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Characterization and origin identification of 2,4,6-trinitrotoluene through its by-product isomers by liquid chromatography–atmospheric pressure chemical ionization mass spectrometry

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Abstract

The by-products of industrial 2,4,6-trinitrotoluene (TNT), including isomers of trinitrotoluene, dinitrotoluene, trinitrobenzene and dinitrobenzene were investigated using liquid chromatography–mass spectrometry (LC–MS), in order to build a profile for the characterization of TNT samples from various origins. LC–MS with atmospheric pressure chemical ionization, in the negative-ion mode, was found to be the most suitable method for this study. The characterization of TNT by the by-product profile was demonstrated on a variety of TNT samples. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Trinitrotoluenes; Nitrotoluenes; Explosives

1. Introduction

Identification and characterization of explosives is of major significance in both forensic analysis of post-explosion residues and in environmental analysis of explosive-contaminated areas [1]. In addition to the type of explosives, it is important to know their country of origin and preferentially their manufacturer. Each manufacturer will produce the explosives with characteristic differences in the type and amount of by-products, impurities and additives, depending on the purity of the raw materials and solvents used and the type of manufacturing processes, thus resulting in a typical profile of by-products, organic impurities and additives.

The production process of 2,4,6-trinitrotoluene (TNT) [2,3], a widely used military explosive, includes toluene formation from benzene and methanol, followed by three steps of nitration: from toluene to mononitrotoluene (MNT), to dinitrotoluene (DNT) and then to 2,4,6-trinitrotoluene (TNT). Nitration is carried out in the presence of nitric and sulfuric acid, followed by crystallization in alcohol or water and washings with sodium sulfite. The three steps of nitration can be carried out as batch processes or as a continuous process.

A typical group of resulting by-products are the isomers of trinitrotoluene (TNT), dinitrotoluene (DNT), trinitrobenzene (TNB) and dinitrobenzene (DNB), whose profile depends on the manufacturing processes (batch or continuous and concentration of acids) as well as the extent of the purification (crystallization and sodium sulfite washing). Other groups are organic impurities originating from sol-

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vents and reagents and additives such as stabilizers, plasticizers and dyes.

Several attempts were made in the past to develop methods for characterization of TNT by determining the presence of various impurities. These included nuclear magnetic resonance [4], liquid chromatography [5], gas chromatography [6], gas chromatography–mass spectrometry (GC–MS) [7]. Another method suggested to differentiate between TNT samples by finding differences in their $^{13}C/^{12}C$ and/or $^{15}N/^{14}N$ isotope ratios [8,9]. Most of these methods could not demonstrate differences in actual TNT samples or had problems of reproducibility.

The main focus of this study is to develop a method of characterization and origin identification of TNT through identification of the corresponding profile of by-products. As the amount of these by-products is very small a highly sensitive and selective analytical method is needed for their unambiguous identification. Liquid chromatography–mass spectrometry (LC–MS) has already been proved to be a suitable technique for the analysis and identification of explosives [10,11]. LC–MS, with atmospheric pressure chemical ionization (APCI), in the negative-ion mode, was found to provide best sensitivity and selectivity for nitroaromatic compounds [12]. In addition, tandem mass spectrometry with collision-induced dissociation (MS–MS-CID) was used for further identification of some of the nitroaromatic isomers.

2. Experimental

2.1. Chemicals and reagents

Standard 2,4,6-TNT (1000 μg/ml in acetonitrile, purity 99%) was purchased from Supelco (Bellefonte, PA, USA). All other five TNT isomers (2,3,4-, 2,3,5-, 2,3,6-, 2,4,5-, 3,4,5-TNT) were provided by Wehrwissenschaftliches Institut für Werk-, Explosiv- und Betriebsstoffe (WIWEB) (Swisttal-Heimerzheim, Germany). 1,2-DNB (purity 99+%), 1,4-DNB (purity 98%), 3,4-DNT (purity 99%), 2,3-DNT (purity 99%), 2,6-DNT (purity 98%) and 2,4-DNT (purity 97%) were purchased from Aldrich (Milwaukee, WI, USA). The 2,5-DNT was provided by the Analytical Laboratory of the Israeli Police Headquarters, Jerusalem. 1,3-DNB (purity 98%) was purchased from Fluka (Buchs, Switzerland) and 1,3,5-TNB (purity 99%) from Supelco. All standards were used without further purification. HPLC-grade methanol and isopropanol (Fisher Scientific, Fair Lawn, NJ, USA) as well as Milli-Q purified water were used throughout the experimental procedures.

