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Analysis of Explosives by Liquid Chromatography–Negative Ion Chemical Ionization Mass Spectrometry

REFERENCE: Parker, C. E., Voyksner, R. D., Tondeur, Y., Henion, J. D., Harvan, D. J., Hass, J. R., and Yinon, J., "Analysis of Explosives by Liquid Chromatography–Negative Ion Chemical Ionization Mass Spectrometry," *Journal of Forensic Sciences*, JFSCA, Vol. 27, No. 3, July 1982, pp. 495–505.

ABSTRACT: On-line high performance liquid chromatography (HPLC)–negative ion chemical ionization (NCI) mass spectrometry has been used for the analysis of explosives mixtures. The liquid chromatography–mass spectrometry (LC/MS) interface used was a commercial direct liquid introduction interface allowing about 1% of the solvent/sample effluent into the ion source. The HPLC mobile phases were acetonitrile/water (50:50) and methanol/water (50:50), which served also as NCI reagent gases. Standard mixtures containing 2,4,6-trinitrotoluene (TNT), cyclonite (RDX), tetryl, and pentaerythritol tetranitrate (PETN), and a military explosive, tetrytol, have been analyzed by the LC/MS system. The minimum detectable amount of TNT was 100 ng injected on column, or approximately 1 ng to the ion source.

KEYWORDS: criminalistics, chemical analysis, explosives, high performance liquid chromatography, negative ion chemical ionization mass spectrometry

The analysis of explosives mixtures has become of major importance in several analytical fields. In the forensic science laboratory, postexplosion analysis of residues is extremely relevant in the criminalistic investigation of a bombing, since it can provide information as to the type of explosive used, its origin, and its manufacture [1]. This analysis is very difficult because only trace amounts of the original material are left on the bombing site. These minute quantities are often highly contaminated with nonexplosive compounds interfering with the analysis.

In the environmental field, the analysis of explosives in water has become of major interest in recent years. One of the main pollution problems in the manufacture of ammunition is the discharge of waste waters produced in the manufacture of explosives into nearby rivers or streams [2]. Other environmental problems are the contamination from explosives in

Presented at the 29th Annual Conference on Mass Spectrometry and Allied Topics, sponsored by the American Society for Mass Spectrometry and held in Minneapolis, MN, during June 1981. Received for publication 10 Dec. 1981; accepted for publication 4 Jan. 1982.

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seawater as a result of obsolete munitions dumps and contamination of underground water resulting from the burial of obsolete ammunition and explosives in the soil. The analysis of trace levels of explosives in polluted water is complicated by the interference of a variety of other organic contaminants present in the water. These two applications have created the need for a specific and sensitive analytical method.

A variety of methods and techniques have been used for the analysis of explosives [1] with various degrees of success. Since the mass spectrometer is a very sensitive and specific device for the analysis and identification of trace quantities of material, it has become a widely used tool for the analysis of explosives [3,4]. To detect explosives in complex mixtures such as debris or polluted water, a separation procedure has to precede the mass spectrometric analysis. Since most explosives are thermally labile compounds, combined gas chromatography/mass spectrometry (GC/MS) cannot be used, as medium or high GC column temperatures are needed.

High-pressure liquid chromatography (HPLC) is a good separation technique for both volatile and nonvolatile compounds and is usually performed with the column at room temperature. It is therefore a suitable method for separation of thermally labile compounds in complex mixtures. HPLC has been used for the analysis of explosives [1,5] in complex mixtures, ultraviolet (UV) absorbance being the detection mode. The combination of the chromatographic separation obtained by HPLC with the highly sensitive and specific detection of mass spectrometry would constitute a powerful technique for the analysis of explosives.

