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Aqueous Recovery from Cotton Swabs of Organic Explosives Residue Followed by Solid Phase Extraction*

REFERENCE: Thompson RQ, Fetterolf DD, Miller ML. Mothershead II RF. Aqueous recovery from cotton swabs of organic explosives residue followed by solid phase extraction. J Forensic Sci 1999;44(4):795–804.

ABSTRACT: A common procedure for processing cotton swabs containing organic explosives residue involves soaking the cotton in acetone or other organic solvent to extract the explosives, followed by direct analysis of the resulting sample solution using chromatography—mass spectrometry (LC- or GC-MS). A water-based procedure was developed to solve problems arising from co-extraction of sample matrix. Common nitro-organic explosives were extracted from cotton into water; the explosives were isolated by solid phase extraction, using a poly-N-vinylpyrrolidone-divinylbenzene sorbent; samples were screened by LC-UV; and the presence of explosives was confirmed by LC- or GC-MS and fast GC-TEA (EGIS).

Explosives residue samples were generated by mixing standards in motor oil on aluminum foil, by detonating four different bombs (C-4, a dynamite, a binary explosive, and TNT) hidden inside suitcases filled with clothing, and by handling a plastic explosive (Semtex H). Ninety-six paired samples were processed by the two procedures (acetone-based and water-based). The water extraction/SPE process was just as effective in recovering organic explosives from cotton swabs, and it better rejected the sample matrix, giving much greater selectivity with all samples except clothing. Water-based samples were screened with high accuracy by LC-UV, and the LC-UV and LC-MS semi-quantitative results were highly correlated.

KEYWORDS: forensic science, explosives residue, organic explosives, water, solid phase extraction, reversed phase liquid chromatography, nitramines, nitroaromatics, nitrate esters, cotton swabs, and mass spectrometry

In the forensic examination of physical evidence for organic explosives, cotton swabs often are used to collect residue from surfaces, such as skin and post-blast debris. Twibell et al. had subjects hold explosives and then wiped the subject's hands with small cotton and viscose wool swabs saturated with solvent (1,2). The swabs were extracted with acetone or ethanol, and the resulting solutions were analyzed without further clean-up. Microgram and larger quantities of explosives were found when sampling was performed soon after contact. Lloyd et al. examined the means of recovering all of the extraction solvent from large cotton swabs, i.e., techniques such as pressing, squeezing, and centrifugation, and found that centrifugation was most efficient by a small margin (3,4). The extraction solvents used in this study were ethanol in one case and isopropanol: water in the other. No significant difference in performance with cotton, viscose, or Acrilan wool swabs were found, and better than 70% recoveries of nanogram quantities of explosives were reported for most of the samples.

For sampling surfaces other than skin, Canadian scientists used Micropore tape (3M Corporation; Minneapolis, MN) or cotton swabs dampened with methanol (5). Subsequent acetone extraction recovered 90% of submicrogram quantities of plastic explosives from a variety of surfaces. German researchers used cotton swabs meant for eye cleansing, wetted and extracted with acetone, to recover residues from steel "witness plates" set up around dynamite bomb sites (6). Most recently, a group from the United Kingdom surveyed a large number of public places for traces of organic explosives (7). They sampled the insides of automobiles, trains, and planes, and many surfaces in transportation depots using cotton swabs soaked in ethanol. In the laboratory, the swabs were extracted with methyl-tert-butyl ether and analyzed by gas chromatography-chemiluminescence.

The literature reviewed above describes procedures for sampling explosives residue that rely on organic solvents to wet and extract cotton swabs. In summary, the procedure is:

Sampling → Extraction with Organic Solvent →

Volume Reduction → LC- or GC-MS Analysis

Organic solvents, such as acetone, are used because explosive compounds dissolve readily in them, but so do many other compounds. This leads to a complex sample matrix that can interfere with the analysis and degrade instrumental performance. This analytical approach involves minimal sample clean-up and relies on chromatography and a very selective detector (e.g., mass spectrometry, chemiluminescence) to avoid interferences.

We have been investigating the reverse approach, i.e., isolating the explosives from the sample matrix during sample processing and making measurements with a less expensive, universal detector (UV absorbance). Separation of analytes and matrix is achieved

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* This is publication 97-10 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 FBI.

Received 19 Aug. 1998; and in revised form 27 Oct. 1998; accepted 27 Oct. 1998.

by use of water instead of organic solvents and the addition of a solid phase extraction (SPE) step. The analytical scheme is:

Sampling → Extraction with Water → SPE → Volume

Reduction → LC-UV Screening → LC- or GC-MS Confirmation

The use of water and aqueous solvents for extraction of organic explosives was studied by Twibell, et al. in 1982 (8). They found that among a variety of organic and aqueous solvents acetone followed closely by water most effectively removed organic explosives from hands. Water provided better detection limits because the baseline noise in the chromatogram was much lower, i.e., the complexity of the matrix was reduced by extracting with water. In situations where submicrogram amounts of explosives residue are present, extracting the sample with a few mL of water results in explosives concentrations of ~0.01 μg/mL. This concentration is well within the limits of solubility of the common organic explosives in water at 25°C (9,10):

PETN	HMX	RDX	Tetryl	
2 μg/mL	5 μ/mL	42 μg/mL	80 μg/mL	
TNT	2,4-DNT	NG	EGDN	
130 μg/mL	270 μg/mL	1500 μg/mL	5000 μg/mL	

Because inorganic explosives residue is water soluble too, separate extraction procedures for organics and inorganics are not necessary. Substitution of an environmentally-friendly solvent (water) for a toxic and flammable one (acetone) is another advantage.

This report describes the application of these procedures to prepared samples in motor oil, samples from several post-blast sites, and samples from persons and automobiles after contact with explosives. Nearly 100 samples, including blanks, were collected and analyzed. Paired samples were obtained in each case or single swabs were divided so that the procedures could be compared. All of the extracts were examined by LC-UV, fast GC-TEA (EGIS), and LC-MS. Some were examined by GC-MS as well. Semiquantitative analysis of the samples is reported, and the selectivities of the two procedures are compared. The potential for LC-UV screening is assessed.

Materials

Reagents—Ultrapure, 18-MΩ water was prepared by passing deionized water through a Maxima UltraPure Water purification device (Elga Ltd.; Bucks, England), and "water" herein implies this high quality water. Organic solvents were of HPLC grade or UV spectrophotometric grade. Mobile phase solvents for liquid chromatography were filtered through a 0.45-μm, nylon membrane.

Automotive motor oil, SAE 10W-40, was purchased at a local food store (Super G brand, Giant Foods). Water saturated with this motor oil was prepared by mixing 100 drops (about 2 g) of oil and 500 mL of water, letting the mixture stand for at least 48 h, and filtering the mixture through a 0.45-μm cellulose acetate membrane to remove globules of undissolved material. It was expected that, in addition to hydrocarbons, the oil-saturated water contained detergents and other polar compounds added to modern lubricants (11). This solution simulated a complex sample and was used to test matrix rejection and measure the selectivity of the SPE cartridges.