Stock solution of each one of the standards was made in 100% methanol. Standard mixtures were prepared by diluting the stock solution to a final concentration of 1 μg/ml (for each standard) in methanol–water (50:50).

2.2. Samples

The TNT samples were provided by the Analytical Laboratory of the Israeli Police Headquarters, Jerusalem, Sandia National Laboratory, Albuquerque, NM, USA, the University of Auburn, Auburn, AL, USA and the Defence Forces Research Institute of Technology, Lakiala, Finland. All samples were first dissolved in methanol, filtered through 0.45-μm membrane filters and then made to final concentrations of 100–500 μg/ml in methanol–water (50:50).

2.3. LC–MS conditions

LC–MS analyses were performed with a Thermo-Finnigan LCQ_{DUO} ion trap mass spectrometer (San Jose, CA, USA), using APCI, in the negative-ion mode. A Finnigan-MAT SpectraSystem HPLC system consisting of a SCM 100 vacuum membrane degasser, a P4000 gradient pump and an AS 3000 autosampler, was coupled to the mass spectrometer. Helium was used as damping and collision gas for the ion trap, while nitrogen served as sheath and auxiliary gas for the ion source.

The discharge current, the vaporizer temperature and the heated capillary temperature were set to 5.0 μA, 420 °C and 180 °C, respectively. For full scan LC–MS–MS-CID analyses, the collision energy was set to 27–34% of 5 V, depending on the respective compound analyzed.

HPLC separations were accomplished with a Restek reversed-phase Allure C_{18} column (150×3.2 mm, 5 μm particle size) (Bellefonte, PA, USA) at a flow-rate of 0.4 ml/min and with sample injection volume of 10 μl. Three mobile phase systems consisting of methanol–isopropanol–water or methanol–water were employed for different groups of standard mixtures. The separation of the six standard TNT isomers was carried out with a linear gradient elution of methanol–isopropanol–water. While the isopropanol percentage was kept constant at 10% throughout the run time, 30% methanol was held for the first 25 min before a linear gradient of methanol from 30 to 85% over 2 min was performed, which was then held for 5 min. This solvent ratio was returned to the initial condition over 2 min and held for additional 3 min. For the standard mixture of three DNB isomers, four DNT isomers (excluding 2,5-DNT) and TNB, the separation was carried out by an isocratic run of methanol–water (57:43). Fig. 1 shows the mass chromatograms of the TNT, DNB, DNT and TNB isomers. When 2,5-DNT was included in the standard mixture of TNB, DNB and DNT, a linear gradient elution with methanol–isopropanol–water was used, while keeping the water percentage constant at 55%. At the beginning of the analysis the solvent ratio of methanol–isopropanol–water (35:15:55) was held for 1 min. Then a change of methanol from 30 to 40% was made over the next 14 min, followed by 2 min of hold time. Subsequently, the solvent ratio was returned to the initial condition over 1 min and held for 2 min.

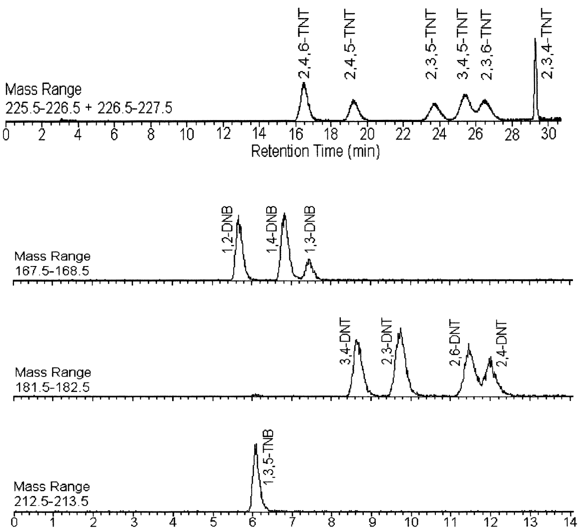

Fig. 1. Mass chromatograms of a standard mixture of six TNT isomers and of mixture of three DNB, four DNT and one TNB isomers in methanol–water (50:50) at a concentration of 1 μg/ml each.

