J Forensic Sci, July 2007, Vol. 52, No. 4
doi: 10.1111/j.1556-4029.2007.00490.x
Available online at: www.blackwell-synergy.com

Désiré Laza,[1] *Ph.D.; Bart Nys,*[1] *Ph.D.; Jan De Kinder,*[1] *Ph.D.; Andrée Kirsch-De Mesmaeker,*[1] *Ph.D; and Cécile Moucheron,*[2] *Ph.D.*

Development of a Quantitative LC-MS/MS Method for the Analysis of Common Propellant Powder Stabilizers in Gunshot Residue*

ABSTRACT: In traditional scanning electron microscopy/energy dispersive X-ray analysis of gunshot residue (GSR), one has to cope more and more frequently with limitations of this technique due to the use of lead-free ammunition or ammunition lacking heavy metals. New methods for the analysis of the organic components of common propellant powder stabilizers were developed based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). A multiple reactions monitoring scanning method was created for the screening of akardite II, ethylcentralite, diphenylamine, methylcentralite, N-nitrosodiphenylamine, 2-nitrodiphenylamine, and 4-nitrodiphenylamine, present in standards mixtures. Five out of seven of these target compounds can be selectively identified and distinguished from the two others with a high accuracy. Samples from the hands of a shooter were collected by swabbing and underwent solid phase extraction prior to analysis. Detection limits ranging from 5 to 115 μg injected were achieved. Results from several firing trials show that the LC-MS/MS method is suitable for the detection of stabilizers in samples collected following the firing of 9 mm Para ammunitions.

KEYWORDS: forensic science, organic gunshot residue, primer ingredients, propellant powder stabilizers, LC-MS/MS, SEM/EDX, solid phase extraction

Current GSR Analysis

When a firearm is discharged, the bullet is expelled together with a cloud of vapors and particles, which are deposited on any object in the area surrounding the firearm. This mixture contains metallic components of the primer as well as the remainder of the unburned and partially burned propellant powder. Sometimes known as "cartridge discharge residues," or "firearms discharge residues," these products of the firearm discharge are commonly called "gunshot residues" (GSRs).

Gunshot residue analysis is routinely carried out in forensic laboratories in different ways according to the chosen objective. It is used to estimate firing distances, to identify bullet holes in clothing and objects, and to determine if a person was involved in a firing incident or was in the neighborhood during the firing of a weapon.

Neutron activation analysis, atomic absorption spectroscopy, inductively coupled plasma mass spectrometry (ICP-MS), and scanning electron microscopy (SEM) detect the presence of the inorganic elements originating from the primer. Combined with energy dispersive X-ray probe (EDX), this last method (SEM/EDX) is now by far the most widespread because it offers the advantage of providing images as well as the chemical composition by the analysis of X-ray spectra of individual GSR particles. In this respect, particles originating from a firearm discharge exhibit characteristic shapes and compositions, which are distinguishable from environmental interferences. Their composition depends on the primer ingredients and consists traditionally of Lead (Pb), Barium (Ba), and Antimony (Sb). More and more "lead-free" or "nontoxic" ammunitions are used, and it is consequently difficult to avoid false negatives when searching for GSR. A solution to overcome this problem may be sought in the detection of organic components of the propellant powders.

Composition of Propellant Powders—Stabilizers as Indicator of GSR

Smokeless propellant powders for small firearms are of two types: single base powders consist of nitrocellulose (NC); double base powders contain NC together with nitroglycerine (NG). Besides those two energetic ingredients, propellant powder composition includes several additives such as gelatinizing agents, flash suppressors, plasticizers, or stabilizers. NC and NG are nitric esters, which are respectively obtained by the nitration of cellulose and glycerine (1). Nitric esters undergo a thermal decomposition initiated by the splitting of ester functional groups and the production of various gaseous molecules (2) of which nitrogen monoxide (NO) and dioxide (NO_2) react as strong oxidizers. This spontaneous decomposition is followed by the production of nitrous and nitric acids, which can accelerate the process of decomposition (3). To inhibit this autocatalytic effect, stabilizers are added to propellant powder formulations. Stabilizers exert their effect by binding the nitrogen oxides (4) and preventing them from reacting with NC or NG. Diphenylamine (DPA) is the most common stabilizer used in single base powders, while ethylcentralite (EC) is added in double base powders, acting as stabilizer as well as gelatinizing agent for NG. Usually, propellant powders contain DPA together with EC. In addition, EC is frequently substituted by methylcentralite (MC) in Chinese ammunitions (5).

[1] National Institute of Criminalistics and Criminology (NICC), Ballistics Section, Chaussée de Vilvorde 98, 1120 Brussels, Belgium.

[2] Faculty of Sciences, Section Organic Chemistry and Photochemistry, Université Libre de Bruxelles, CP 160/08, Brussels, Belgium.

*Financial support provided by the Belgian Federal Office for Scientific, Technical and Cultural Affairs.

Received 11 Feb. 2006; and in revised form 30 July 2006 and 24 Feb. 2007; accepted 4 Mar. 2007; published 12 June 2007.