Solutions of Explosives—Stock solutions of common energetic compounds (2,4-DNT, EGDN, HMX, NG, PETN, RDX, Tetryl, and TNT) at 100 or 1000 mg/L in acetonitrile were obtained commercially (Radian International Corporation; Austin, TX). Standard mixtures of the eight explosive compounds, at 1 and 10 mg/L of each compound, were prepared by volumetric dilution of the stock solutions with 50% acetonitrile:50% water. Working standards, containing 0.125 or 1.25 mg/L of each explosive, were prepared by volumetric dilution (50.0 μL diluted to 400 μL) of the standard mixtures with 50% acetonitrile:50% water. Laboratory samples were prepared by adding μL volumes of one or the other of the standard mixtures, containing ng quantities of each explosive compound, to 25 mL of water or oil-saturated water or to several drops of motor oil.

Solid Phase Extraction (SPE) Cartridges—Three SPE cartridge systems were obtained commercially and were compared. Porapak R_{DX} cartridges (Waters Corporation; Milford, MA) contained 500 mg of polyvinylpyrrolidone-divinylbenzene copolymer in a 6-mL plastic body. Oasis brand cartridges (Waters Corporation; Milford, MA) contained 60 mg of polyvinylpyrrolidone-divinylbenzene copolymer (30-μm particles) in a 3-mL plastic body. The Oasis sorbent contained a slightly higher fraction of vinylpyrrolidone than the Porapak R_{DX} sorbent. SDB-XC disk cartridges (3M Corporation; Minneapolis, MN) did not have a bed of sorbent like the other two systems, but rather contained a 7-mm diameter, 0.5-mm thick, Teflon disk that was impregnated with 7.5 mg of styrene-divinylbenzene copolymer (12-μm particles). The disk was retained at the bottom of a 3-mL plastic cartridge. The SPE tubes were processed manually using a 16-port vacuum manifold (Alltech Associates, Inc.; Deerfield, IL).

Swabs for Sampling—A box of sterile cotton balls meant for use in first aid (Q-tip brand, Cheesebrough-Ponds USA Co.; Greenwich, CT) was purchased at a local food store. The average cotton ball was a 2-cm sphere weighing 400 mg. The cotton balls were cleaned by soaking first in water for two hours and then in acetonitrile for 2 h. Most of the solvent was squeezed from the cotton prior to drying the cotton balls at 85°C for 18 h. The clean and dry swabs were stored inside a polyethylene bag at room temperature.

The type of samples and handling of the sample swabs are reviewed in Table 1. Not more than 24 h in advance of use, a cotton ball was wetted with <1 mL solvent and placed inside a 10-mL plastic syringe (Becton-Dickinson; Singapore) that was used without pretreatment. The syringe was capped to protect the swab from contamination. The damp cotton ball was removed with forceps at the sampling site, rubbed against surfaces to collect residue, and then returned to the same syringe for transport back to the labora-

TABLE 1—*Samples and cotton swabs.*

Sample Type	Organic Explosives	# Samples	Wetting Agent	Swabs
Simulated w/ Motor Oil	All	8	0.75 mL I:W*	Divided
C-4 Bomb	RDX	20	0.50 mL water or acetone	Whole
Dyno Unigel Bomb	EGDN, NG	18	0.50 mL water or acetone	Whole
Kinestik Bomb	None	8	0.75 mL I:W	Divided
TNT Bomb	TNT	17	0.75 mL I:W	Divided
Contact with Semtex	PETN, RDX	25	0.50 mL I:W	Divided

* 80% isopropanol: 20% water (I:W).

tory. Whole swabs were used in instances where parallel samples were collected, e.g., half of a suitcase part was swabbed with water and the other half swabbed with acetone. More commonly, one swab was used to collect explosives residue from each piece of evidence, the swab was cut in half (through the soiled portion of the cotton), and each portion was processed separately. Except for the sampling step, blank cotton swabs were treated in an identical fashion.

Explosives Residue Samples

Simulated Samples—Samples were prepared in the laboratory in the following manner. On a clean piece of aluminum foil were placed 4 drops of motor oil and a few μL of the standard mixture of explosives. A wetted cotton ball was used to wipe up the pool of oil containing explosives and to wipe the surface of the foil. The cotton was cut in half with clean scissors, and each piece was placed inside a 10-mL syringe for subsequent processing.

Suitcase Bombs—To obtain samples from an actual explosion, 0.5 pounds of C-4, a military explosive containing RDX, were placed inside a hardcover (plastic) suitcase (1.5′ × 2′ × 8″) filled with used clothing (cotton and cotton-polyester blends), and 0.5 pounds of Dyno Unigel, a commercial dynamite containing NG and EGDN, were placed inside a similar hardcover (plastic) suitcase filled with used clothing (cotton and cotton-polyester blends), and then both were detonated by FBI explosives experts in late April 1997. The fuse was comprised of several parts: starter cap—time fuse (black powder core)—Primadet Nonel (Al, HMX)—blasting cap (black powder, PETN). Witness plates (~1.5′ tall × 1′ wide) of cardboard covered with aluminum foil and of drywall were attached to wooden posts and positioned 1.0 m, 2.5 m, and 5.0 m from the suitcase/bomb. The suitcase was laid flat on the ground on top of plywood with a plastic tarp below. Examination of the post-blast site showed suitcase parts and clothing scattered over a 10-m radius from the seat of the explosion. Sections of clothing were shredded, burned, and melted during the blast. The suitcase was separated into pieces of inner and outer shell; the typical size of the pieces was ~100 cm^2. An exterior half-shell of the suitcase, only slightly damaged, was found at the dynamite site. A badly damaged and bent, but nearly intact, exterior half-shell of the suitcase was found at the C-4 site.

Equal areas of each of the witness plates (6 plates in all), an old newsbox placed at the dynamite bomb site, pieces of the plastic tarp, and a few other items were swabbed with solvent-wetted cotton balls to collect any explosives residues present. At each blast site, most of the pieces of the suitcase were collected, and the pieces were placed inside a thick, plastic garbage bag. In the laboratory, sampling was performed in duplicate on equal areas of debris with solvent-wetted cotton balls. Similarly, most of the clothing was collected and placed inside a separate plastic bag. In the laboratory, 3 in. × 3 in. squares from unburned areas of cloth were cut from the clothing for testing.

To obtain more samples, one tube of a binary explosive (Kinestik), a commercial explosive containing solid ammonium nitrate and liquid nitromethane, was placed inside a hardcover (plastic) suitcase filled with clothes (as described above), and 0.5 pounds of TNT, a cast commercial explosive, were placed inside a hardcover (plastic) suitcase filled with clothes (as described above), and then both were detonated by FBI explosives experts in late May 1997. The sites of the explosions were sand pits where smokeless powder pipe bombs routinely were exploded. The fuse was comprised of several parts: starter cap—time fuse (black powder core)—Detcord (PETN). Witness plates (12 in. tall × 9 in. wide) of cardboard covered with aluminum foil or polyethylene food wrap were attached to wooden posts and positioned 1.0 m, 2.0 m, and 3.0 m from the suitcase/bomb. The suitcase was placed flat on the ground on top of a plastic tarp.