Several on-line liquid chromatograph/mass spectrometer (LC/MS) systems have been described [6-10] and successfully used for a variety of applications [11,12]. An off-line combination of HPLC and mass spectrometry for the analysis of explosives has been reported [13]. Explosives mixtures were separated by HPLC and fractions were collected and then introduced by solid probe into the mass spectrometer. This method is tedious and requires a human interface to collect the HPLC fractions, evaporate the solution to dryness, redissolve it in another solvent, and introduce the new solution into the probe cups. These steps are not amenable to continuous monitoring of explosives in environmental media.

In this paper we describe results obtained from interfacing an HPLC with a mass spectrometer and using the combined LC/MS system for the analysis of explosives mixtures [14].

Experimental Method

Equipment

The mass spectrometer used in this research was a Finnigan 3300 chemical ionization (CI) mass spectrometer previously modified for negative ion CI operation [15] connected to a Finnigan/Inco 2300 data system. The HPLC consisted of two Waters 6000A pumps (Waters Associates, Milford, MA), a Waters 660 Solvent Programmer, and a Perkin-Elmer LC-55 Variable Wavelength UV detector (Perkin-Elmer, Norwalk, CT).

The columns were RP-18 and RP-8 reversed-phase columns, 10- μ m particle size, 4.6 mm by 10 cm length (Brownlee Labs, Inc., Santa Clara, CA). Mobile phases were acetonitrile/water (50:50) and methanol/water (50:50). Eluant flow rate was 1 mL/min and the UV detector was set at 254 nm.

The LC/MS interface was an unmodified direct liquid insertion probe LC/MS interface [15] (Hewlett-Packard, Palo Alto, CA), which is a variable split-type interface. The probe inlet system of the mass spectrometer (6.3-mm [$1/4$ -in.] inside diameter) was replaced by a 12.7-mm ($1/2$ -in.) inside diameter direct probe inlet system. A "desolvation chamber" [16] was threaded into the direct probe inlet adaptor plate of the Finnigan ion source. The LC/MS interface probe slid into the desolvation chamber, maintaining source tightness for CI. The droplets of the jet were vaporized in the desolvation chamber, and the eluant gas mixture then entered the CI source and was subsequently ionized. No modifications of the pumping system were necessary.

Samples and Solvents

Pure explosives standards (2,4,6-trinitrotoluene [TNT], cyclonite [RDX], tetryl, and pentaerythritol tetranitrate [PETN]) were supplied by the Israel Police Headquarters in Jerusalem and the military explosive tetrytol by the North Carolina State Bureau of Investigation in Raleigh.

High resolution mass spectrometry was done using a VG Micromass ZAB-2F mass spectrometer operated at 10 000 resolving power.

Solvents used were HPLC grade acetonitrile and methanol (Fisher Scientific Co., Fairlawn, NJ). These were filtered through a 0.5- μm filter (Millipore Corp., Bedford, MA). The water used for the mobile phase solution was HPLC grade (J. T. Baker Chemical Co., Phillipsburg, NJ) and was filtered through a 0.45- μm filter (Millipore Corp., Bedford, MA).

Procedure

Negative ion chemical ionization (NCI) was found to be more sensitive than positive ion CI mass spectrometry for the investigated compounds and was therefore chosen as the preferred ionization mode. The LC mobile phase functions in the ion source as an electron energy moderator, and the mode of ionization is probably electron capture and dissociative electron capture.

A TNT-LC mobile phase solution, continuously flowing through the LC and into the mass spectrometer, was used to tune the system for maximum sensitivity. The flow rate in the LC was 1 mL/min. Since the interface probe will continuously split 1% of the eluate from the HPLC column through a 5- μm orifice into the ion source [15], the flow rate through the mass spectrometer will be 10 $\mu\text{L}/\text{min}$. The tuning also included the adjustment of the jet length of the interface probe and adjustment of position of the interface probe with respect to the ion source. Injected volume into the HPLC was 1 to 10 μL .

