Anal. Chem. **2003,** *75,* 731−735

Trace Analysis of Peroxide-Based Explosives

Rasmus Schulte-Ladbeck,[†] Peter Kolla,[‡] and Uwe Karst*,[†]

Department of Chemical Analysis and MESA$^+$ Research Institute, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands, and Bundeskriminalamt, KT 16, 65173 Wiesbaden, Germany

The first method for quantitative trace analysis of peroxide-based explosives is described. A reversed-phase high-performance liquid chromatography method with post-column UV irradiation and fluorescence detection for the analysis of triacetone triperoxide (TATP) and hexamethylene triperoxide diamine (HMTD) has been developed. After separation, the analytes are degraded photochemically to hydrogen peroxide, which is subsequently determined on the basis of the peroxidase-catalyzed oxidation of *p*-hydroxyphenylacetic acid to the fluorescent dimer. This two-step reaction scheme in combination with the respective blanks (photochemical reactor switched off) provides for high selectivity. The limits of detection were 2×10^{-6} mol/L for both TATP and HMTD, respectively. The method has been applied to the analysis of real samples.

In the last years, an increasing number of incidents regarding the use of triacetone triperoxide (TATP) and hexamethylene triperoxide diamine (HMTD) were reported. These explosives were used in conjunction with drug crimes,[1] amateur chemist accidents,[2] and terrorism.[3,4] With more descriptions of the simple synthesis being available to the public in various media, the number of the accidents is steadily rising.

TATP is the substance predominantly involved in these cases, as it is easy to synthesize and the starting materials are readily available. Wolffenstein first prepared TATP in the 19th century.[5] He also proposed a structure (see Figure 1a), which could later be confirmed.[6] The dimer of acetone peroxide (see Figure 1b)[7] is frequently observed as a byproduct of the synthesis, especially when sulfuric acid is used as a catalyst. In some cases, a tetramer of acetone peroxide may also be present.[8] As an explosive, TATP is almost as powerful as TNT, but extremely sensitive to impact, friction, and temperature changes, especially if stored as a dry product. TATP is one of the most sensitive explosives known. This is one of the reasons why it is not applied for any commercial or military purposes. Another reason is the tendency of TATP to sublime within a few days.[9] Due to the dangerous properties and the frequent use, analytical methods for the determination of TATP are required to unambiguously identify the explosive at high concentrations in unknown substance mixtures and in traces after explosions.

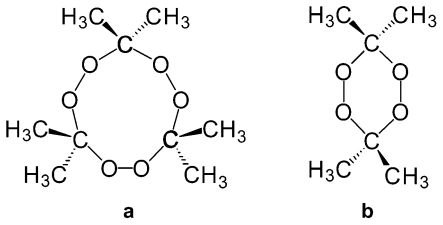

Figure 1. Structures of triacetone triperoxide (a) and the dimer of acetone peroxide (b).

Today, the methods most frequently used for the identification of TATP are IR spectroscopy or chemical ionization mass spectrometry (CI-MS).[1,2,10] There is no significant absorption in the UV spectrum, and TATP is not fluorescent. One method for the qualitative trace analysis of the peroxide by GC/MS was described in ref 11. In this method, a fragment of TATP (m/z 43 $[CH_3 - CO]^+$) is observed after separation using gentle GC conditions including on-column injection and electron impact ionization. However, as m/z 43 is commonly observed for several important fragments, e.g., $[C_2H_5N]^+$, $[CHNO]^+$, and $[C_3H_7]^+$, this method significantly lacks selectivity when applied to complex matrixes. To our knowledge, there are no methods published for quantitative trace analysis of TATP.

HMTD was first synthesized by Legler in 1881.[12] It is not as sensitive to impact as TATP, but nevertheless it is a dangerous explosive. To our knowledge, there is no reported application of HMTD as a commercial or military explosive. The structure of HMTD was first proposed by Bayer and Villiger[13] (see Figure 2a). An alternative structure was described by von Grisewald[14] (Figure 2b). Today, Bayer's proposal is the most acknowledged one.[15] HMTD can be identified by means of either IR spectroscopy or CI-MS.[10,16] As for TATP, there is no significant absorption within

* Corresponding author. E-mail: u.karst@ct.utwente.nl.
[†] University of Twente.
[‡] Bundeskriminalamt.