3. Results and discussion

3.1. TNT, DNT, DNB and TNB standard mixtures

The full scan negative-ion APCI mass spectra of the TNT isomers provided limited information on their identification, due to lack of fragment ions, despite the different abundance ratios of *m/z* 226 ($[M-H]^-$) to *m/z* 227 (M^-), observed for different TNT isomers. Therefore, MS–MS-CID analyses were performed. The *m/z* 227 of 2,4,6-TNT produced major fragment ions at *m/z* 210 (–OH, *ortho* effect, loss of 17 mass units) and *m/z* 197 (–NO, loss of 30 mass units). While the MS–MS-CID of *m/z* 227 of 3,4,5- and 2,3,4-TNT produced major daughter ions at *m/z* 197 and *m/z* 181 ($-NO_2$, loss of 46 mass units), the fragmentation of 2,4,5-, 2,3,5- and 2,3,6-TNT was characterized by a single daughter ion at *m/z* 197.

It is notable that only in CID of the symmetric configuration, 2,4,6-TNT, was an *ortho* effect observed, resulting in the ion at *m/z* 210. Although 2,4,5-, 2,3,5-, 2,3,6- and 2,3,4-TNT also possess a nitro group *ortho* to the methyl group, no loss of OH was observed in their CID mass spectrum. In positive ion electron ionization (EI) of TNT isomers [13] the *ortho* effect was observed in all but 3,4,5-TNT, as expected. The differences in results are due to the different ionization modes (negative ion APCI and positive ion EI) and hence different levels of energy involved in the formation of the TNT isomer ions. The MS–MS-CID results of *m/z* 227 of the individual TNT isomers are summarized in Table 1. Results show that HPLC separation, combined with MS and MS–MS data enable the identification of the TNT isomers.

The mass spectra of DNB, DNT and TNB isomers were all characterized by the molecular ions, namely, *m/z* 168 for DNB, *m/z* 182 for DNT and *m/z* 213 for TNB. All three DNB isomers demonstrated the same fragmentation pattern in the MS–MS-CID analysis. Among the DNT isomers, only 2,4-DNT was distinguished from others by showing an OH

Table 1
MS–MS-CID data of TNT isomers

Parent ion		Daughter ions		Tentative identification
m/*z*	ion	*m*/*z*	%	
2,4,6-TNT				
227	M^-	210	100	$[M-OH]^-$
		197	48	$[M-NO]^-$
		181	5	$[M-NO_2]^-$
		167	4	$[M-2NO]^-$
		151	5	$[M-NO_2-NO]^-$
		137	13	$[M-3NO]^-$
2,4,5-TNT				
227	M^-	197	100	$[M-NO]^-$
		181	2	$[M-NO_2]^-$
2,3,5-TNT				
227	M^-	197	100	$[M-NO]^-$
3,4,5-TNT				
227	M^-	197	49	$[M-NO]^-$
		181	100	$[M-NO_2]^-$
2,3,6-TNT				
227	M^-	197	100	$[M-NO]^-$
		181	10	$[M-NO_2]^-$
2,3,4-TNT				
227	M^-	197	60	$[M-NO]^-$
		181	100	$[M-NO]^-$
		151	3	$[M-2NO]^-$