Propellant powders for conventional ammunitions invariably contain NC and at least one stabilizing compound. NC quantitatively constitutes the major ingredient, but is also used in lacquers, varnishes, celluloid films, printing, and pharmaceutical industries (6). Based on the comparison of molecular masses, Lloyd has analyzed and differentiated the higher molecular mass NC often used in propellants. After discharge of the firearm, the NC can be hydrolyzed and may not be distinguishable from the NC originating from environmental sources (7). This makes the detection of isolated NC, in the absence of other propellant powder components, not relevant for organic GSR analysis. As a stabilizer is a systematic part of each type of propellant powder formulation, its identification in a sample can be considered as an alternative to the detection of the NC. Identifying a component as GSR necessitates accurate information on its occurrence in the daily environment. To our knowledge, there is no data concerning the centralites (EC and MC). Conversely, DPA constitutes the most widely used stabilizer, particularly in single base propellant powders. However, it is also used, for its antioxidant properties, in the production of dyes, explosives, plastics, pharmaceuticals, and rubber (4,7). It is mainly used in agricultural sector for the control of superficial scald in apples and pears. Its detection without any of its nitrated derivatives cannot be considered of higher significance. The nitrated derivatives consist of nitro- or nitroso-compounds. They can result from the degradation of the DPA itself or originate from residual intermediary products in chemical industries. However, there is no information on the occurrence of this simultaneous detection.

Studies on the reaction mechanisms of the degradation of DPA during the aging of smokeless powder have been published (3,4,8–11). These mechanisms (involving N-nitrosation and denitrosation of the secondary amine of the DPA and C-nitration of the aromatic ring by electrophilic substitutions) lead to the formation of various reaction products. The plausible first stages of the pathways of the conversion are shown in Fig. 1. While aging, an increased number of hydrogen atoms of aromatic rings could be substituted and the ultimate stage should be the formation of the 2,2′,4,4′6,6′-hexanitrodiphenylamine, which was experimentally observed after 70 h of nitration/nitrosation of DPA at 120°C by NG (4). The occurrence of derivatives of DPA in aged propellant powders depends on the temperature of storage. At this temperature, the first step of the nitration is the formation of N-nitrosodiphenylamine (N-NO-DPA), followed by simultaneous C-nitration and N-denitrosation. The C-nitration occurs most likely at the para position.

Our screening of the composition of the widespread ammunitions in Europe by the use of LC/PDA (reverse phase liquid chromatography coupled to UV-detection) shows that the primary degradation products of DPA frequently encountered are essentially N-NO-DPA, 4-NO_2-DPA, and 2-NO_2-DPA. Therefore, those derivatives have been added to the list of chosen target compounds. Akardite II (AK II), which is frequently used as a stabilizer in double base

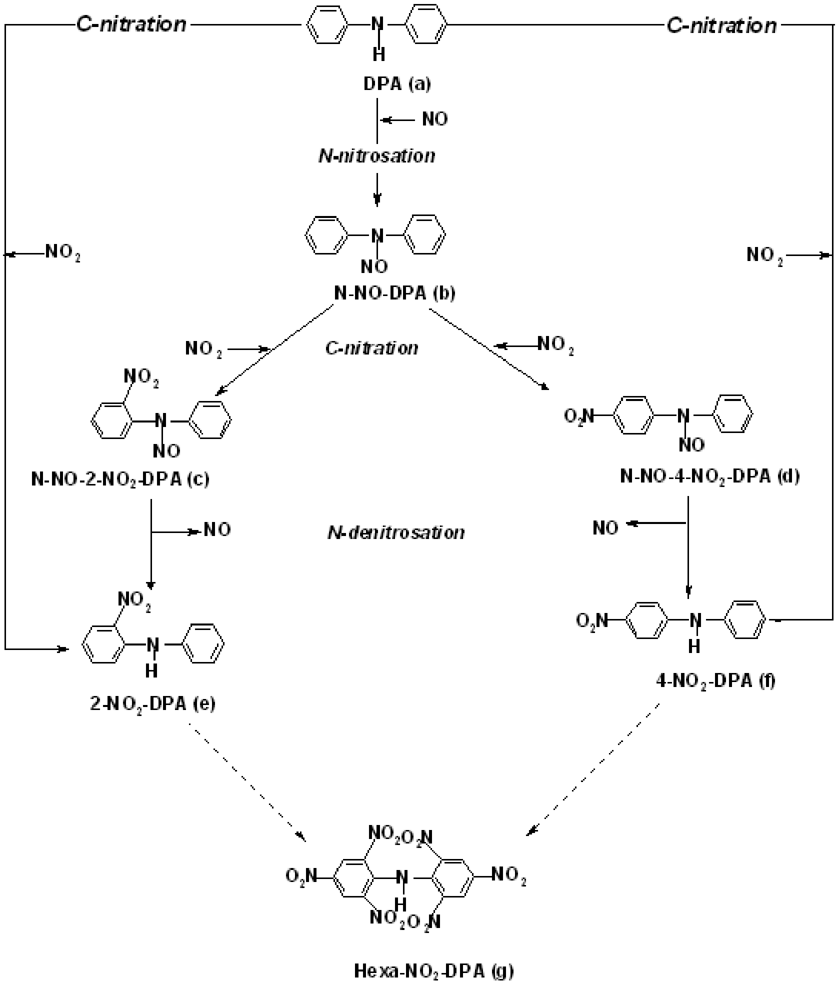

FIG. 1—*Decomposition pathways of DPA (a) leading successively to the formation of the N-NO-DPA (b), N-NO-2-NO_2-DPA (c), N-NO-4-NO_2-DPA (d), 2-NO_2-DPA (e), 4-NO_2-DPA (f), and hexa-NO_2-DPA (g).*