(1) White, G. M. J. *Forensic. Sci.* **1992**, *37*, 652−656.
(2) Evans, H. K.; Tulleners, F. A. J.; Sanchez, B. L.; Rasmussen, C. A. J. *Forensic. Sci.* **1986**, *31* (3), 1119−1125.
(3) *The Independent*, London 1996; October 8, p 7.
(4) Cooper, R. T. *Los Angeles Times*; 2001; December 29, p A12.
(5) Wolffenstein, R. *Chem. Ber.* **1895**, *28*, 2265−2269.
(6) Groth, P. *Acta Chem. Scand.* **1969**, *23* (4) 1311−1329.
(7) Bayer, A.; Villiger, V. *Chem. Ber.* **1900**, *33*, 858−860.
(8) Jiang, H.; Chu, G.; Gong, H.; Qiao, Q. D. *J. Chem. Res.* **1999**, *4*, 288−289.
(9) Bellamy A. J. J. *Forensic. Sci.* **1999**, *44* (3), 603−608.
(10) Zitrin, S.; Kraus, S.; Glattstein, B. *Proceedings of the International Symposium on the Analysis and Detection of Explosives*; U.S. Government Printing Office: Washington, DC, 1984; pp 137−141.
(11) Arai, H.; Nakmura, J. *4th IAFS Tokyo 1996 Fires and Explosions*; 1996; pp 209−211.
(12) Legler, L. *Chem. Ber.* **1881**, *14*, 602−604.
(13) Bayer, A.; Villiger, V. *Chem. Ber.* **1900**, *33*, 2479.
(14) von Grisewald, C.; Siegens, H. *Chem. Ber.* **1921**, *54*, 490.
(15) Schaefer, W. P.; Fourkas, J. T.; Tiemann, B. G. *J. Am. Chem. Soc.* **1985**, *107*, 2461−263.

10.1021/ac020392n CCC: $25.00
Published on Web 01/15/2003

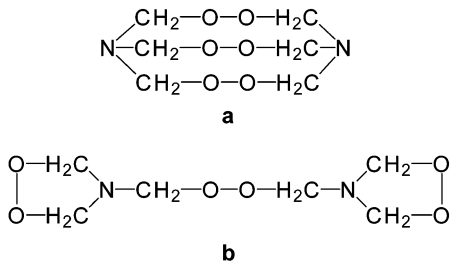

Figure 2. Structures of hexamethylene triperoxide diamine as suggested by Bayer (a) and Grisewald (b).

the UV range, the compound has no fluorescence characteristics, and no sensitive method for quantitative trace analysis of HMTD is currently available.

EXPERIMENTAL SECTION

Safety Note. TATP and its homologues as well as HMTD are extremely dangerous materials, which may lead to severe and spontaneous explosions under impact, friction, and temperature changes. The synthesis of these substances may only be carried out by highly qualified personnel, under the use of appropriate safety measures (reinforced goggles and gloves, splinter-proof vessels, protective shield, etc.) and in small quantities. For this work, the substances were produced according to literature procedures (see below) in quantities not exceeding 100 mg. Working with larger amounts of the substance strongly increases the danger associated with spontaneous explosions.

Reagents. All chemicals were purchased from Aldrich Chemie (Steinheim, Germany), Merck (Darmstadt, Germany), Sigma (Deisenhofen, Germany), and Fluka (Neu-Ulm, Germany) in the highest quality available. Acetonitrile for HPLC was Merck gradient grade. The horseradish peroxidase was purchased from Sigma (Deisenhofen, Germany) (EC 1.11.1.7).

Instrumentation. HPLC-DAD-Fluorescence Instrumentation and Analysis. A liquid chromatographic system consisting of the following components (all from Shimadzu, Duisburg, Germany) was used: two LC-10AS pumps, degasser GT-154, SPD-M10Avp diode array detector, RF-10AXL fluorescence detector, SIL-10A autosampler, software Class LC-10 version 1.6, and CBM-10A controller unit. The injection volume was 10 μL. The column material was Merck LiChroSpher RP18; particle size 5 μm; pore size 300 Å; column dimensions 250 mm × 3 mm. For UV irradiation, a low-pressure mercury lamp with 6-W power was used.

For most isocratic HPLC separations, a solvent mixture consisting of 60% acetonitrile and 40% water at a flow rate of 0.6 mL/min was used. As an exception, the chromatogram shown in Figure 7 was obtained by applying an isocratic flow of 75% acetonitrile and 25% water. For the separation of real samples with a complex matrix, a binary gradient of acetonitrile and water may be employed. For the applications described, a gradient was not necessary for separation.