Examination of the post-blast site showed damage similar to that described above for the C-4 and dynamite detonations. The witness plates (6 plates in all), pieces of the plastic tarp, the hands of the bomb tech, and a few other items were swabbed with solvent-wetted cotton balls to collect any explosives residues present. The swabs were carried to and from the scene inside 10-mL syringes with caps. At each blast site, most of the pieces of the suitcase were collected, and the pieces were placed inside a thick plastic garbage bag. In the laboratory, some of the debris was sampled with solvent-wetted cotton balls. All of the cotton swabs were cut in half with clean scissors, and the half-swabs were placed inside syringes for subsequent processing. At the blast site, most of the clothing was collected and placed inside a separate plastic bag. Clothing was sampled in the laboratory by taking a whole cotton shirt and soaking it in 200 mL of solvent (water or acetone) for 10 min. Then the solvent was squeezed out of the cloth, and 10 mL (water) or 5 mL (acetone) was taken for further processing and analysis.

Handling a Plastic Explosive—Four persons were asked to squeeze a small ball of Semtex H, then to rub their hands together, and finally to unlock and drive their cars for a couple of minutes. Two of the subjects wiped their hands on a paper towel before entering their vehicles. Wetted cotton balls were used to collect residue in each case from the driver-side door handles (both inside and outside), from the steering wheel, gear shift, and dashboard, from the car keys, and from the driver's hands. Samples also were taken inside of the car before and a few days after the contact with the plastic explosive.

Extracting Explosives from Cotton Swabs

Extraction with Acetone—To extract explosives residue from a swab, acetone was drawn through the tip of the syringe up to the 5.5-mL graduation to cover the cotton ball with solvent. The filled syringe stood at room temperature for 10–15 min, and then the acetone was pushed from the syringe and collected in a glass sample tube. The cotton was pressed tightly to the bottom of the syringe to remove the last few drops. Samples that were very cloudy or otherwise showed a large amount of particulate were centrifuged for 10 min, and the supernatant was taken for further processing.

The sample solution was evaporated to a few μL at 65°C under ~500 mL/min nitrogen gas flow, and 50% methanol:50% water was added to bring the volume to ~400 μL. In some cases, the clear acetone solution turned cloudy upon volume reduction and/or addition of methanol:water. Instrumental analysis followed.

Extraction with Water—To extract explosives residue from a swab, water was drawn through the tip of the syringe up to the 5.5-mL graduation to cover the cotton ball with solvent. The filled syringe stood at room temperature for 10–15 min, and then the water was pushed from the syringe and collected in a glass sample tube. The cotton was pressed tightly to the bottom of the syringe to remove the last few drops. Another 5.5 mL of water was drawn into the syringe to rinse the cotton, and the water was immediately added to the same sample tube. A sample volume of ~11 mL was generated in this way. Samples that were very cloudy or otherwise

TABLE 2—*Automated SPE procedure and Benchmate II settings for oasis cartridge.*

General
Liquid-driven dispensing; Dispense rate = 60 mL/min
Reagent autowash volume = 1.00 mL
Air push following liquid dispensing; Air push rate = 18 mL/min
Conditioning
10 mL methanol; 10 mL methanol; 10 mL methanol; 10 mL water
Flow rate = 5.4 mL/min
Loading Sample
Initial sample volume in 1st tube ≈11 mL
Syringe filled with sample; Aspiration rate = 60 mL/min
Syringe emptied into SPE cartridge; Flow rate = 2.4 mL/min
Sample volume = 9.2 mL (Measured volume = 9.2 mL)
Sample collected in 2nd tube after passing through SPE cartridge
Washing
3 mL 50% methanol: 50% water; Flow rate = 1.8 mL/min
Eluting Explosive Compounds
1.5 mL methanol (Measured volume = 1.3 mL); Flow rate = 1.2 mL/min
Eluate collected in 3rd tube that contains 0.15 mL water (added manually)

The total time for this procedure was 33 min per sample.

showed a large amount of particulate were centrifuged for 10 min, and the supernatant was taken for further processing.

Matrix components were removed and the organic explosives concentrated by automated, solid phase extraction (SPE) using Oasis cartridges and a Benchmate II robot (Zymark Corp.; Hopkinton, MA). The optimized procedure developed in this laboratory is described in Table 2. The SPE eluate was evaporated to a few μL at 65°C under ~500 mL/min nitrogen gas flow, and then 50% methanol: 50% water was added to bring the volume to ~400 μL. In recovery studies, 10 μL of an internal standard solution (I.S.; 2,3-dimethyl-2,3-dinitrobutane; 15 μg/mL acetonitrile) were added to correct for variation in final volume. Instrumental analysis followed. Final results for the water-extracted samples were corrected for the fact that only 9.2 mL of the ~11 mL sample were processed by the robot.

Analysis of Soils

Five samples of soil were collected from the test range where the experimental bombs were detonated. Surface and subsurface samples were taken from the first and second sand pits where the Kinestik and TNT tests had been previously conducted, respectively. A fifth sample was collected between the pits of the red clay soil. Soil samples (20 g) were slurried in 40 mL of acetonitrile in 125 mL erlenmeyer flasks and sonicated for 2 h (maximum temperature of 41°C). The samples were then allowed to settle overnight (~15 h) in the dark. The solvent was decanted into 50 mL centrifuge tubes and spun for 10 min at 1100 rpm. A 10 mL aliquot was diluted with deionized water to 200 mL (5% acetonitrile: 95% water) for manual solid phase extraction using the Oasis SPE procedure.

Instrumental Analysis

Liquid Chromatographic—Photodiode Array (LC-UV) Detection—Standard solutions and sample extracts were analyzed by LC-UV. The instrument included an autosampler (model 717 plus, Waters Corporation; Milford, MA), a high-pressure pump and low-pressure solvent mixer (model 600E multisolvent delivery system and model 600 controller, Waters), a photodiode array detector (model 996, Waters), and a computerized data acquisition and instrument control system (NEC Image 466 PC running v.2.15 Millennium software, Waters). Twenty-μL injections were made. The mobile phase was a methanol:water gradient mixture—50% methanol for 13 min, increase to 75% methanol at 18 min, hold for 5 min, return to 50% at 28 min—flowing at 0.80 mL/min and sparged continuously with 30 mL/min helium. Each run lasted 42 min to ensure that all matrix components were washed from the column. The separations were achieved in a 4.6 mm × 150 mm, Supelcosil LC-18-DB column packed with 5-μm particles (Supelco, Inc.; Bellefonte, PA). The nitrate esters (EGDN, NG, PETN, and internal standard) were determined at 210 nm and the nitramines and nitroaromatics at 240 nm.

EGIS Detection—The EGIS system is comprised of a short-column gas chromatographic system coupled to a thermal energy analyzer (Thermedics Inc.; Chelmsford, MA). The thermal energy analyzer is a very specific detector and is based on pyrolysis of NO_2-containing molecules to give nitrogen oxide species, reaction of these gaseous products with ozone, and monitoring of the chemiluminescence of the excited product, NO_2 (12,13). Injections (1 μL) were made onto the coils of the sampling device.