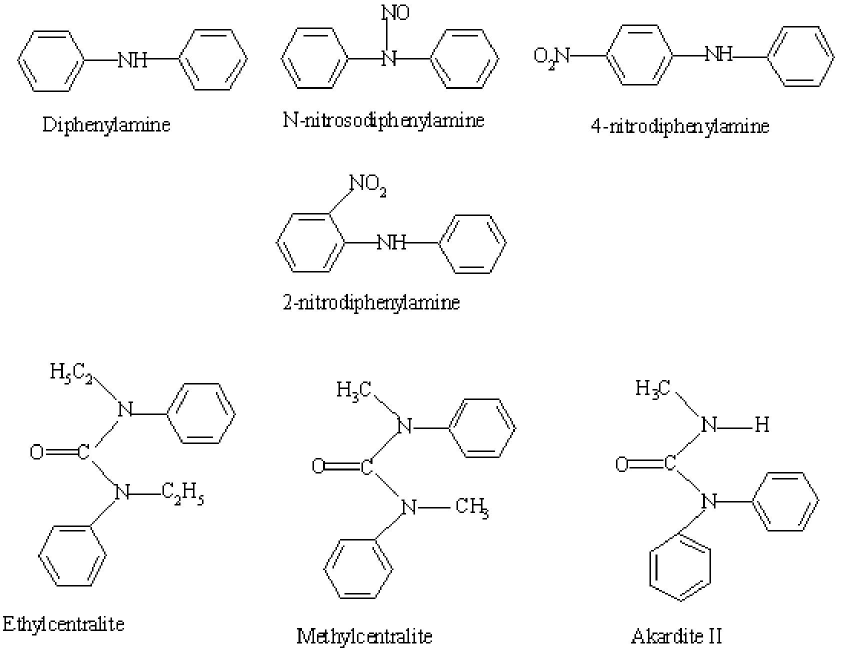

FIG. 2—*Molecular structures of the target compounds.*

propellant powders for rockets, has also been studied. Fig. 2 presents the molecular structures of the target compounds.

Review of Analytical Techniques Applied to the Analysis of GSR

The characterization of stabilizers in gun propellants is largely described in literature (12–16), and the comparison of propellant powder composition with its GSR has been reported for some types of ammunition (17–19). The applicability of analytical methods for the detection of GSR depends both on the sensitivity (determination of concentrations at trace levels) and on the selectivity (separation of different compounds in a mixture).

Gas chromatography (GC) is widely used for the analysis of explosives in many forensic laboratories (20,21) and can be applied successfully to the detection of smokeless powder components (22).

Gas chromatography–mass spectrometry (GC/MS) and high-performance liquid chromatography with a pendant mercury drop electrode (HPLC/PMDE) (23) have been applied for the analysis of NG, 2, 4-dinitrotoluene (2, 4-DNT), DPA, EC, and MC.

Gas chromatography/thermal energy analyzer (GC/TEA) and ion mobility spectrometry (IMS) are frequently used as a part of technologies for the detection of explosives (24,25). In the TEA-technique, the IR light emitted by electronically excited NO_2^- groups is detected. This explains the common use of the GC/TEA for the detection of explosives containing NO_2^- groups and in the analysis of propellant constituents such as NG, DNT, and nitrode-rivatives of stabilizers. During tests we performed in our laboratory, IMS turned out to be easy to use, but the detection of GSR constituents other than NG did not give satisfactory results.

Micellar electrokinetic capillary electrophoresis (26–28) has been used to separate and identify organic GSR as well as explosive constituents such as ethylene or diethylene glycol dinitrate, NG, trinitoluene (TNT), and others. If well optimized, this technique constitutes a selective tool. Coupled to an UV-detector, its efficiency could be affected by the low sensitivity of such a type of detector.

HPLC-MS/MS for the Analysis of Organic GSRs

High performance liquid chromatography coupled to tandem mass spectrometry (HPLC/MS/MS) constitutes a powerful technique for the analysis of organic residue traces because it combines high specificity with high sensitivity (6). Wu et al. have developed a "tandem MS method" for the determination of DPA and its nitrated derivatives in unburned smokeless gunpowder (29). Experiments were carried out using a flow-injection system, which enables a direct injection of samples into the mass spectrometer. To this purpose, a chromatographic column was installed before the sample injector providing a constant solvent flow rate and con sequently stabilizing the ion current. DPA, N-NO-DPA, 4-NO_2-DPA, 4-nitrosodiphenylamine (4-NO-DPA), and 2, 4-dinitrodiphenylamine (2, 4-2NO_2-DPA) were used as standards. Optimizations of the tandem MS conditions as well as the quantitative analyses were performed using standard solutions in methanol. The limits of detection were 5.9, 2.5, and 11.7 nmol/L, respectively, for DPA, N-NO-DPA, and 4-NO_2-DPA, as detected in propellant gunpowder samples. The authors suggested the use of this method for the analysis of GSR and have analyzed recovery tests from hands. In this respect, a standard solution of a target compound was spread on the hand of a person and left to evaporate. The hand was subsequently wiped with a cotton swab soaked in methanol. The sample preparation consisted of squeezing the swab using a medical syringe followed by a dilution of the resulting aliquot with methanol. The same group has undertaken a similar approach for the analysis of MC in propellant gunpowder as well as in samples collected from the hands of shooters.