Results and Discussion

Three explosives mixtures were examined in this study: a standard mixture of 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX) and 2,4,6-*N*-tetranitro-*N*-methylaniline (tetryl); a standard mixture of TNT, RDX, and PETN; and a military explosive, tetrytol, containing tetryl and TNT as explosives ingredients. The development of the methodology for each mixture was based on optimal performance of the combined LC/MS technique and trying to keep the HPLC parameters constant when analyzing various mixtures.

RDX and Tetryl

An RP-18 reversed-phase column was used with acetonitrile/water (50:50) as mobile phase. A 10- μL solution of the explosives mixture in acetone at a concentration of 100 ng/ μL was injected into the column. Ion source temperature was 185°C. For better vaporization of the sample/solvent and lower water condensation on the cooler parts of the source, a higher temperature is desired. However, for less thermal fragmentation of the sample lower temperatures should be used. For each sample the best compromise must be determined.

Figure 1 shows the LC chromatogram obtained from the UV detector and the total ion current (TIC) mass spectrometer trace. The mass spectrometer was scanned from mass to charge ratio m/z 100 to 400 at 4 s per scan; therefore the TIC trace does not include the ions of the mobile phase nor sample ions in the mass range below m/z 100. Thus, the acetone peak appears on the UV trace but does not appear on the TIC trace.

Figure 2 shows the LC-negative ion CI mass spectra of the two compounds. The NCI mass spectrum of RDX (molecular weight MW 222) contains two abundant ions: m/z 129, $[\text{M}-\text{H}(\text{NO}_2)_2]^-$, and m/z 102, $[\text{M}-\text{CH}_2\text{N}(\text{NO}_2)_2]^-$. These two ions are part of the reported

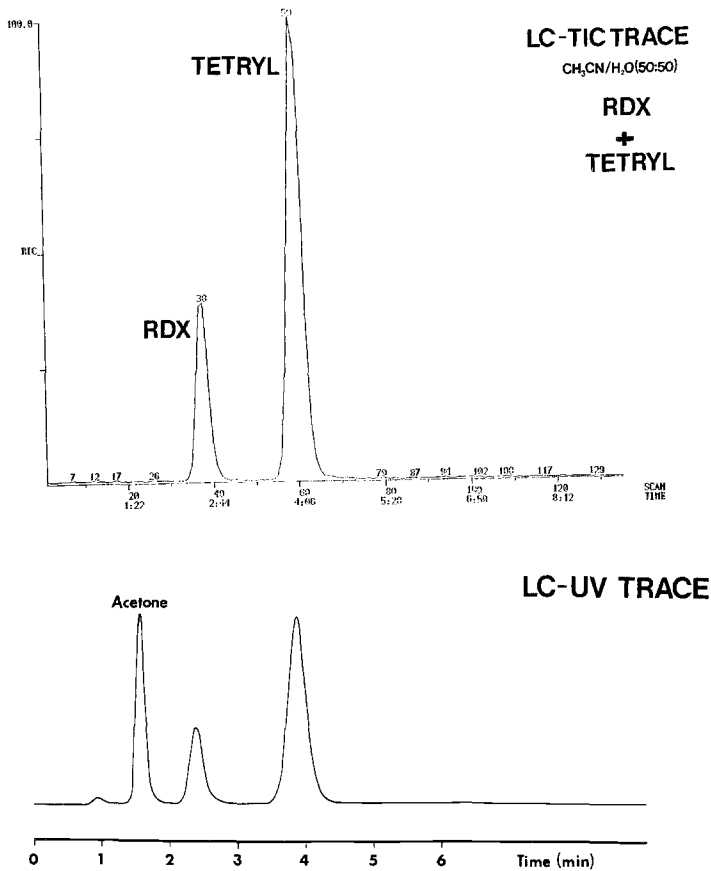


FIG. 1—TIC and UV traces of LC chromatogram of RDX plus tetryl.