Fluorescence Spectroscopy. For optimization of excitation and emission wavelengths, an Aminco Bowman Series 2 luminescence spectrometer from Polytec (Waldbronn, Germany) was used.

Synthesis. The synthesis of TATP and HMTD was done as described in the literature.[5,15] For this work, the synthesis was carried out to obtain 100 mg of the explosives in case of quantitative yield of the product. For safety precautions, refer to the Safety Note above. Excess amounts of the explosives can be destroyed according to ref 9.

Experimental Setup. Postcolumn Derivatization. The postcolumn derivatization setup is shown in Figure 3. Immediately after the separation of the analytes, the latter were irradiated with UV light of 254 nm. A 25-m knitted Teflon tubing (inner diameter, 0.3 mm) was wrapped around the UV lamp and was used as a reaction loop. After decomposition of the analytes to hydrogen peroxide, two HPLC pumps were introduced to deliver the derivatization reagents 1 (a solution of 205.0 mg (1.18 mmol) of K_2HPO_4 and 7.6 mg (0.05 mmol) of *p*-hydroxyphenylacetic acid (pHPAA) in 100 mL of water) and 2 (a solution of 8.4 mg of horseradish peroxidase in 100 mL of water). Both reagents were added with a flow rate of 0.2 mL/min, giving an overall flow of 1 mL/min. The following reaction loop was an 8-m knitted Teflon tubing coil (inner diameter, 0.3 mm) which was heated to 40 °C. The excitation wavelength of the fluorescence detector was set to 324 nm and the emission wavelength was 405 nm.

RESULTS AND DISCUSSION

As TATP and HMTD cannot readily be analyzed by gas chromatography because of their thermolability, and HPLC with UV/visible detection is not possible because of the lack of chromophoric groups, alternative strategies for the analysis of the peroxide-based explosives were investigated. In general, liquid chromatographic separations on reversed-phase stationary phases appear to be promising for these compounds. At least for HMTD, it could be expected that protonation of the tertiary nitrogen atoms should lead to pseudomolecular ions which could be detected in liquid chromatography/mass spectrometry (LC/MS). For TATP, a respective prognosis is more difficult. Both common soft ionization techniques in LC/MS, electrospray ionization and atmospheric pressure chemical ionization, were applied. In both cases, however, no signal was observed even for high concentrations of the analytes (investigated concentration range: 1 μmol/L−1 mmol/L) in the full scan and selected ion monitoring modes and in the positive and negative ionization modes, respectively.

The focus has therefore been directed on the attempt to decompose the peroxides to hydrogen peroxide and to determine hydrogen peroxide by an enzyme-catalyzed reaction under formation of a chromophor or fluorophor. As general approaches, decomposition could be carried out by heat, by strongly acidic pH conditions, or by ultraviolet irradiation. While the first two methods proved to be inefficient in initial tests, photolysis of the peroxides was performed successfully.

Based on these data, a reversed-phase separation of the peroxides with a two-step postcolumn reaction scheme was developed. The complete system is depicted in Figure 3. After separation on a RP-18 column (for details, see Experimental Section), UV irradiation at 254 nm was used in order to decompose the explosives to hydrogen peroxide. Kettrup et al. used a similar setup for the analysis of various peroxides.[17] For the analysis of

(16) Suelzle, D.; Klaeboe, P. *Acta Chem. Scand.* **1988**, *A24*, 165−170.

(17) Hong, J.; Maguhn, J.; Freitag, D.; Kettrup, A. *Fresenius J. Anal. Chem.* **1998**, *361*, 124−128.

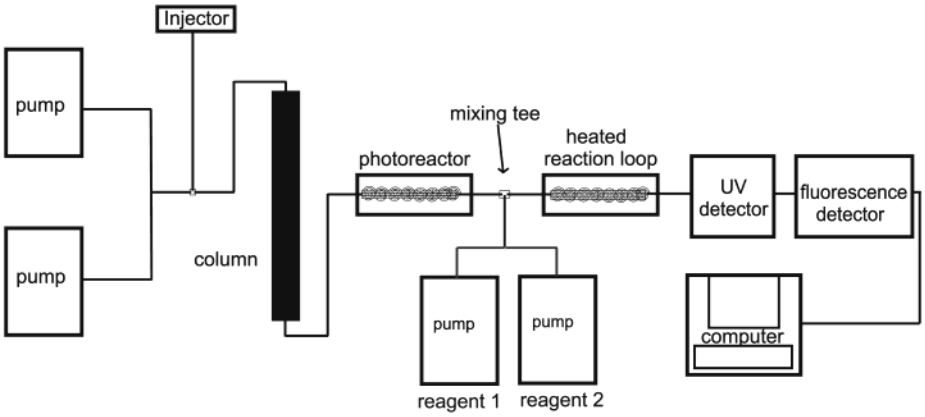

Figure 3. HPLC setup with photoreactor and enzymatic postcolumn derivatization.