In this work, our approach takes into consideration a number of relevant parameters when it comes to taking samples. The protocol needs to take into account the nature of the substrate. All sources of contaminations must be prevented, ranging from the crime scene over the actual sampling material to the laboratory environment (30). This implies the need for making of new sample collection kits, the validation of the method, and a continuous quality control of analytical procedures. The organic GSR kits should be suitable for the collection of samples from the human skin (surface of the hands and the face of a person suspected of crime with firearms or a victim) as well as the collection of compounds from objects situated in the neighborhood of the shooting incidence. By the development of a sample preparation protocol based on the solid phase extraction (SPE) the sensitivity of detection has been increased, at least, by 10-fold. This was obtained in comparing the limit of detection of standards components successively analyzed with and without a previous SPE processing.

In addition, a target compound is determined by a retention time (resulting from the chromatographic separation) in conjunction with the identification of specific ions by MS. This increases the selectivity and consequently reduces the risk of obtaining false positive results.

Experimental

Apparatus and Reagents

Experiments were carried out with a Quattro Micro triple quadrupole mass spectrometer incorporating a Z-spray source (Waters, Milford, MA) and coupled to an Alliance 2695 HPLC system (Waters) through an Electrospray probe interface. The resulting LC-MS/MS system was controlled by "MassLynx" software. The mass spectrometer was attached to a "Peak Scientific" nitrogen generator and to a stand-alone argon source. The main working parameters for the mass spectrometer were the following: ionization mode, positive electrospray (ESI^+); capillary (kV), 3.20; cone voltage, 10–45 V (component dependent); extractor (V), 2; RF lens (V), 0.1; source temperature (°C), 120°C; desolvation temperature (°C), 350°C; cone gas flow (L/h), 120; desolvation gas flow (L/h), 360; multiplier (V), 650; gas cell Pirani pressure (mbar), 1×10^{-4}.

Diphenylamine, N-NO-DPA, 2-NO_2-DPA, 4-NO_2-DPA, AK II, and pure formic acid (FA) were purchased from Sigma Aldrich (Bornem, Belgium). EC and MC were kindly supplied by PB Clermont, a Belgian gunpowder manufacturer. A concentrated solution (1000 g/L) of each target component was prepared in isopropyl alcohol, stored in the refrigerator, and kept in the dark. Cotton swabs "STELLA 61" were used (Stella, EtsJ. Delrivière, Waterloo, Belgium). They were cleaned by rinsing successively with water, acetone, and isopropyl alcohol followed by an overnight drying at 80°C. Centrifugation tubes with a capacity of 50 mL (25 mL with an inserted filtering tube) were purchased from Alltech (Lokeren, Belgium). A vacuum manifold (Waters) and lots of SPEC C18 cartridges (Varian) for solid-phase extraction were used. The vacuum manifold was connected to a vacuum pump working with compressed air (Pneuvano, Wommelgem, Belgium), allowing for a vacuum pressure up to 10^6 Pa (10 bar). This manifold has the capacity to process up to 20 samples simultaneously.

Sample Collection and Preparation

In the experiments, we have performed sets of five shooting, with different makes of firearms. The used ammunitions are listed in Table 1. A set of firing trials was carried out once a week and careful attention was paid to minimize the risk of contamination. At the beginning of the firing sequence prior to all other activities of the shooter, the venting system in the shooting room was turned off. A same shooter performed all of the shooting, and the firearm was not cleaned between firings. The shooter was first asked to wash his hands with soap and tap water and wipe them with a clean kitchen paper. A blank sample was taken and five ammunition rounds were afterwards discharged. Immediately after each firing, the thumb and forefinger back area of the shooting hand were thoroughly swabbed by means of cotton swabs moistened with isopropyl alcohol/water, 75/25% (v/v). Isopropanol was used as a solvent as it is less toxic than methanol. The dirty swab was separately put in a filtering centrifugation tube, and 3.2 mL of dissolving solvent (isopropyl alcohol/water [75/25%]) was subsequently added. The tube was then centrifuged and the resulting aliquot was diluted five times with de-ionized water. This dilution constitutes a crucial requirement in our sample preparation protocol, which involves a SPE technique as a tool for concentration and purification of samples (31). For this purpose, a set of SPEC C18 cartridges, containing octyldecyl-bonded phase sorbents, were fitted to the vacuum manifold and conditioned by rinsing successively with 250 μL of isopropyl alcohol and with the same volume of de-ionized water. A total of 5000 μL of the aqueous samples were subsequently loaded, and the liquid phase was pumped out. The sorbents were afterwards rinsed with 250 μL of de-ionized water and dried. The compounds of interest were eluted in 200 μL of acetonitrile/water/methyl alcohol: 80/10/10% (v/v). To optimize the precision and the accuracy of the final analysis, the level of the vacuum was controlled during the extraction procedures. The vacuum level was maintained at 2 inch Hg (7 kPa) during conditioning procedures, while the loading step was performed at 2–5 inch Hg (7–17 kPa). A vacuum level of 10 inch Hg (33 kPa) was used for drying the sorbent bed before the elution with a vacuum level of 2 inch Hg of the components of interest. A completed elution was achieved by increasing the final pressure level up to 7 inch Hg