Table 2
MS–MS-CID data of DNT, DNB and TNB isomers

Parent ion		Daughter ions		Tentative identification
m/*z*	ion	*m*/*z*	%	
3,4-DNT				
182	M^-	152	100	$[M-NO]^-$
2,3-DNT				
182	M^-	152	100	$[M-NO]^-$
2,6-DNT				
182	M^-	152	100	$[M-NO]^-$
2,4-DNT				
182	M^-	165	100	$[M\ -OH]^-$
		164	9	$[M-H_2O]^-$
		152	45	$[M-NO]^-$
		137	10	$[M-NO-CH_3]^-$
		135	23	$[M-HNO_2]^-$
2,5-DNT				
182	M^-	152	100	$[M-NO]^-$
1,2-DNB				
168	M^-	138	100	$[M-NO]^-$
1,4-DNB				
168	M^-	138	100	$[M-NO]^-$
1,3-DNB				
168	M^-	138	100	$[M-NO]^-$
1,3,5-TNB				
213	M^-	183	100	$[M-NO]^-$

loss (*ortho* effect) to produce a daughter ion at *m*/*z* 165 in addition to the daughter ion at *m*/*z* 152, which is common to the other DNT isomers. The MS–MS data of the DNB, DNT and TNB isomers are listed in Table 2.

3.2. TNT samples

Based on the above results obtained for the TNT, DNB, DNT and TNB standards, TNT samples from different and similar origins were analyzed in order to test their characteristic profile of by-products. It was found that the gradient elution program used in the separation of TNT isomers was best suitable for the analysis of these TNT samples which were mainly characterized by the combination of 1,3-DNB, 2,4-DNT, 1,3,5-TNB and various TNT isomers. Figs. 2–8 show the mass chromatograms of the analyzed TNT samples from different sources. The identity of chromatographic peaks with a minimal signal-to-noise ratio of 3 was confirmed by either full scan MS or MS–MS-CID.

Fig. 2 shows the mass chromatograms of two Russian TNT samples, which demonstrate the same by-product profile (2,3,4-TNT, 2,4-DNT and 1,3,5-TNB), leading to the conclusion that they originate from same manufacturer. Fig. 3 shows the mass chromatograms of two Hungarian samples demonstrating a similar by-product profile of 2,3,6-TNT, 2,3,4-TNT and 1,3,5-TNB. The mass chromatograms of two TNT samples originating from two landmines of the same type (Fig. 4) show the presence of 2,4,5-, 2,3,6- and 2,3,4-TNT. The absence of 2,4-DNT in one of the samples could be attributed to evaporation of the more volatile 2,4-DNT, if this landmine was buried for a long time in the ground.

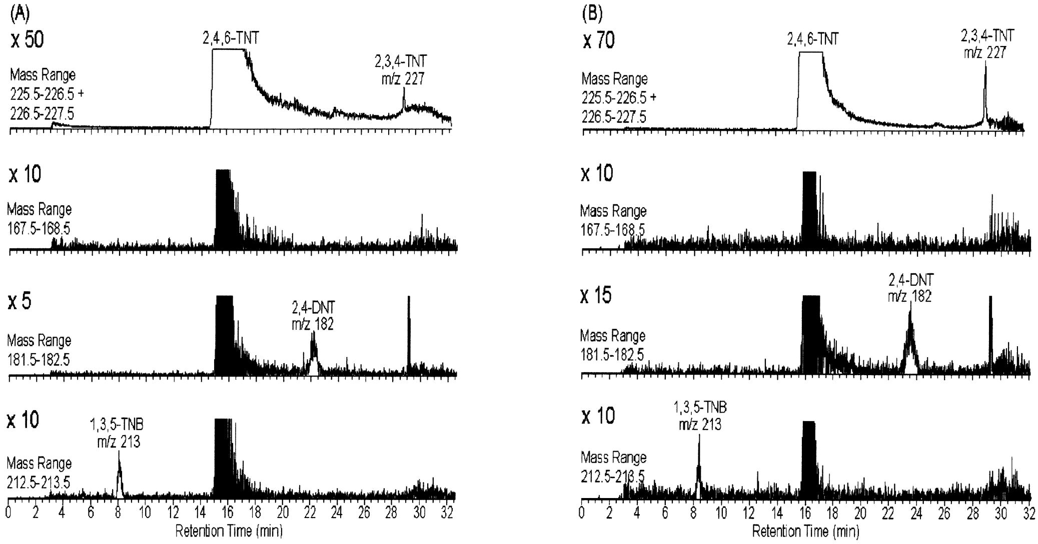

Fig. 2. LC–APCI-MS mass chromatograms of two TNT samples from Russia [(A) 200 μg/ml and (B) 100 μg/ml, in methanol–water (50:50)].