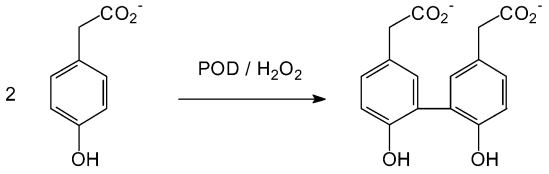

Figure 4. Oxidation of *p*-hydroxyphenylacetic acid to the fluorescent dimer by means of peroxidase (POD) and hydrogen peroxide.

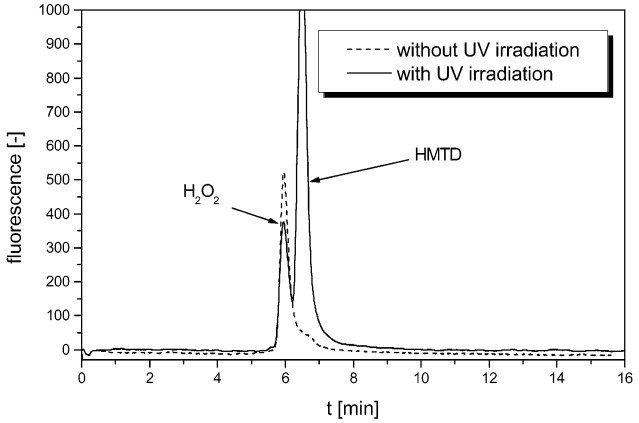

Figure 5. Chromatogram after separation of HMTD and derivatization after and without UV irradiation. The concentration of the HMTD solution was 1×10^{-3} mol/L. Composition of the mobile phase was 65% acetonitrile and 35% water.

the formed product, the reaction of H_2O_2 with pHPAA under peroxidase (POD) catalysis was used, thus yielding highly fluorescent and easily detectable species. This approach is an established detection technique for hydrogen peroxide.[18−21] The fluorescent dimer of pHPAA is formed in the presence of peroxides and POD (see Figure 4), which is then detected at an excitation wavelength of 324 and an emission wavelength of 405 nm. The limits of detection are 2×10^{-6} mol/L for both TATP and HMTD, respectively. The limits of quantification are 6×10^{-6} mol/L for both TATP and HMTD, respectively. The linear range covers two decades starting from the limits of quantification to 5×10^{-4} mol/L for HMTD and to 1×10^{-3} mol/L for TATP, respectively. Regarding the concentration 2×10^{-5} mol/L the relative standard deviation (RSD) ($n = 4$) for HMTD was 3.1% and for a concentration of 5×10^{-4} mol/L the RSD ($n = 4$) was 2.1%. The RSD ($n = 4$) for TATP at a concentration of 8×10^{-6} mol/L was determined as 4.1%, and for a concentration of 5×10^{-4} mol/L the RSD ($n = 4$) was 1.9%.

As the UV light is essential for the detection of the peroxides, there is an additional possibility, apart from the retention time and fluorescence selectivity, to identify the nature of the presumed HMTD/TATP peak. Turning the UV light on and off in different runs, the peroxide peaks are also literally switched on and off, thus giving an additional proof that the peaks are the peroxides in question and not interfering compounds reacting with the pHPAA without photochemical decomposition.

With respect to the photoreactor, there is a special point of interest to be taken into consideration: The length of the knitted capillary had to be carefully optimized, as the irradiation at 254 nm slowly decomposes the generated hydrogen peroxide. Therefore, a compromise between full decomposition of the analytes and degradation of the H_2O_2 generated had to be chosen, to obtain the optimum limits of detection. This effect becomes obvious when the same samples are quantified once with and once without UV irradiation. Figures 5 and 6 show the chromatograms of a HMTD and a TATP sample, with and without UV irradiation. With the UV irradiation turned on, both HMTD and TATP could be easily detected. Additionally, a peak from residual H_2O_2 resulting from the synthesis could be observed. Without photochemical treatment, this signal is significantly higher, thus clearly demonstrating the destructive effect of UV light toward hydrogen peroxide. In Figure 6, the presumed peak of the acetone peroxide dimer is also present. Similar to the TATP, it was only observed in the presence of UV irradiation which may further confirm the assumption that it is the dimer compound. Within the dominating TATP peak, a shoulder can be seen that is presumed to be the tetramer of the acetone peroxide.