TABLE 1—*Characteristics of the used ammunitions.*

No.	Ammunition	Caliber	Weight	Country	Production	Lot no.
1	Dynamit Nobel Sintox	9 mm Para	6 g	Germany	1996–1997	61MR
2	Federal Ballisticlean	9 mm Para	100 grains	USA	1995	BC9NTI
3	Speer & Lawman	9 mm Para	124 grains	USA	2002	K16F22
4	Fiocchi (gas check)	9 mm Para	123 grains	Italy	2003	4012249–1379
5	Fiocchi	9 mm Para	123 grains	Italy	2002–2003	3903033/3416
6	Dynamit Nobel (DN) Geco	7.65 mm Browning	73 grains	Germany	2000	46SU 3320
7	Fiocchi	7.65 mm Browning	73 grains	Italy	2003	41055039–1471
8	DN RWS	7.65 mm Browning	73 grains	Germany	Unspecified	49QM
13	MFS	7.65 mm Browning	71 grains	Hungary	2003	Unspecified
9	Sellier & Bellot	7.65 mm Browning	73 grains	Czech Republic	Unspecified	MC27
10	CCI	.22 LR	40 grains	USA	Unspecified	B10R13
11	DN RWS	.22 LR	40 grains	Germany	2002	57SV4
12	Fiocchi	.22 LR	40 grains	Italy	Unspecified	2510125
14	Remington	.22 LR	40 grains	USA	Unspecified	1500
15	Winchester	.22 LR	40 grains	USA	Unspecified	ACD16 F03

(25 kPa). As the vacuum pressure varied, the flow rate varied accordingly and was not specifically determined.

To determine calibration curves, standard mixtures in the concentration range from 0.4 to 150 nmol/L were prepared in isopropanol/water (75/25%). A total of 3.2 mL of each mixture was poured into a filtering centrifugation tube containing a clean swab and subsequently centrifuged. The aliquot underwent the same treatment as the samples collected from the hands of the shooter. In addition, solutions containing 500 nmol/L of each standard compound were prepared in acetonitrile/water (50/50%) for the optimization of the MS/MS detection parameters.

LC Conditions

In order to achieve separation of components, a gradient reverse phase chromatographic method was developed. The method involved the use of an Xterra MS C18 column (3.5 μm, 2.1 × 150 mm; waters) and a ternary mobile phase (acetonitrile/methanol/water). The mobile phase was acidified by 0.1% (v/v) of FA and had a flow rate of 200 μL/min. The temperature was kept at 30°C. An injection volume of 20 μL was used for the qualitative and quantitative analyses. The gradient chromatography program contained eight entries, as described in Table 2.

TABLE 2—*Gradient chromatography program.*

Time (min)	Solvent A (%)	Solvent B (%)	Solvent C (%)	Flow (μL/min)	Curve
	40.0	10	50.0	200	1
3	36.6	10	53.4	200	6
10	35.0	10	55.0	200	6
11	10.0	10	80.0	200	6
13	10.0	10	80.0	200	6
14	40.0	10	50.0	250	6
19	40.0	10	50.0	250	6
20	40.0	10	50.0	200	1

Solvent A: water + 0.1% (v/v) of concentrated formic acid; solvent B: methanol + 0.1% (v/v) of concentrated formic acid; solvent C: acetonitrile + 0.1% (v/v) of concentrated formic acid.

Results and Discussion

Optimization of the Tandem Mass Spectrometer (MS/MS) Detection

A solution containing 500 nmol/L of a target compound (described by a molecular formula "M") was injected into the mass spectrometer by "infusion" (a direct injection) using a 250 μL Hamilton syringe, with a flow rate of 10 μL/min. The formation of ions in gas-phase was achieved using a positive electrospray ionization (32) leading to the formation of positively charged species, which could be a molecular ion, a radical ion, or a protonated molecular ion. In this work, the protonated molecular ions $[M + H]^+$ were chosen as precursor ions and their presence was determined by scanning the mass-to-charge ratios (m/z) in a range including the m/z of the precursors. Depending on the target compound, the cone voltage was adjusted to maximize the intensity of signals of the precursor. In these conditions, AK II, MC, EC, DPA, N-NO-DPA, and the two isomers of nitrodiphenylamine were identified with m/z value of 227, 241, 269, 170, 199, and 215, respectively. The precursors were subsequently fragmented by collisions-induced decomposition in the collision cell (which was filled with Argon gas, 6.0 ultraplus, at 0.35 Pa), followed by the scanning of the product ions. During this process, usually called "daughter scan," the collision energy was adjusted to optimize the signals for the most abundant product ions. Depending on the molecular structure of the precursor, the number and the characteristics of the product ions varied. An illustration can be seen from Fig. 3, which is composed of two spectra obtained from the product scan of the protonated 4-NO_2-DPA and 2-NO_2-DPA, with collision energy of 25 eV. The two precursors have a same value of m/z equal to 215. We can observe that the first precursor gave markedly a product ion with an m/z of 168, while the second precursor led to the formation of most intensive signal corresponding to an m/z of 180. A precursor can consequently be decomposed into numerous products ions. In these experiments, we associated each precursor with a single product ion that provided the most sensitive and reproducible signal. An m/z value of 92, 134, 120, 93, 169, 168, and 180 was respectively attributed to the product ion of the precursor AK II, MC, EC, DPA, N-NO-DPA, 4-NO_2-DPA, and 2-NO_2-DPA. We already have observed that each of the target compounds can be specifically identified by one couple of precursor and product ions.