NCI mass spectrum of RDX with isobutane as moderating reagent [17], which includes also higher mass ions. As there was no published NCI mass spectrum of tetryl (MW 287), we recorded a high-resolution NCI mass spectrum of tetryl using a VG Micromass ZAB-2F mass spectrometer with methane as moderating gas. Although the moderating gases are different, negative ions formed in the LC/MS and high-resolution mass spectrometers and having the same nominal mass are assumed to have similar chemical structure. In the methane negative ion chemical ionization spectra the ion at m/z 242 was due primarily to the ^{13}C contribution from the ion at 241 (241.0206 = measured; 241.0208 = calculated for $\text{C}_7\text{H}_5\text{N}_4\text{O}_6$; 242.0245 = measured; 242.0242 = calculated for ^{13}C $\text{C}_6\text{H}_5\text{N}_4\text{O}_6$). The m/z 242 ion in the LC/MS spectra may arise from an ion-molecule reaction caused by the solvent-reagent gas.

The ion at m/z 225 was assigned to loss of NO_3 from the molecular anion (225.0222 = measured; 225.0259 = calculated for $\text{C}_7\text{H}_5\text{N}_4\text{O}_5$). The ion at m/z 213 was a doublet and is assigned to loss of $\text{C}_2\text{H}_4\text{NO}_2$ and loss of CNO_3 (212.9898 = measured; 212.9895 = calculated for $\text{C}_5\text{HN}_4\text{O}_6$ and 213.0213 = measured; 213.0259 = calculated for $\text{C}_6\text{H}_5\text{N}_4\text{O}_5$).

The ion at m/z 207 was measured 207.0159 ($\text{C}_7\text{H}_3\text{N}_4\text{O}_4 = 207.0154$). However, it was observed as part of a multiplet. The relative intensities of these ions varied from run to run. The ions at m/z 194 and 181 and 182 were also multiplets, and their relative intensities changed considerably from run to run.

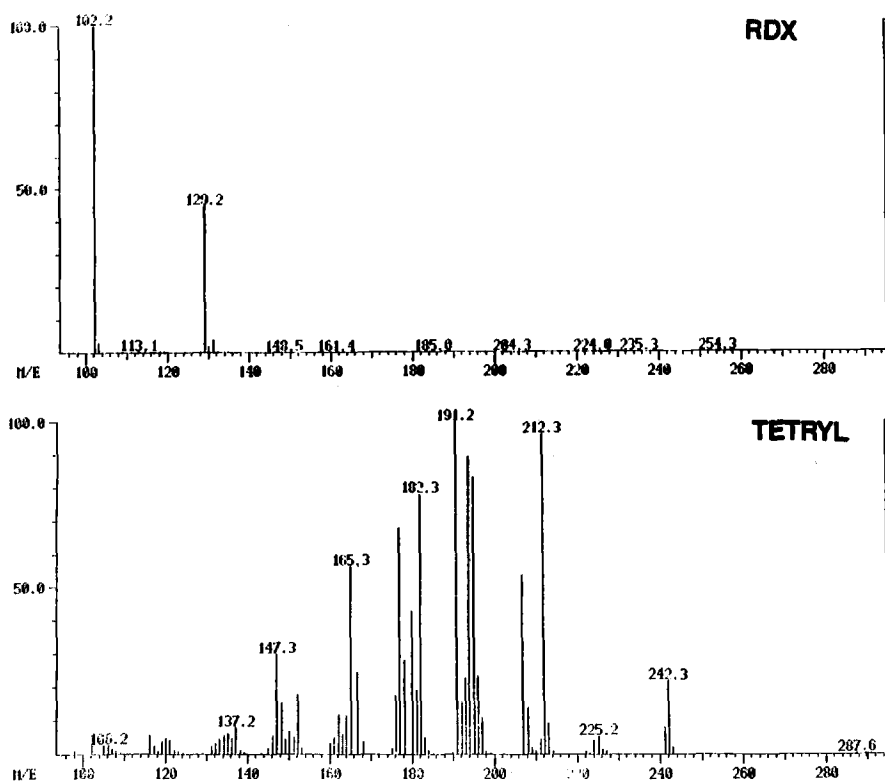


FIG. 2—LC/NCI mass spectra of RDX and tetryl. Source temperature: 185°C.