Real Samples. For simulation of indoor explosion real samples, residues of a house cleaning procedure obtained by a vacuum cleaner (901, 58 mg, mainly dust) was collected and spiked with unpurified TATP (4.95 mg) and HMTD (3.70 mg). The sample was subsequently eluted with acetonitrile (35 mL), filtered, and finally injected into the HPLC system. Figure 7 shows that HMTD, TATP, and the TATP dimer could be separated and

(18) Lazrus, A. L.; Kok, G. L.; Lind, J. A.; Gitlin, S. N.; McLaren, S. E. *Anal. Chem.* **1985**, *57*, 917−922.
(19) Lazrus, A. L.; Kok, G. L.; Lind, J. A.; Gitlin, S. N.; Heikes, B. O. *Anal. Chem.* **1986**, *58*, 594−597.
(20) Sukugawa, H.; Kaplan, I. R. *Atmos. Environ.* **1987**, *21*, 1791−1798.
(21) Meyer, J.; Büldt, A.; Vogel, M.; Karst, U. *Angew. Chem.* **2000**, *112*, 1510−1512.

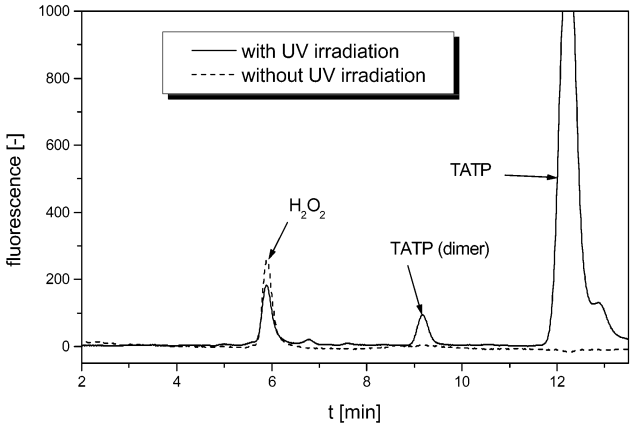

Figure 6. Chromatogram after separation of TATP and derivatization after and without UV irradiation. The concentration of the TATP solution was 1×10^{-3} mol/L. Composition of the mobile phase was 65% acetonitrile and 35% water.

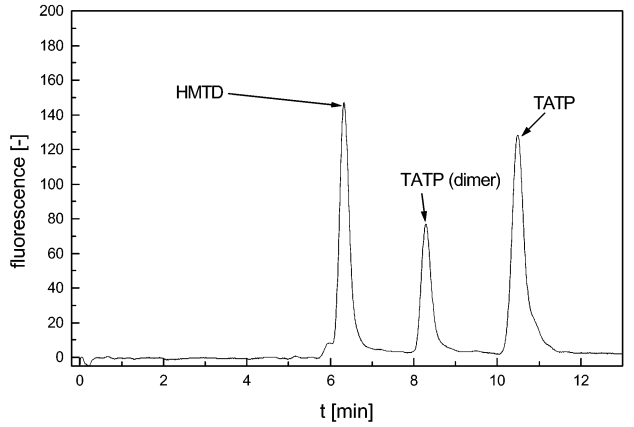

Figure 7. Chromatogram of a household dust sample spiked with TATP ($\sim 1 \times 10^{-3}$ mol/L) and HMTD ($\sim 7 \times 10^{-4}$ mol/L). Composition of the mobile phase was 75% acetonitrile and 25% water.