Liquid Chromatographic—Mass Spectrophotometric (LC-MS) Detection—A liquid chromatograph (model 1090, Hewlett-Packard Company; Palo Alto, CA) with autosampler was interfaced to a Finnigan quadrupole mass spectrometer (model TSQ 700, Finnigan MAT; San Jose, CA). The mobile phase, a mixture of methanol and 1.0 mM ammonium nitrate, was pumped at 0.3 mL/min and operated in gradient mode—50% methanol at the start, linear ramp to 90% methanol at 4 min, and immediate return to 50% at 5 min. Five-microliter injections were made onto a Hypersil ODS column, 2.1 mm × 100 mm, packed with 5 μm particles. The atmospheric pressure ionization interface was operated in electrospray mode (spray voltage = 4.5 kV, capillary temperature = 200°C), and the MS was in negative ion mode, scanning from 220 to 385 amu with a scan time of 1 sec. Nitrate ion adducts of the nitramine and nitrate ester compounds were formed in the interface (14), and the detector signal was recorded as selected ion traces.

Gas Chromatographic—Mass Spectrophotometric (GC-MS) Detection—GC-MS analysis was conducted with a Finnigan GCQ (Finnigan MAT; San Jose, CA), a gas chromatograph coupled to a quadrupole ion trap mass analyzer. The GC was operated in splitless injection mode at an injector temperature of 150°C, a split vent time of 1.0 min, and an injection volume of 1.0 μL. The analytical capillary column was a 15 m × 0.25 mm, 0.25 μm film, DB-5MS fused silica column (J&W Scientific; Folsom, CA), and the separation was carried out under temperature-programmed conditions—35°C for 1 min, increase to 225°C at 20°C/min, and hold for 1 min. The mass analyzer was set to negative chemical ionization mode with methane as reagent gas and a manifold pressure of 4×10^{-4} torr. The source temperature was 150°C, and the transfer line from the GC was heated to 225°C. The full-scan mass range was 45 to 300 amu with a scan time of 1 sec.

Miscellaneous Equipment—Both fixed- and variable-volume pipettors (pipetman, Rainin Instrument Company; Woburn, MA) were used for delivering small volumes of liquids. Eluates and other solutions were reduced in volume by blowing nitrogen at ~500 mL/min over the liquid, held in 12 mm × 75 mm, clear

glass culture tubes (Fisher Scientific; Pittsburgh, PA) and heated to 65°C (multi-blok heater, VWR Scientific Products; South Plainfield, NJ).

Calculation of Analyte Recovery—Percent recovery of each of the explosive compounds was determined for the volume reduction and the solid phase extraction steps from the LC-UV data. Peak areas given by injection of working standards, containing internal standard (STND area and IS1 area respectively), by injection of sample blanks, containing internal standard (BLNK area and IS2 area respectively), and by injection of samples, containing internal standard (SAMP area and IS3 area respectively), were measured. The percent recovery of each sample component was found by the formula: $100 \times \{(\text{SAMP} / \text{IS3}) - (\text{BLNK} / \text{IS2})\} / (\text{STND} / \text{IS1})$.

Results and Discussion

Reversed Phase Liquid Chromatographic (LC-UV) Analysis—Many of the reported LC separations of organic explosives have employed a C_{18} stationary phase and a methanol:water mobile phase (15–18), and the same was used here to separate the explosives and internal standard (I.S.) in under 20 min (Fig. 1). Baseline resolution of the nitramines and nitroaromatics was achieved at 240 nm. Quantitation of the nitrate esters, monitored at 210 nm, was somewhat problematic. While the internal standard (2,3-dimethyl-2,3-dintrobutane; I.S.) and PETN were well resolved, the NG peak overlapped slightly with the TNT peak and EGDN coeluted with RDX. Peak areas for EGDN only could be estimated with considerable uncertainty. Peak area was linear with the amount of injected explosive over the range 0.5 − 50 ng.

Volume Reduction Step—The evaporation of acetonitrile-water and methanol-water solutions was studied to determine if any of the explosives were volatilized or decomposed during this step. The volatility of the energetic compounds varies over eight orders of magnitude from $<10^{-10}$ torr (vapor pressure of HMX at 20°C) to 0.050 torr (vapor pressure of EGDN at 20°C). EGDN and NG are most likely to be lost at higher temperatures. About 3 mL of acetonitrile solution containing 10–500 ng of each explosive were reduced in volume to <200 μL. Then the I.S. and water were added, and the solution was analyzed. Analyte peak areas were normalized to the I.S. peak area in each chromatogram. Loss of explosives as high as 10–20% of the original amounts was observed, and most of the loss occurred after the volume dropped below 1 mL. Addition of 0.3 mL of water to the 3 mL of acetonitrile solution prior to solvent evaporation maximized the average recovery (96 ± 5%), including NG but excepting Tetryl (70%). The loss of Tetryl was ascribed to decomposition. Evaporation of 1 mL of methanol solution mixed with 0.1 mL of water also gave high average recoveries (95 ± 5%), NG and Tetryl included. Addition of larger volumes of water or mixtures of acetonitrile-water and methanol-water in azeotropic ratios did not significantly improve the recoveries.

Solid Phase Extraction (SPE) Step—The SPE process typically includes the following steps: conditioning/cleaning the sorbent with organic solvents; loading the sample on the cartridge, retaining the analytes along with some of the matrix; washing the sorbent with weak eluent to remove matrix components; drying the cartridge; and eluting the analytes with stronger eluent. Many experimental variables—e.g., liquid flow rate through the cartridge, solvent-sorbent contact time, nature of the solvents, drying time, sorbent dryness, sample concentration, and sample volume—affect the performance of an SPE cartridge. Likewise, characteristics of the cartridge—e.g., the nature of the resin, resin surface area, resin bed design, and cartridge dimensions—are important factors.

Optimized, manual procedures (Table 3) were developed for each of three commercial SPE cartridges: Oasis, Porapak R_{DX}, and

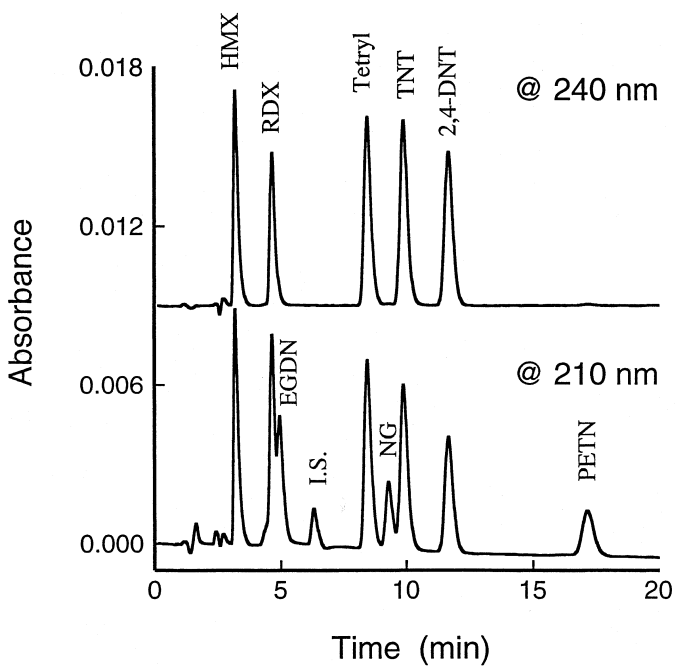

FIG. 1—*LC-UV (210 and 240 nm) chromatograms of a standard mixture of organic explosives. The injection volume contained 25 ng of each explosive compound. The upper chromatogram is offset on the absorbance axis by +0.009 units.*