The thermally unstable nature of this sample introduces some uncertainties into the nature of the fragment ions observed. Source temperature, sample pressure, and the nature of the reagent gas have a large influence on the mass spectrum observed.

Tetrytol

An RP-18 reversed-phase column was used with methanol/water (50:50) as mobile phase. A 10- μ L solution of the explosive in acetonitrile (approximately 1 mg/mL) was injected into the column. The ion source temperature was 130°C. Figure 3 shows the LC chromatogram as obtained on the UV detector and as a TIC trace. The mass spectrometer was scanned from m/z 100 to 400. Figure 4 shows the LC/NCI mass spectra of the two explosive components of tetrytol, tetryl and TNT. The tetryl NCI spectrum is similar to the one in Fig. 2. The NCI mass spectrum of TNT (MW 227) contains the same ions as the NCI spectrum of TNT with isobutane as reagent [17] but at different relative abundances. The most abundant ions are at m/z 227[M]⁻, m/z 210[M-OH]⁻, m/z 197[M-NO]⁻, m/z 180[M-HNO₂]⁻, and m/z 167[M-2NO]⁻.

TNT + RDX + PETN

An RP-8 reversed-phase column was used with methanol/water (50:50) as mobile phase. A 10- μ L solution of the explosives mixture in methanol, at a concentration of 1.2 μ g/mL

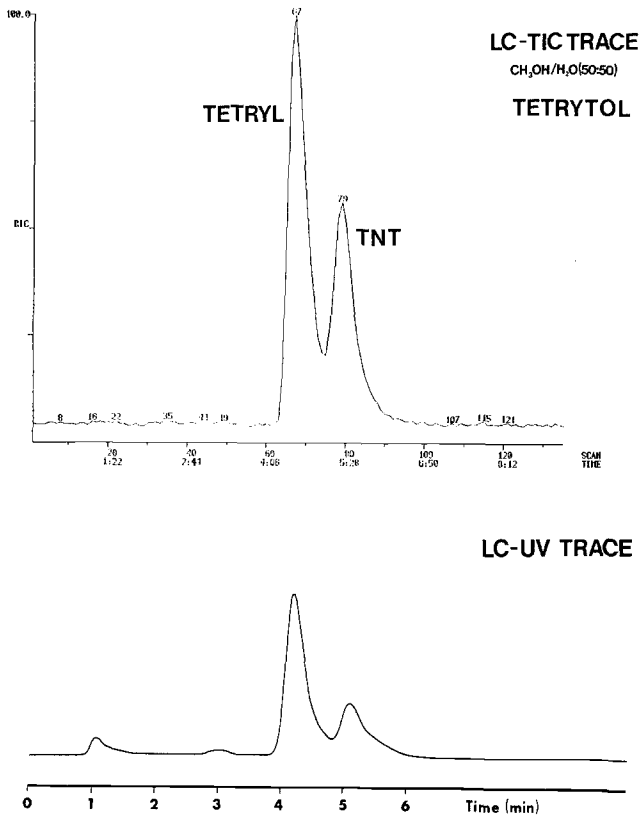


FIG. 3—TIC and UV traces of LC chromatogram of tetrytol.

with proportions of TNT/RDX/PETN as 1:1:10, was injected into the column. The source temperature was 180°C. Figure 5 shows the LC chromatogram as obtained on the UV detector and as a TIC trace. The UV absorbance peak of PETN is very low because of its low absorbance in the region above 215 nm [18]. The mass spectrometer was scanned from m/z 50 to 400 at 4 s per scan.