Additional TNT samples analyzed included a sample from China (Fig. 5) with a profile of 1,3-DNB, 2,4-DNT and 1,3,5-TNB, a sample from the UK (Fig. 6) with a profile of 2,3,6- and 2,3,4-TNT, 2,4-DNT and 1,3,5-TNB, a sample from Israel (Fig. 7) with a profile of 2,3,4-TNT and 1,3,5-TNB and a sample from Canada (Fig. 8), which was characterized by 2,3,4- and 3,4,5-TNT, 2,4-DNT and 1,3,5-TNB isomers.

Analysis of a 4-times crystallized TNT sample (Fig. 9) showed no presence of any of the by-product isomers.

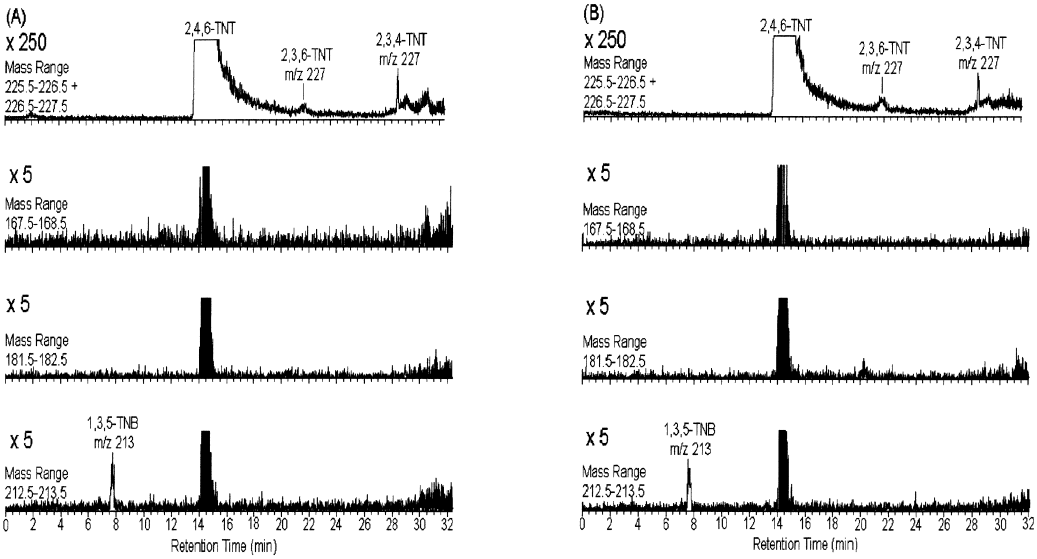

Fig. 3. LC–APCI-MS mass chromatograms of two TNT sample from Hungary [(A) and (B) 200 μg/ml in methanol–water (50:50)].