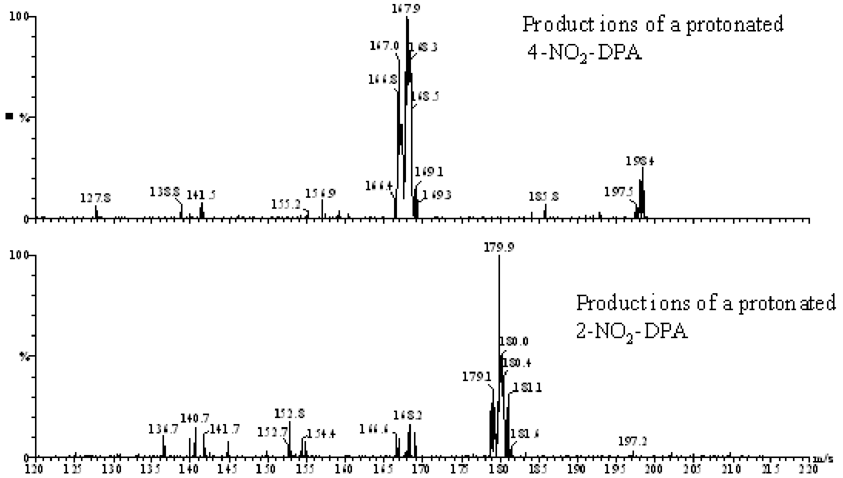

FIG. 3—*Mass spectra obtained from the product scan of the protonated 4-NO_2-DPA and 2-NO_2-DPA, with collision energy of 25 eV.*

Our ultimate goals were to set up multiple reaction monitoring (MRM) detection conditions and finally to develop an MRM method for qualitative and quantitative analyses. In this detection mode, both quadrupoles operate statically: Q_1 is focused on a specific precursor and Q_2 on a product ion. The MRM detection mode consists of a combination of two or more single ion monitoring scans concurrently performed with a single stage mass spectrometer. When dealing with a stand-alone tandem mass spectrometer (uncoupled with as HPLC instrument, for example), the use of an MRM detection mode could increase the sensitivity by reducing the noise interferences as well as the selectivity by matching at least two different reactions. Because the fragmentation of the precursor constitutes the single source for the product ion, the MRM detection mode enables the determination of the presence of precursor ions in samples and thus the identification of the corresponding target compounds.

TABLE 3—*MRM method.*

Compound	Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision energy (eV)	Channel
DPA	170	93	37	25	1
N-NO-DPA	199	169	15	12	2
4-NO_2-DPA	215	168	25	23	3
2-NO_2-DPA	215	180	25	17	4
AK II	227	32	27	17	5
MC	241	134	25	15	6
EC	269	120	25	15	7

A dwell time of 0.14 sec was needed for the scanning of each transition, including an inter-channel delay of 0.05 sec, all the transitions were scanned within a cycle time of 1.33 sec; 541 scans were performed during a total acquisition time of 720 sec (12 min). Cycle time: 1.33 sec. Retention window /total acquisition time (sec): 720 sec.

AK II, akardite II; DPA, diphenylamine; EC, ethylcentralite; MC, methylcentralite.

For these experiments, the MRM method deals with seven target precursors and is composed of seven transitions, which are defined as the association of a precursor to its specific product ion. As shown in Table 3, transitions are scanned one after another by ascending order of the m/z values of precursors. As an example, the transition "170 > 93" of DPA is first scanned during a dwell time of 0.14 sec, followed by automatic signal processing and storage of the data in channel 1. A delay of 0.05 sec (inter-channel delay) is respected between two successive transitions. This adds up to a cycle time of 1.33 sec for the seven transitions. The

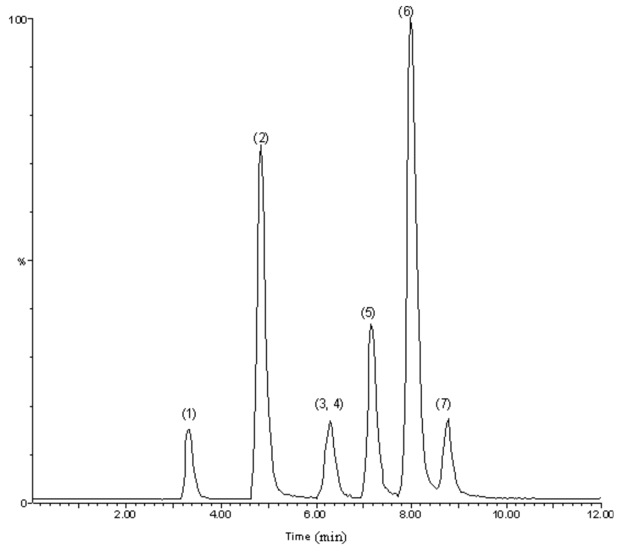

FIG. 4—*A typical MRM chromatogram (TIC versus the time) obtained by the injection of 20 μL of a standard sample containing 150 nmol/L of each target compound. Peak "(1)", "(2)", "(4)", "(5)", and "(6)" correspond to AK II, MC, DPA, EC, and 2-NO_2-DPA, respectively. The peak "(3 and 4)" results from the co-elution of N-NO-DPA with 4-NO_2-DPA.*

