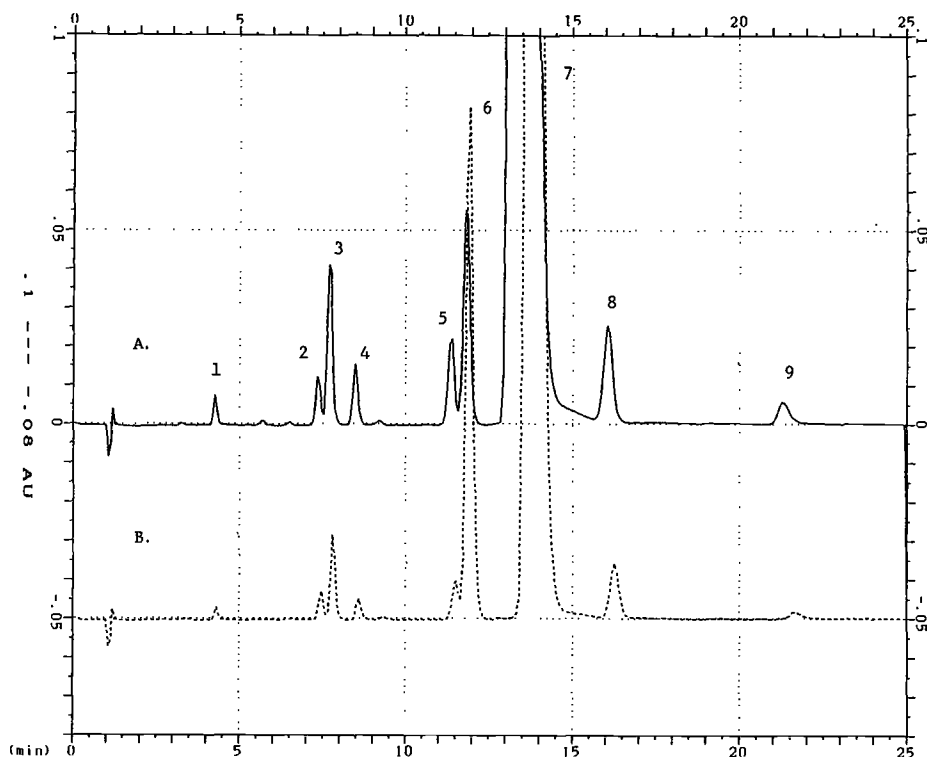


FIG. 6—Comparison of (a) a known and (b) a questioned sample of TNT. Peak identification: (1) 2,6 TNT, (2) unk4, (3) 2,4 DNT, (4) unk1, (5) unk2, (6) 2,3,4 TNT, (7) 2,4,6 TNT, (8) 2,4,5 TNT, (9) unk3.

grams. The ability to do this type of analysis is critical since many of these components elute in the same general time span, and since the absolute retention times varied with column age. Examination of the cluster analysis information reveals that many of these samples were very similar to each other. Upon closer examination of the chromatograms themselves, it is clear that discrimination between the samples can depend upon the presence or absence of just a few minor peaks. Further analysis of the clustering information will await the receipt of samples of known origin. Such samples will help to establish the link between manufacture and impurities. This study provides the ground work for such an analysis.

One particular example of the utility of this method is in the comparisons between samples. Figure 6 shows an example of two TNTs which were recovered at different times from the same suspect terrorist group. The question raised was whether these two samples were chemically related? Visual analysis of the data reveals that although the number and types of impurities are the same, sample b contains a significantly larger amount of the 2,3,4 TNT isomer. Cluster analysis of the data, Fig. 5, places the two samples, d and f, in the same generic group. Thus, while the two TNT samples cannot be said to be identical, the fact that their profiles contain the same types of impurities provides a possible relationship between them.

References

- [1] TM 9-1300-214, US Army, pp. 8.72–9.91.
- [2] Gehring, D. G. and Shirk, J. E., "Separation and Determination of Trinitrotoluene Isomers by Gas Chromatography," *Analytical Chemistry*, Vol. 39, No. 11, Sept. 1967, pp. 1315–1318.
- [3] Kriegel, G., "Qualitative and Quantitative Identification of Powder and Explosive Components Using High Performance Liquid Chromatography," Report R 111/85, Institut Franco-Allemand de Recherches, Saint-Louis, France, 27 June 1985.
- [4] Characterization and Identification of TNT, Report 120-83, Bundesinstitut für Chemisch-Technische Untersuchungen Beim Bundesamt für Wehrtechnik und Beschaffung, Germany, 27 April 1983.
- [5] Basch, A. and Kraus, S., "Analysis and Characterization of Military-Grade Trinitrotoluene by Gas Chromatography," *Journal of Forensic Sciences*, Vol. 24, No. 4, 1979, pp. 870–874.
- [6] Nissenbaum, A., "The Distribution of Natural Stable Isotopes of Carbon as a Possible Tool for the Differentiation of Samples of TNT," *Journal of Forensic Sciences*, Vol. 20, No. 3, July 1975, pp. 455–459.
- [7] Gehring, D. G., "Identification of Impurities in Trinitrotoluene by Nuclear Magnetic Resonance Spectrometry," *Analytical Chemistry*, Vol. 42, No. 8, July 1970, pp. 898–902.
- [8] Kelso, A. G., "The Importance of Sample Environment in the Gas Liquid Chromatographic Analysis of Trinitrotoluene," Technical Note 375, Materials Research Laboratories, Ascot Vale, Victoria 3032, Australia.
- [9] Sanders, P. J., "Separation of Nitration By-Products in Commercial Grade Trinitrotoluene by High Performance Liquid Chromatography," Report No. MLR-TN-464, Materials Research Laboratories, Ascot Vale, Victoria, Australia, 1982.
- [10] *HPLC Solvent Reference Manual*, J. T. Baker Chemical Co., Phillipsburg, NJ, 1985.
- [11] Urbanski, T., "Chemistry and Technology of Explosives," Vol. 1, Pergamon Press, New York, 1964, pp. 345–394.

Address requests for reprints or additional information to
Bruce R. McCord, Ph.D.
Forensic Science Research Unit
FBI Laboratory
FBI Academy
Quantico, VA 22135