Figure 6 shows the LC/NCI mass spectra of RDX, TNT, and PETN. The mass spectra of RDX and TNT are similar to the spectra of RDX in Fig. 2 and TNT in Fig. 4. The peaks at m/z 49 and 63 are cluster ions from the solvent and correspond to $[(\text{CH}_3\text{OH} + \text{H}_2\text{O}) - 1]^-$ and $[(\text{CH}_3\text{OH})_2 - 1]^-$. The NCI mass spectrum of PETN (MW 316) contains only one ion at m/z 62, $[\text{ONO}_2]^-$, which is the base ion peak in the NCI mass spectrum of PETN with isobutane as reagent [17]. However, the isobutane-NCI spectrum also contains a series of higher-mass ions.

Sensitivity Measurement

A sensitivity measurement was made using an RP-18 reversed-phase column with acetonitrile/water (50:50) as mobile phase. A 100 ng/ μL solution of acetonitrile was prepared. Amounts of 5, 3, and 1 μL were injected into the column, and NCI mass spectra were recorded. Figure 7 shows the total ion current and the characteristic ions in the TNT mass

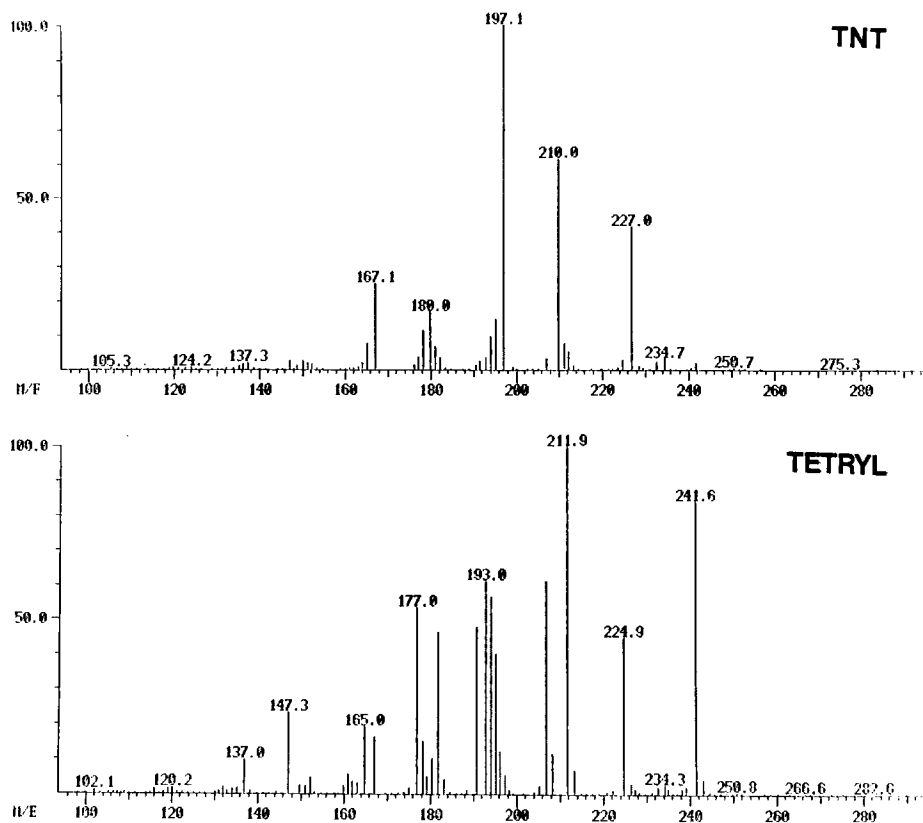


FIG. 4—LC/NCI mass spectra of TNT and tetryl. Source temperature: 130°C.

spectrum after injection of 3 and 1 μL of the solution. Signal-to-noise ratio at an injection of 1 μl of the solution, which is 100 ng of TNT, was better than 3:1. Scan range was 100 to 400 AMU at 4 s per scan. As the split ratio, which is the relative amount of solution jetting out of the interface probe into the mass spectrometer, is approximately 1% [16], the amount of TNT entering the ion source is 1 ng for 1 μL injected into the column.