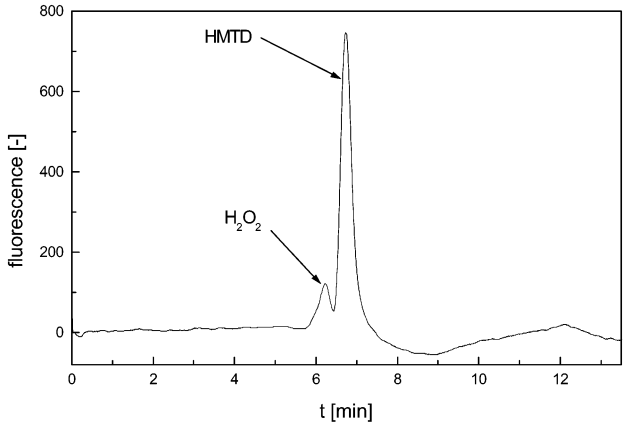

Figure 8. Chromatogram of a real sample from parts of a metal box destroyed by an explosion of HMMTD. Composition of the mobile phase was 65% acetonitrile and 35% water.

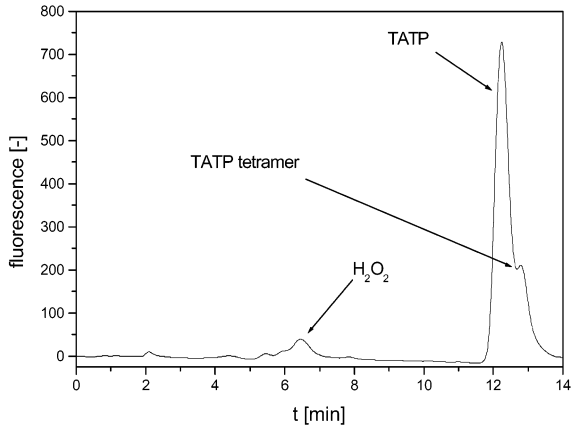

Figure 9. Chromatogram of a real sample from parts of a paper box, which was destroyed by an explosion of TATP. Composition of the mobile phase was 65% acetonitrile and 35% water.

detected. The peak of the tetramer is not present in this chromatogram. The reason for the appearance of the tetramer in only some of the product mixtures needs further investigation.

The critical peak pair in this chromatogram is hydrogen peroxide and HMTD. The concentration of H_2O_2 is very low, compared to the other substances. Therefore, the separation and quantification of the HMTD was not adversely effected by a higher concentration of acetonitrile in the mobile phase. Seventy-five percent acetonitrile in the mobile phase compared to 65% organic solvent in the mobile phase of the other measurements considerably accelerates the separation.

Subsequently, parts of metal and paper boxes, which had been destroyed through explosions of TATP and HMTD, respectively, were analyzed. All samples were stored in airtight bags prior to analysis, mainly to avoid sublimation of TATP and the acetone peroxide dimer. The materials were washed with acetonitrile (50 mL), and the solution was filtered with standard filter paper and injected into the HPLC (see Figures 8 and 9). Figure 9 shows no dimer because the synthesis of TATP was carried out without sulfuric acid in this case. This is also an additional indication that the peak at ~8.5 min, observed in the earlier chromatograms, is the dimer and not the tetramer.

HMTD could be determined without problems. It was detectable immediately after the explosion and even after two months of storage in a refrigerator at 4 °C. The investigated samples contained HMTD concentrations significantly exceeding the limits of quantification of the method (Figure 8).

In contrast to HMTD, the debris of a suspected TATP explosion site should be analyzed as fast as possible. As TATP is prone to sublimation, most of the trace residues will be evaporated after a few days. TATP standing at room temperature will lose as much as 3% of its weight during 2 days. If there is a catalyst like sulfuric acid present, the loss may increase up to 50% during the same time period.[5] This has a serious effect on all measurements regarding TATP. TATP could be detected after 2 weeks of storage without problems (Figure 9). After a storage time of 2 months in an airtight bag at 4 °C, however, the TATP concentration was significantly reduced.

CONCLUSIONS

With this work, the first quantitative trace analytical method for the analysis of HMTD and TATP was developed. Both analytes could be separated by means of RP-HPLC and were very sensitively and selectively detected by fluorescence spectroscopy. The intrinsic selectivity of the postcolumn derivatization/

fluorescence method is responsible for the very low level of interferences when real samples were investigated. Therefore, it is possible to analyze postexplosion sites and to find remains of these two explosives. This includes sites where no visible amounts of TATP or HMTD are found. In addition, illegal manufacturing sites could be identified with this method. With 2×10^{-6} mol/L for both compounds, the limits of detection are sufficient for the analysis of real samples from explosion sites.

ACKNOWLEDGMENT

Financial support by the German Minister of the Interior (Berlin, Germany) is gratefully acknowledged.

Received for review June 17, 2002. Accepted November 18, 2002.

AC020392N