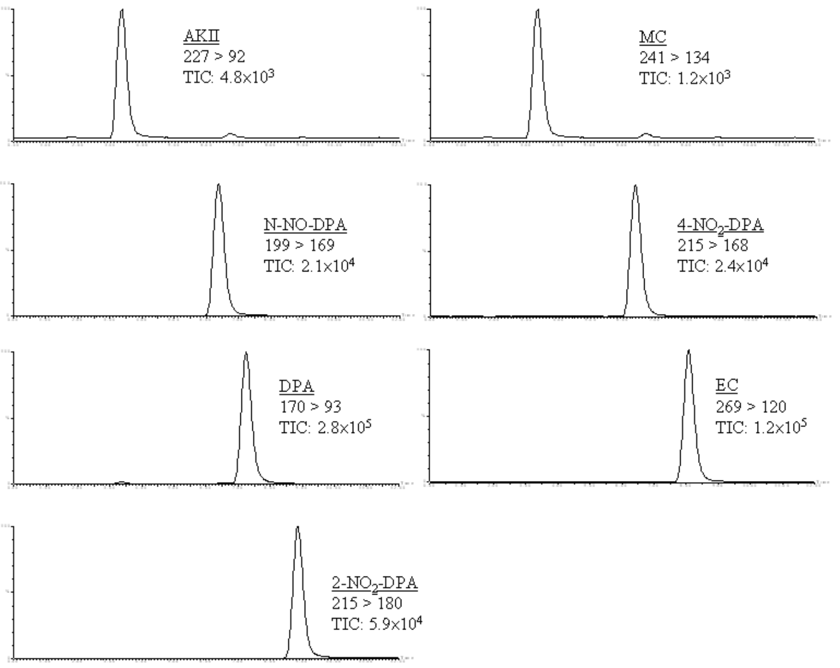

FIG. 5—*Extracted ion chromatograms; all target compounds can be differentiated by MRM transitions, as given in Table 3.*

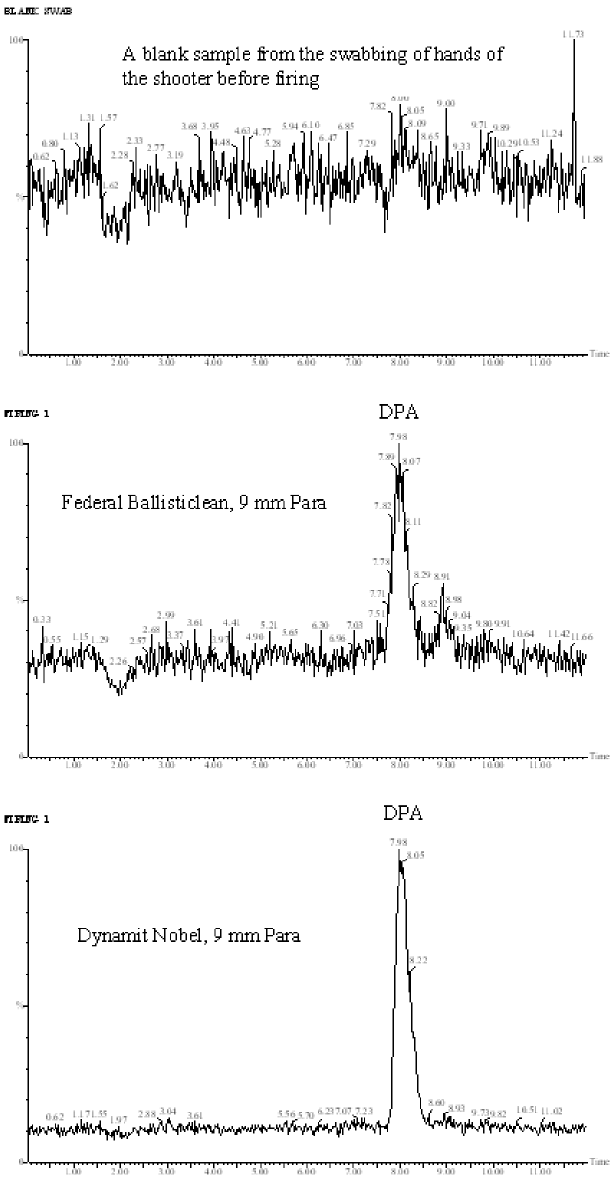

FIG. 6—*Chromatograms obtained by LC-MS/MS (MRM) analysis of samples collected from the shooting hands.*

spectrometer, coupled to the HPLC instrument, performs 541 scans for a retention window (a total acquisition run-time) of 12 min (720 sec). Data are recorded as intensity versus time. The total ion current chromatogram, i.e., the sum of noise and signals, can be displayed and the m/z values of the peaks are determined. Usually, the software (MassLynx) automatically performs this assignment by the comparison of the signals with the time at which Q_1 was statically focused on an m/z value of a precursor ion and Q_2 on an m/z value of the product ion.

Validation of the LC-MS/MS (MRM) Method

Qualitative analyses were performed by successive injections of standard mixtures containing DPA, N-NO-DPA, 4-NO_2-DPA, 2-NO_2-DPA, EC, MC, and AK II. As shown in Fig. 4, a typical MRM chromatogram consists of six peaks instead of seven, because N-NO-DPA co-elutes with 4-NO_2-DPA. By reversed-phase chromatographic separation, 2-NO_2-DPA has a later retention time than 4-NO_2-DPA, its structural para isomer. Indeed, the NO_2-group in ortho position could bind the hydrogen atom of the secondary amine and would become less favorable for polar interactions with the mobile phase, making the entire molecule of 2-NO_2-DPA less polar than the para isomer. Consequently, the two compounds would exhibit a different affinity for the nonpolar surface of the stationary phase and can thus be separated by their retention times. Figure 5 shows the extracted ion chromatograms for all the MRM transitions. In spite of the chromatographic co-elution and the fact that the m/z of product ions differ only by 1 unit, N-NO-DPA and 4-NO_2-DPA were well distinguished. This distinction was made possible because of successful matching of the precursor ion and the product ion. Chromatographic retention times constitute supplementary information. In summary, the LC-MS/MS method allowed the selective identification of DPA, 2-NO_2-DPA, EC, MC and AK II and the distinction with a high accuracy of N-NO-DPA, 4-NO_2-DPA, contained in the analyzed mixtures.