Conclusions

The data presented demonstrate the simplicity of combining an HPLC with a CI-quadrupole mass spectrometer into an on-line LC/MS, with commercially available instruments and interface.

The usefulness of LC/MS for the separation and analysis of explosives has been demonstrated in a few examples of standard and military explosives mixtures, including nitro compounds, a nitramine, and a nitrate ester. The NCI mass spectra of explosives were similar when methanol/water and acetonitrile/water mobile phases were used. However, they contained fewer high-mass ions than the published NCI mass spectra with isobutane as reagent.

More complex mixtures are now being investigated to substantiate the use of this method for the analysis of explosives for forensic science and environmental analysis applications.

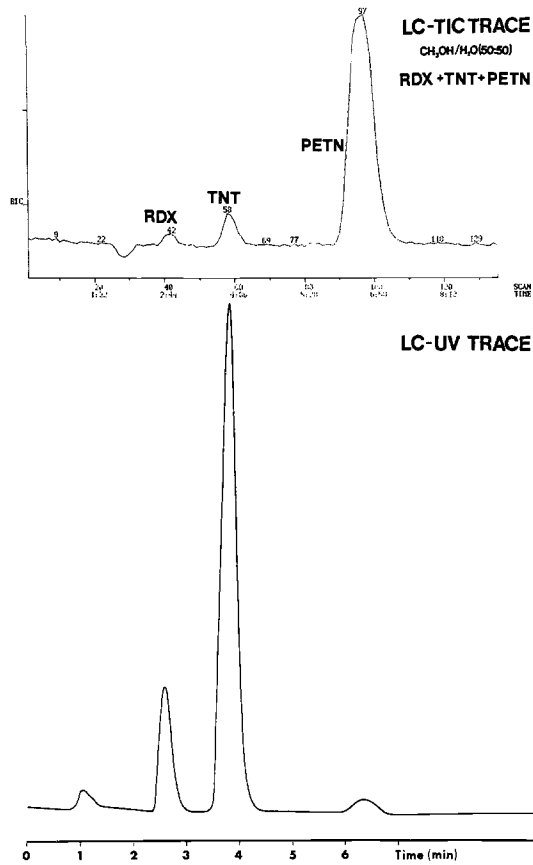


FIG. 5—TIC and UV traces of LC chromatogram of TNT plus RDX plus PETN.