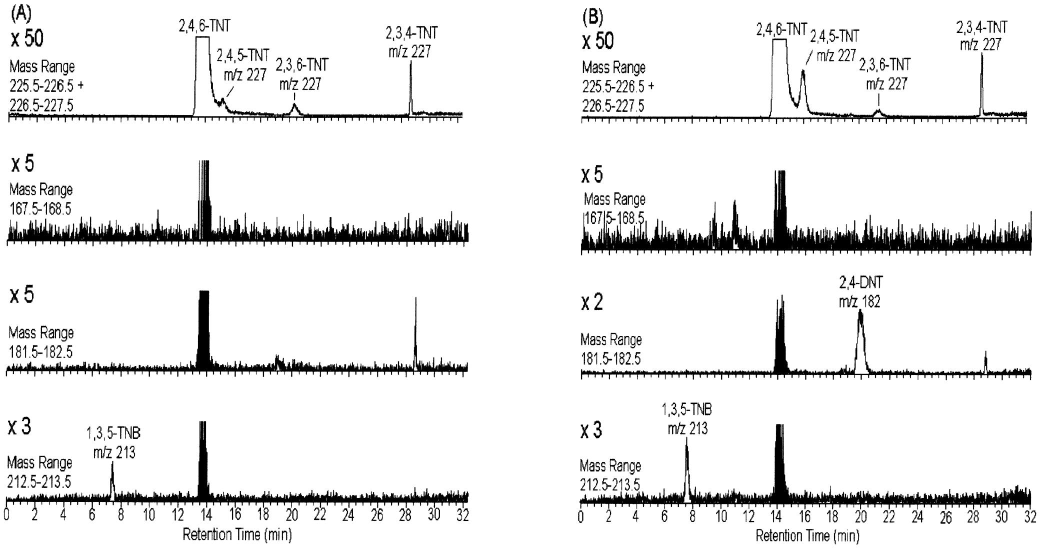

Fig. 4. LC–APCI-MS mass chromatograms of two landmine TNT samples [(A) and (B) 200 μg/ml in methanol–water (50:50)].

4. Conclusion

The by-product profiles obtained demonstrate that LC–APCI-MS in the negative ion mode, with back-up of MS–MS-CID for positive identification, is a promising technique for the characterization and origin identification of TNT samples through their TNT, DNB, DNT and TNB isomer profile. These isomers are by-products originating from the manufacturing process of TNT, and their combined profile is different for samples from different manufacturing plants. The mass chromatograms of the 4-times

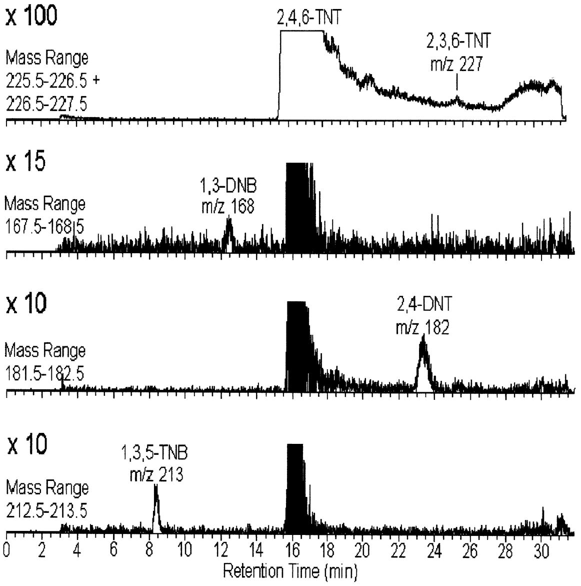

Fig. 5. LC–APCI-MS mass chromatograms of TNT sample from China [200 μg/ml in methanol–water (50:50)].

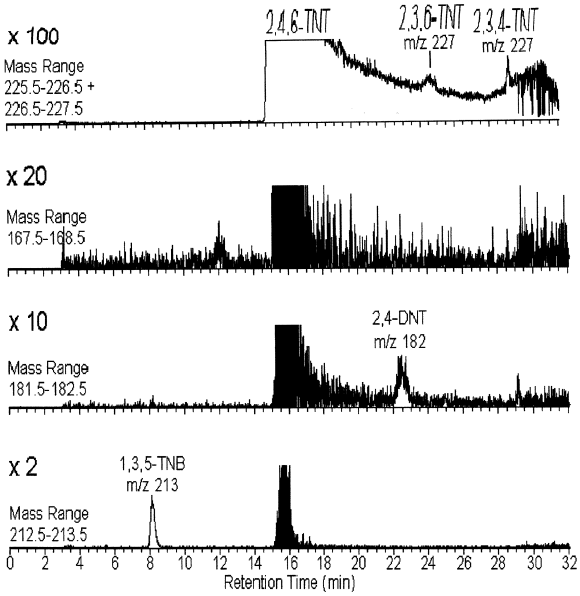

Fig. 6. LC–APCI-MS mass chromatograms of TNT sample from the UK [200 μg/ml in methanol–water (50:50)].