TABLE 3—*Optimized procedures for the solid phase extraction of organic explosives from water.*

	Waters 6-mL Porapak R_{DX} Cartridge
Condition	15 mL acetonitrile @ 4 mL/min; 25 mL water @ 4 mL/min
Load	25 mL sample @ 2 mL/min
Wash	10 mL 50% methanol:50% water @ 3 mL/min
Dry	5 min @ full vacuum
Elute	3.00 mL acetonitrile @ <1 mL/min
	Waters 3-mL Oasis Cartridge
Condition	3 × 10 mL methanol @ 5 mL/min; 10 mL
Water	@ 5 mL/min
Load	25 mL sample @ <2 mL/min
Wash	3 mL 50% methanol:50% water @ 2 mL/min
Dry	5 min @ full vacuum
Elute	1.00 mL methanol @ <2 mL/min
	3M 3-mL SDB-XC Disk Cartridge
Condition	2 × 3 mL acetone @ 5 mL/min; 2 × 3 mL acetonitrile @ 5 mL/min
	3 mL methanol @ 4 mL/min; 10 mL water @ 3 mL/min
Load	25 mL sample @ 3 mL/min
Wash	3 mL water @ 3 mL/min
Dry	10 min @ full vacuum to completely dry the extraction disk
Elute	0.50 mL 90% methanol:10% water @ <2 mL/min

SDB-XC. These procedures provided recoveries of the set of explosives (50 − 500 ng of each compound) from 25 mL of water ranging from 80 to 110%. Recoveries were not significantly different among the three SPE systems nor at different levels of explosive. Table 4 provides specific results for Oasis. The lower recovery of HMX was ascribed to small losses during the wash step and was expected since HMX showed the weakest retention by the sorbents. All three SPE cartridges were quite effective in extracting explosive compounds from a simulated complex matrix. To 25 mL of oil-saturated water were added 50 or 500 ng of each organic explosive. Processing these samples with each of the SPE systems and LC analysis of the eluates showed recoveries ranging from 63% to 103% for the set of explosives at 50 ng and from 70 to 120% at 500 ng. Specific results for Oasis are given in Table 4, and representative chromatograms are given in Fig. 2. Comparison of Figs. 1 and 2 gives visual proof that the matrix rejection and analyte recovery were quite good.

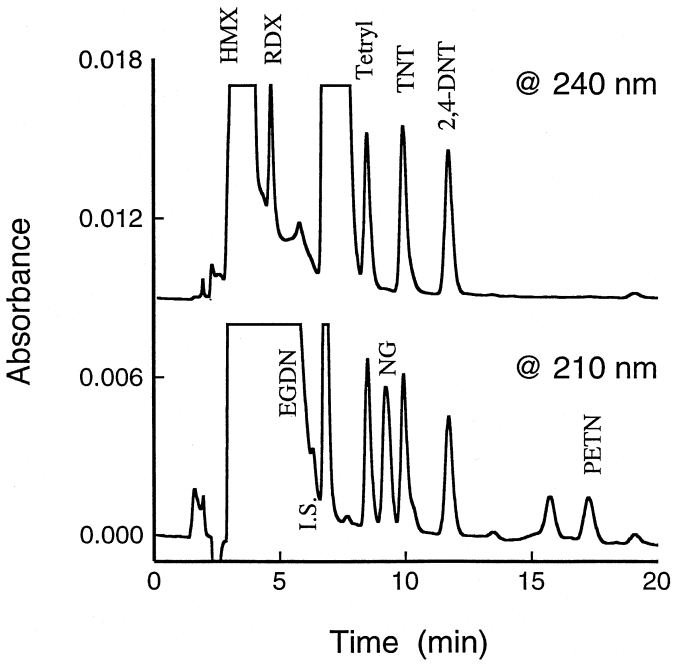

FIG. 2—*LC-UV (210 and 240 nm) chromatograms of an eluate produced by solid phase extraction (Oasis cartridge) of oil-saturated water containing organic explosives (500 ng of each compound). At 100% recovery the injection volume would contain 25 ng of each explosive compound. The upper chromatogram is offset on the absorbance axis by +0.009 units.*

All of the tested SPE systems were acceptable for use in the clean up of samples containing explosives residue. The Porapak R_{DX} cartridge, because it contained the largest amount of sorbent, best rejected the matrix and best retained explosives in samples prepared with oil-saturated water. On the other hand, the SDB-XC cartridge, with its small sorbent mass, was the cleanest and required the smallest volume of eluent. Despite these advantages, the Oasis cartridge was chosen for further work, because it had the highest selectivity, as defined by the smallest difference in eluent strength between the wash solution and eluent, was the most economical and was easily automated. The optimized, automated extraction procedure (Table 2) gave excellent recoveries for a mixture of explosives dissolved in water, similar to the results from the manual procedure. On average, 92% of the explosives in the range of 50 ng to 500 ng per 10 mL water were recovered. The Oasis cartridges had a large sample capacity; when samples containing 10 μg to 100 μg of RDX and TNT in 10 mL water were processed, neither of the explosive compounds was found in the water (sample solvent) that had passed through the SPE cartridges. Blank chromatograms showed the Oasis cartridges to be quite clean. A few small, but significant, background peaks at 210 nm did not interfere with measurements of NG or PETN, and the minimal background at 240 nm caused no problems for quantitation of the nitramines and nitroaromatics.

Recovery of Explosives from Cotton Balls—Rinsing commercial cotton balls with water and acetonitrile, followed by thorough oven drying, gave cotton that was adequately clean for sampling explosives residue. The blank chromatograms at 240 nm are nearly flat and at 210 nm contain only a few peaks (Fig. 3a). Acetone extracts of the cotton often became cloudy with fine particulate as the volume was reduced to 400 μL, and the chromatogram showed evidence of several impurities. The water extract had much less contamination.

Small amounts of working standards, containing 50 to 500 ng of each organic explosive, were added directly to whole cotton swabs. The cotton balls were wafted in the air for a few seconds to promote solvent evaporation, and then they were processed either by water extraction/SPE or acetone extraction. With water the overall average recovery was only 57%, indicating that a large fraction of the explosives remained bound to the cotton or in the small amount of liquid not squeezed out of the swab. Using warm water (up to 55°C) and extended times (up to 1 h) did not significantly change the recovery efficiency. With acetone the recoveries were low as well, averaging only 51%. Certainly further study of this step is warranted; the recovery process needs to be optimized, perhaps by using a material other than cotton and using centrifugation to recover more of the extraction fluid.

TABLE 4—*Percent recoveries using the Oasis solid phase extraction cartridge.*

	HMX	RDX	Tetryl	NG	TNT	DNT	PETN
			From Water				
7 trials @ 50 ng*	83 ± 8	101 ± 7	96 ± 6	110 ± 10	103 ± 7	93 ± 7	106 ± 7
4 trials @ 500 ng	84 ± 7	94 ± 7	99 ± 7	108 ± 8	98 ± 7	94 ± 6	102 ± 7
			From Water Saturated with Motor Oil				
5 trials @ 50 ng	†	70 ± 19	80 ± 12	†	86 ± 7	90 ± 10	63 ± 9
3 trials @ 500 ng	†	95 ± 17	100 ± 12	83 ± 6	89 ± 5	96 ± 5	88 ± 5

* Amount of each explosive added to 25-mL volume of solvent.
† Coeluting compounds made it impossible to quantitate this explosive.