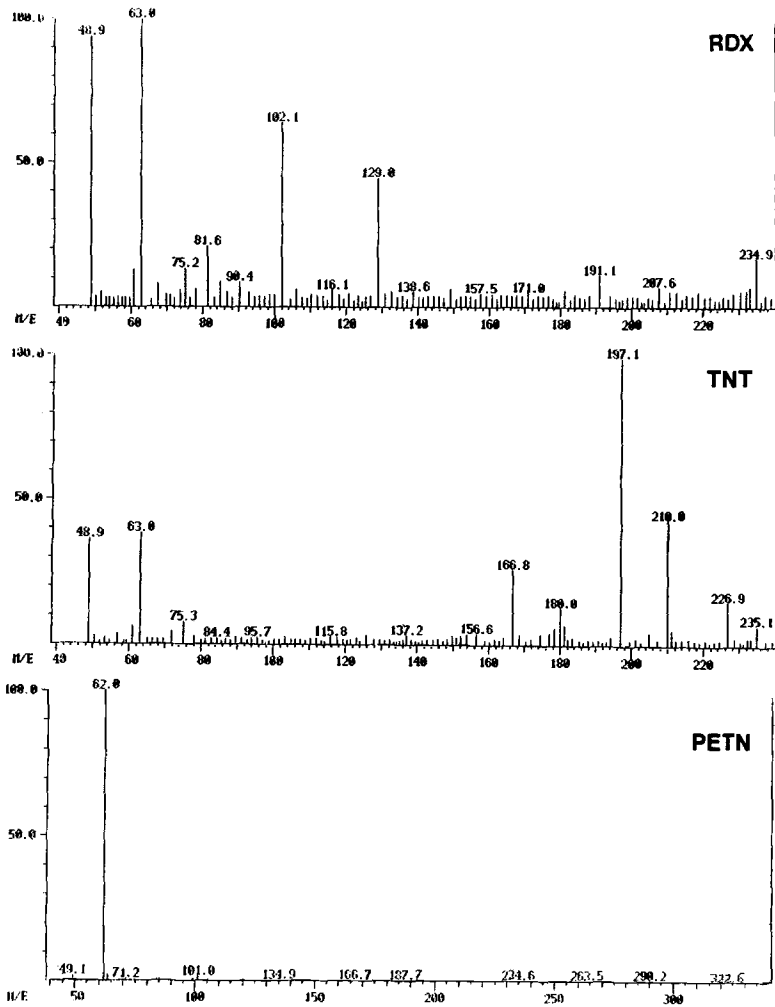


FIG. 6—LC/NCI mass spectra of RDX, TNT, PETN. Source temperature: 180°C.

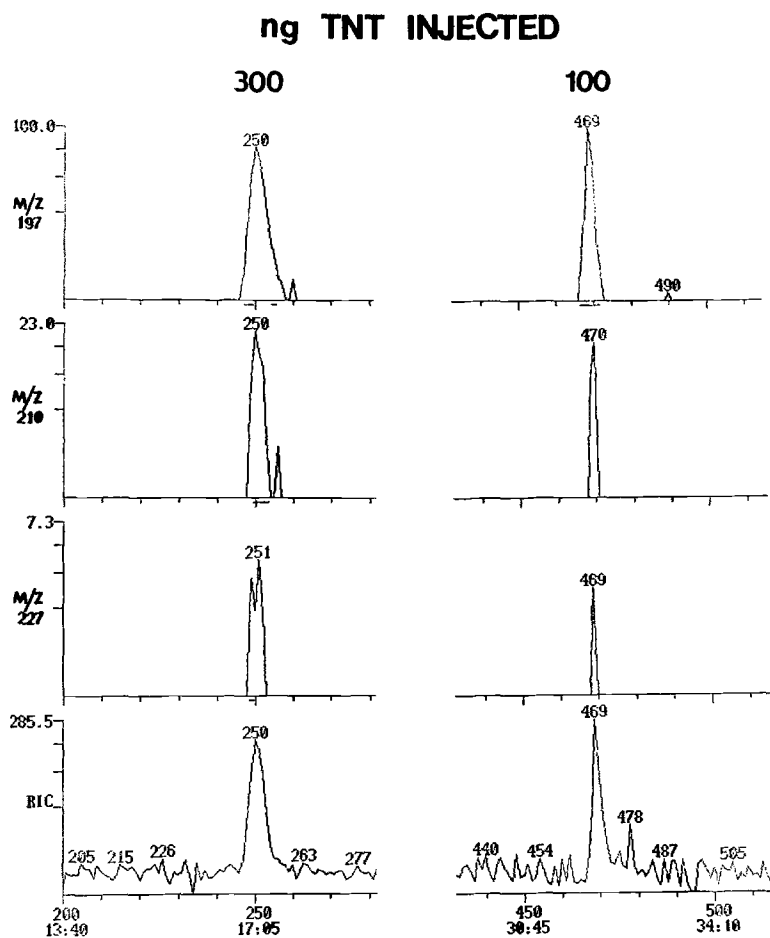


FIG. 7—Sensitivity measurement of TNT using acetonitrile/water (50:50) as mobile phase. Source temperature: 185°C.

Acknowledgments

We thank the Israel Police Headquarters in Jerusalem and the North Carolina State Bureau of Investigations in Raleigh for supplying the explosives. We thank Robert G. Hall for the construction of the probe inlet system and the desolvation chamber.

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