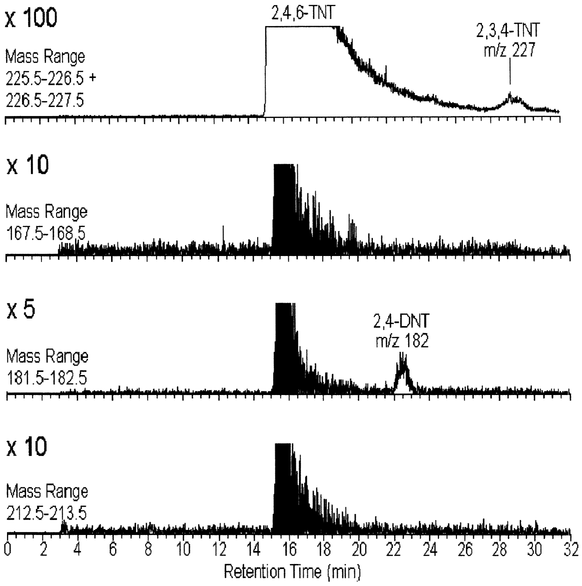

Fig. 7. LC–APCI-MS mass chromatograms of TNT sample from Israel [200 μg/ml in methanol–water (50:50)].

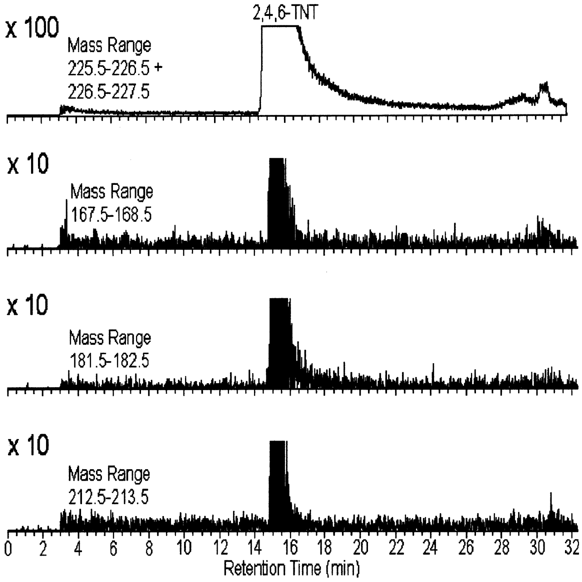

Fig. 9. LC–APCI-MS mass chromatograms of 4-times crystallized TNT sample [100 μg/ml in methanol–water (50:50)].

crystallized TNT sample prove that thorough purification of TNT can eliminate all the by-products. Such purification is not being done in the manufacturing of TNT because of economic reasons. Another possible reason is that the isomer impurities have an effect on the melting point and on the crystallization properties and hence on the casting properties of TNT [14]. Pure 2,4,6-TNT has a tendency to crystallize in a needlelike fashion which could cause voids and cracks in shell and grenade filling.

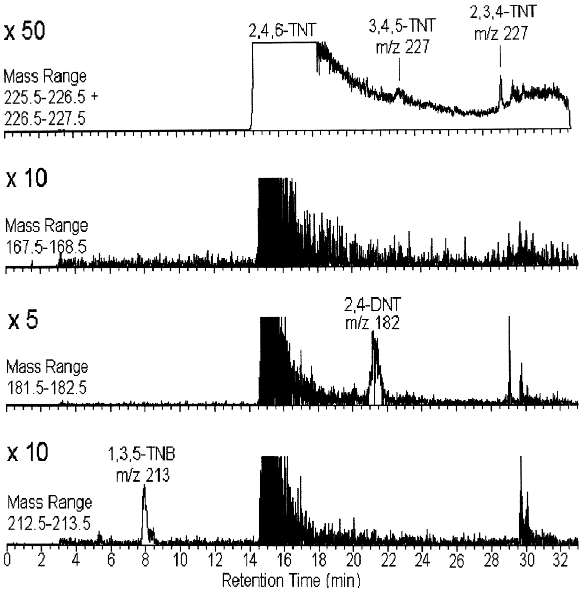

Fig. 8. LC–APCI-MS mass chromatograms of TNT sample from Canada [500 μg/ml in methanol–water (50:50)].

A collection of standard TNT samples from manufacturers is being accumulated in order to become the basis of a database of isomer by-product profiles, which will be used to determine the origin of TNT samples.

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