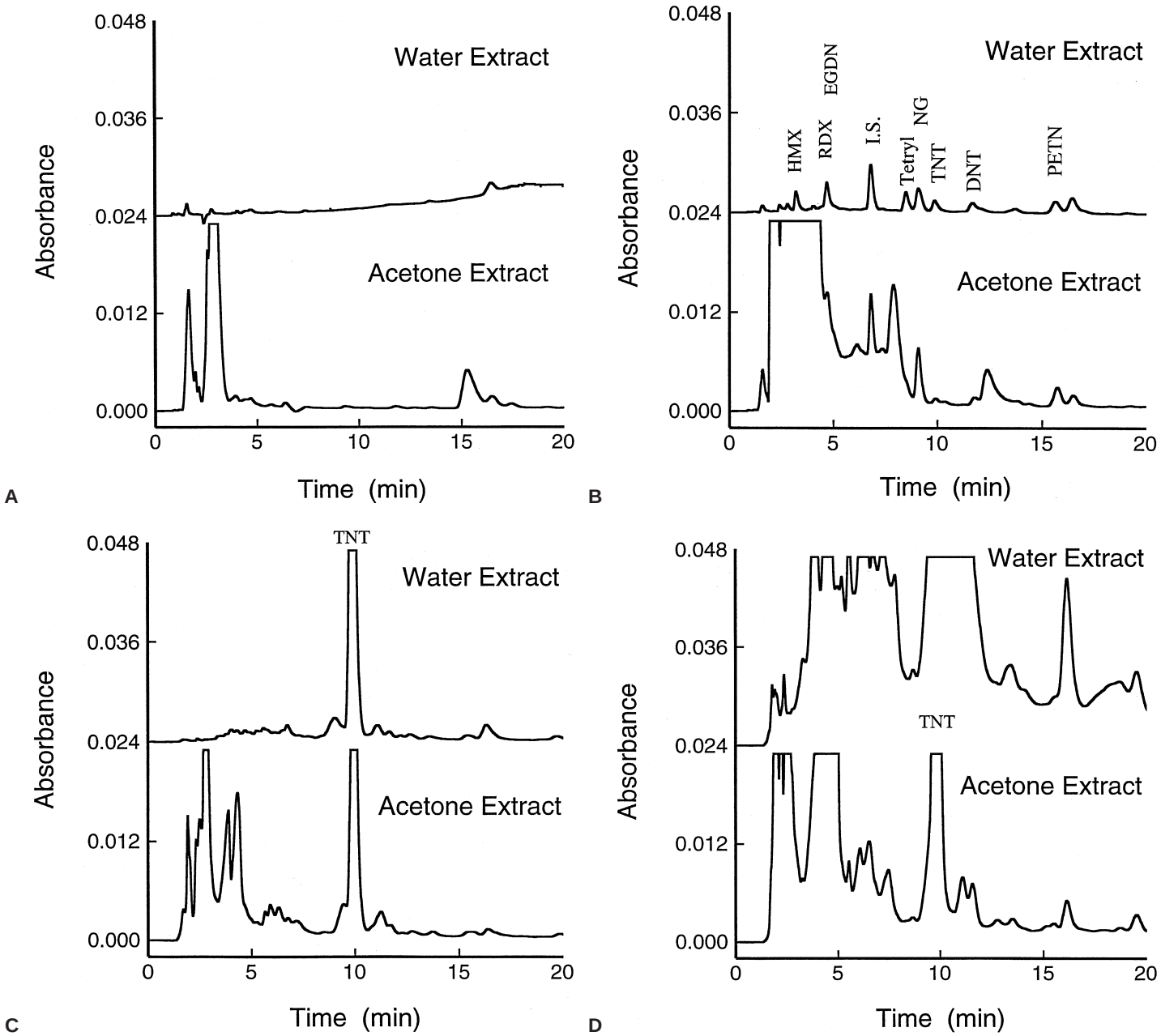

FIG. 3—*LC-UV (210 nm) chromatograms of samples derived from: (a) clean cotton swab (blank); (b) cotton swab containing 80 mg of motor oil and 600 ng of each explosive compound; (c) cotton swab containing residue from a small piece of suitcase shell found after detonating TNT; (d) cotton swab containing residue from a section of clothing found after detonating TNT. Each swab was cut in half prior to processing. The upper chromatograms (representing water extracts) are offset on the absorbance axis by +0.024 units.*

Adding 4 drops of motor oil to the cotton swab reduced the amount of explosives ultimately collected. Average recoveries for the set of explosives fell to 45% using the aqueous procedure and 43% using acetone. One can calculate that ~20% of the explosives remained in the oil and were lost to the analysis. The losses were largest for the nitroaromatic explosives, compounds that have the highest octanol-water partition coefficients (9). Representative chromatograms are shown in Fig. 3b.

Semiquantitative Analysis of Samples—Semiquantitative results based on the LC-MS data (GC-MS for EGDN) were calculated for each pair of samples. The results were semiquantitative rather than quantitative, because the calculated sample concentrations varied over several orders of magnitude, the instrumental response was nonlinear over the measurement range, and only a two-point calibration was performed. The concentration of explosive in the injected volume was estimated on the basis of the standard response, and the amount of explosive in the original whole or half swab was calculated, and the logarithm of this number was recorded. The amounts of explosives found in the sample swabs varied over 5 orders of magnitude, from <10 ng to >10 μg for both acetone- and water-processed samples. A plot of the semiquantitative results for those samples processed by water extraction/SPE, was made (Fig. 4). Only the values for the major components of each bomb are shown.

In Fig. 4, the plot symbol shape (square, circle, or diamond) represents the amount of explosive (high, moderate, or near zero/zero respectively) estimated by LC-UV analysis. The position of the plot symbol on the Y-axis indicates the amount of explosive estimated by GC- or LC-MS analysis. A black dot inside a plotted symbol ("bullet hole") indicates that the explosive was also detected by fast GC-TEA (EGIS). Many of the swabs, including blank swabs that were part of each set of experiments, showed no traces of explosives by mass spectrometry, and for these samples a symbol was placed at log(y) = −4. A conservative definition of a sample positive for explosive was used: a positive sample was one in which an explosive was detected by both mass spectrometry and the EGIS instrument. Therefore, positive samples are shown in Fig. 4 by symbols off the x-axis and including a "bullet hole."

For the first set of samples (C-4 and dynamite bombs), a pair of swabs, one wetted with acetone and one with water, was used to collect residue from each piece of evidence; each cotton ball was wiped across half of the surface. Next, the two swabs were processed separately, one with acetone and the other with water and SPE. A difference in the number of swabs that were positive for explosives was found between the acetone- and water-treated swabs, and it is likely that much of this difference was due to the nature of the wetting solvent. Specifically, water alone was not very effective in collecting residue on cotton. In sampling the second set of bombing sites (Kinestik and TNT bombs), isopropanol:water-wetted cotton swabs were used exclusively. One cotton ball was used to sample each piece of evidence, and then the swab was cut in half. The two half-swabs were processed separately, one with acetone and the other with water and SPE. The use of identical swabs for sampling resulted in similar numbers of positives.

Observations about specific samples at the suitcase bomb sites are instructive. The least amount of explosives residue was found at the site of the C-4 detonation, and the traces of RDX were most concentrated near the seat of the explosion—on the plastic tarp, on the plywood, and on the bottom cover of the suitcase. Residue from the newsbox placed at the dynamite site was found to contain not only NG, but RDX, HMX, and TNT. This was not surprising since the newsbox had been used in the past for demonstrations, stored in a shed with many types of explosives, and handled by the bomb technicians. EGDN was found only on about half the evidence on which was found NG, and the ratio of EGDN to NG varied widely on the pieces positive for both. None of the samples collected from the witness plates at 5 m (C-4 and dynamite sites) were found to contain explosives. In response, the plates were placed closer to the suitcases in the second set of explosions in May. Acetone-rinsed clothing gave more positives for NG and EGDN in comparison to the water-based processing. The greatest amount of explosives residue was collected at the site of the TNT detonation. One suitcase part was covered with >500 μg TNT and a much smaller amount of 2,4-DNT, a contaminant/breakdown product of TNT. Positive samples were randomly distributed among the witness plates, except that more positives were collected from the aluminum foil wrap than from the polyethylene wrap. A couple of shirts tested positive for TNT, and one was positive for PETN, which was used in the detonating cord.

The Kinestik bomb should not have produced residue containing organic high explosives, yet nitroglycerin was found on a third of the swabs. This result likely was due to contamination from soil on the test range where smokeless powder had been burned and NG had been found before (13). Analysis of additional soil samples from the sand pits provided corroboration. Surface and subsurface samples of the sand pit at the Kinestik blast site were found by LC-UV to contain 6.8 μg/g and 3.0 μg/g of NG respectively. The second sand pit (TNT blast site) had lower levels of NG: 2.7 μg/g and 0.12 μg/g in the surface and subsurface samples respectively. LC-UV screening and GC/MS analysis did not indicate any other explosives in the sand pit samples. A soil sample from outside the sand pits in the vicinity of the C-4 and dynamite detonations did not indicate any explosives residue. Another source of potential contamination was the bomb technicians. Their hands were swabbed, and microgram quantities of PETN, RDX, DNT, and TNT were discovered.

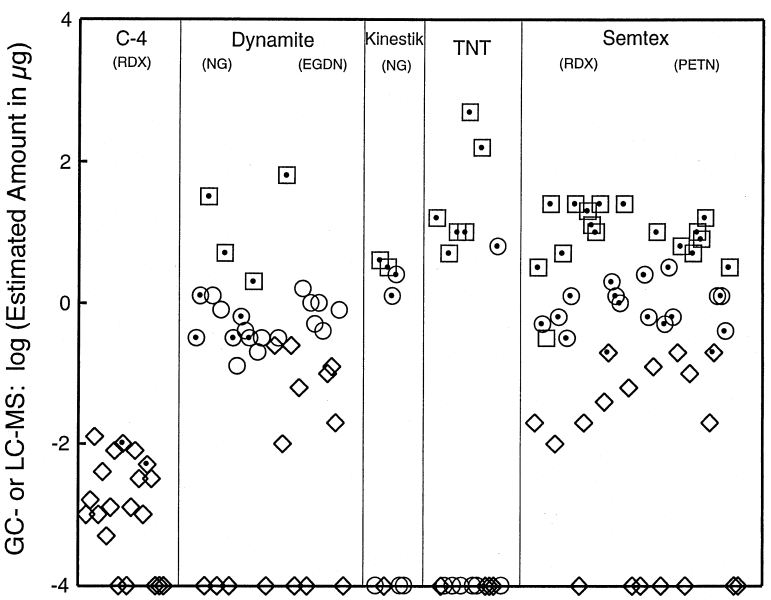

FIG. 4—*Semiquantitative results by LC-UV, GC- or LC-MS, and EGIS for samples generated by the detonation of explosives (C-4, dynamite, Kinestik, TNT) or handling Semtex and processed by water extraction/SPE. The shape of a plot symbol indicates the UV screening result: square (>2 μg of explosive on the piece of evidence); circle (>0.2 μg); diamond (<0.2 μg). The position of the plot symbol on the y-axis indicates the amount of explosive estimated by mass spectrometry. A "bullet hole" through the symbol indicates that the explosive compound was detected by EGIS.*

Skin was also a sampling surface in the Semtex H handling experiments. Almost all of the wipes taken after the subjects handled the plastic explosive tested positive for PETN and RDX, with about equal numbers of positives for water-processed and acetone-processed swabs. Again the similar numbers likely are due in part to the use of a common wetting agent (isopropanol: water) and dividing the swabs after sampling. Several hundred μg of RDX and lesser amounts of PETN were found on the hands of all four subjects, even the two persons who wiped their hands on a paper towel prior to sampling. The ratio of RDX to PETN varied widely, and the most concentrated samples also showed traces of HMX. The amount of explosives found decreased in the order of decreasing surface area: hands; combined steering wheel, dashboard, gear shift; interior driver's side door; car keys. In every case, wipes collected three days after exposure to Semtex were negative, indicating excellent collection efficiency by swabbing and/or poor persistence of the explosive compounds. Swabs taken of the interior of the subjects' automobiles (combined driver's side door, steering wheel, dashboard, and gear shift) before contact with Semtex also were negative for explosives except for a single positive swab. Because the owner of this automobile was the person conducting the Semtex experiments, contamination of the automobile or of the cotton during handling was suspected as the source of the RDX and PETN.

Comparison of the Water- and Acetone-Based Results—The LC-MS (GC-MS for EGDN) results for the nearly 100 pairs of samples are plotted in Fig. 5. Samples that had no traces of explosives were plotted on one or both axes, at log(x or y) = −4. A best-fit line is superimposed on the off-axis data and has the form: log y = (0.89 ± 0.05) log x + (0.12 ± 0.06). The y-intercept of the log-log plot is identical to the logarithm of the average ratio (acetone/water) of the results; the average ratio was 1.3 ± 0.4. This implies that the acetone-derived samples contained slightly more explosive than the water-derived samples, but the difference was not significant. The slope of the log-log plot is less than 1 and, thus, indicates that the bias toward acetone was greater at lower sample concentrations. This may have been due in part to the use of separate, differently-wetted swabs in the first two bombing cases, bombs that produced decidedly smaller amounts of explosives residue. In conclusion, water extraction/SPE processing produced the same results (not significantly different) as the more common procedure that relied on acetone.

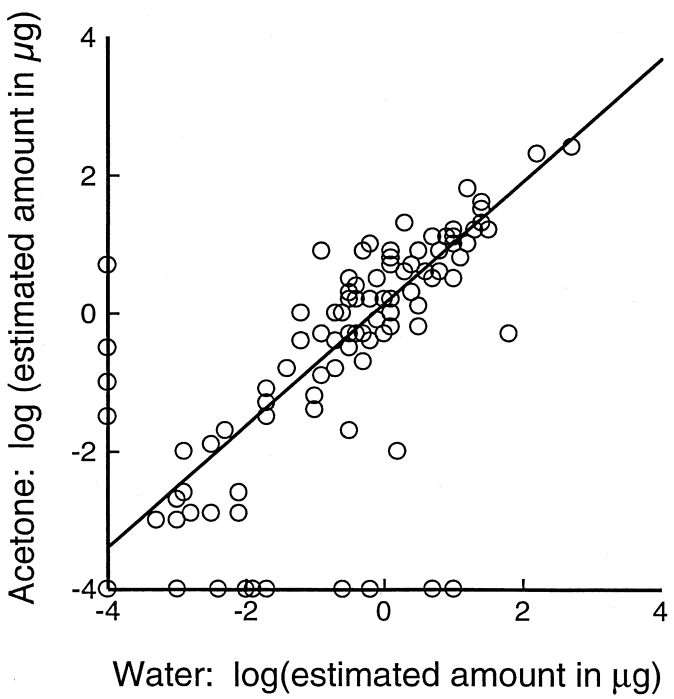

FIG. 5—*Comparison of the semiquantitative results from water-based analysis and acetone-based analysis. The straight line is drawn through the off-axis data and has the form: log y = (0.89 ± 0.05) log x + (0.12 ± 0.06).*

Sample Screening by LC-UV—Nearly all of the LC-UV results showed that extraction of cotton swabs with water, followed by SPE, produced samples that were largely free of significant matrix components. Screening for positive samples was possible with the water-based procedure because of the lower solubility of matrix components in water and the rejection of many soluble matrix components by solid phase extraction. This was in stark contrast to samples prepared by extraction with acetone. Figure 3b shows a typical result for the extraction of explosives from motor oil. In this experiment 10 drops of motor oil and an explosives mixture containing 600 ng of each of the explosives were spread on a clean aluminum foil sheet. The simulated sample was wiped up with a damp cotton ball, the swab was cut in half, and the halves were processed by the two methods. The acetone extract contained large amounts of early-eluting matrix components that made it difficult or impossible to detect small quantities of explosives. Quantitation was easily performed on the water-extracted sample.

Swabs from suitcase parts found at the suitcase bombing sites were processed and gave uncomplicated chromatograms. A typical set is shown in Fig. 3c and was the pattern for almost all of the swabs that were tested. The acetone extract contained some matrix components that interfered with the identification of the early-eluting explosives, HMX, RDX, and EGDN. Although TNT was easily identified, an overlapping peak made accurate quantitation difficult. The water/SPE extract was cleaner, and TNT quantitation was uncomplicated. In contrast, clothing samples proved problematic for both acetone- and water-based processing. Figure 3d demonstrates the difficulty in identifying by LC-UV TNT or any explosive compound at moderate to low concentrations on clothing. Further study is needed to identify the source of contamination and to develop a more selective procedure for processing clothing samples.

LC-UV screening of water/SPE-processed samples was quite accurate. Samples that contained moderate or high amounts of explosive (>0.2 μg; circles and squares in Fig. 4) based on LC-UV analysis were called positive screens; those estimated to have lower amounts were called negative screens (diamonds in Fig. 4). Nearly 80%, 103 of 131, of the LC-UV screens were confirmed by both mass spectrometry and EGIS testing, and all but one of the 29 samples estimated by LC-UV to have high amounts of explosive were confirmed as positives. Only four of the samples had negative screens that were unconfirmed. More common were samples that had positive screens, but in which explosives were not detected by EGIS alone (11%) or not detected by both mass spectrometry and EGIS (7%). In the former case, the errors likely were the result of high detection limits for some explosives on the EGIS instrument, and in the latter case the errors likely were caused by misidentification of coeluting matrix components (background LC peaks with similar retention times) as explosive compounds.

The correlation between the UV screening results and the mass spectrometry results was strong, as shown in Fig. 6. A best-fit line

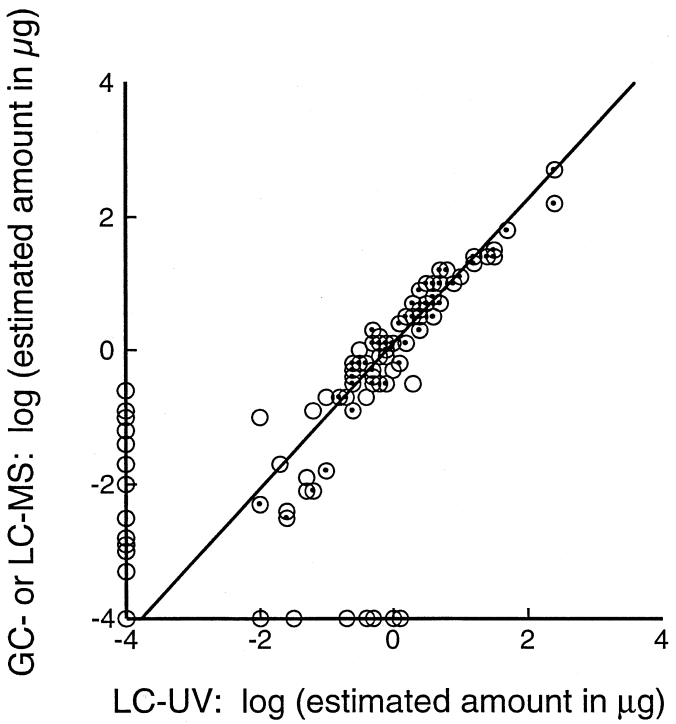

FIG. 6—*The nearly 1:1 relationship between the semiquantitative results from LC-UV analysis and GC- or LC-MS analysis. A "bullet hole" through the symbol indicates positive identification of the explosive compound by mass spectrometry and EGIS testing. The straight line is drawn through the off-axis data (both positives and negatives) and has the form: log y = (1.08 ± 0.04) log x + (0.04 ± 0.04).*

is superimposed on the off-axis data and has the form: log y = (1.08 ± 0.04) log x + (0.04 ± 0.04). The y-intercept of the log-log plot is identical to the logarithm of the average ratio (MS/UV) of the results; the average ratio was 1.1 ± 0.2. The slope of the log-log plot is slightly greater than 1. This means that LC-UV measurements slightly underestimated the amounts of explosives particularly at the higher levels. However, the differences were not significant.

Conclusions

The new method was easily automated and may directly handle residues derived from both inorganic and organic explosives. The water extract, after passing through the SPE cartridge, could be analyzed for inorganic ions, but this has yet to be demonstrated in practice. While the SPE step took an additional 30 min, the difference in time required for volume reduction (30 min conventionally and 10 min in the new procedure) was a balancing factor. Consequently, sample throughput was similar for the two methods.

Water extraction followed by SPE is an effective process for treating organic explosives residue on cotton swabs for subsequent analysis by liquid chromatography. Because interferences are reduced significantly in most cases, reliable screening of samples by LC-UV is facilitated.

Acknowledgments

RQT is grateful for the generous financial support by Oberlin College and its sabbatical leave program and by the Federal Bureau of Investigation. The assistance of personnel from the FBI Materials and Devices Unit in the preparation and detonation of the explosive devices and of Dennis Maslanka, FBI FSRTC, in sampling of some of the bomb sites is greatly appreciated.

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