Quantitative analyses were performed using straight lines obtained from an automatic least-squares curve fitting (33), which consists of plotting the absolute MRM transition responses (peak areas) versus the concentrations (nmol/L) of each target molecule. A series of experiments were conducted to determine the stability of the calibration curves. Results showed that the correlation coefficient varied from one component to another. Its estimated value is 0.991 and 0.993 respectively for AK II and MC, 0.995 for DPA, N-NO-DPA, and 4-NO_2-DPA, and 0.997 for EC and 2-NO_2-DPA. Experiments were repeated eight times within a period of 4 months (one set of experiments every 2 weeks), using freshly prepared standard solutions.

The repeatability of the method was verified through replicate extractions and analyses of a mixture containing approximately 5 nmol/L of target compounds. The results obtained from 30 trials showed that the retention times of "extracted ion chromatogram" peaks fluctuated with an averaged relative standard deviation (RSD) of 0.45%. The concentration was measured with a precision of <17% (RSD), except for AK II (29%). The high values of the RSD resulted, in part, from the influence of the SPE procedures. Indeed, we have performed the same repeatability study without the SPE procedures and have observed that the precision was improved. The corresponding RSD varied from 10% to 15%, which constitutes an acceptable range for a method applied to the analysis of trace compounds at such a low concentration. Also, these experiments allowed increasing the correlation coefficient up to 0.999.

The recovery of the completed method (i.e., including the sample preparation and the chromatographic separation) was studied. With an initial concentration equivalent to 1 pg/μL and an injection volume of 20 μL, the recovery obtained from the analysis of 30 standard samples was of 57% to 89%. At these concentration levels and a signal to noise ratio (S/N) of 3 (34), the limits of detection were 0.29, 0.27, 0.34, and 0.21 nmol/L for DPA, N-NO-DPA, 4-NO_2-DPA, and 2-NO_2-DPA, respectively. EC as well as MC had a limit of detection of 0.07 nmol/L. A limit of detection of 1.3 nmol/L was determined for AK II. Converted into the equivalent amount of target compounds injected onto the column, the limits of detection were of 5, 6, 20, 27, 32, 34, and 115 μg injected onto the column for EC, MC, 2-NO_2-DPA, N-NO-DPA, 4-NO_2-DPA, DPA, and AK II, respectively. The method was thus found very sensitive for the analysis of centralites (EC and MC). Further investigations on the recovery of SPE procedures showed that the SPEC C18 cartridges poorly retain AK II and explained the relative low sensitivity of detection for this compound.

Analysis of Stabilizer Residues in Samples Collected from the Hands of a Shooter

The concentration of the target compounds in samples collected from the hands of a shooter was determined. Values were dependent on the target compound as well as the fired ammunition and varied within the range from 0 to 50 nmol/L. The results exhibited an uncertainty level of >50% (RSD). The results of the analyses of samples collected by swabbing into the inside of expelled cartridges showed similar fluctuations. Thus, the quantitative analysis of GSRs is affected by the parameters involved in firing process, such as the type of the ammunitions and weapons used and, most likely, the recovery of the combustion reactions. The high level of uncertainties of quantitative results should consequently be considered as independent of the analytical method. Moreover, the number of firings per set of trials is low for a confident determination of the RSD value.

In addition, a qualitative comparison of ammunition types was performed. To this purpose, an average concentration obtained from the five shots was compared to the corresponding limit of detection (nmol/L). The result was expressed as a "ratio of detection" (ROD). A target compound should be detectable when the value of ROD is larger than 1. DPA was detected in all of the GSR samples. Indeed, LC-MS/MS chromatograms exhibited a peak of DPA with retention of 7.9 min, as illustrated in Fig. 6. The ROD value depends on the caliber and the type of the ammunition. Samples from Federal Ballisticlean 9 mm Para, RWS 7.65 mm Browning, and Dynamit Nobel Sintox 9 mm Para exhibited ROD values higher than 100 units, whereas ROD of 11 and 42 were respectively measured in samples obtained from the firing of Fiocchi ammunitions of 9 mm Para (ZP and Gas Check 9) and MFS 7.65 Browning. This value was found <6 units in samples from Speer & Lawman 9 mm Para, Dynamit Nobel 7.65 mm Browning and from all ammunitions of .22 Long, as described in Table 4.

N-NO-DPA was the most frequently detected DPA derivative. It was observed in 73.3% of the examined ammunition types: Fiocchi-ZP, Fiocchi Gas Check, Speer & Lawman and CCI .22 Long did not exhibit a significant value of ROD. N-NO-DPA was remarkably detected in samples from Winchester .22 Long, with a ROD value higher than that of DPA. This would mean that the propellant powder was already aged and most of the initial amount of DPA would have been converted into N-NO-DPA. In addition, N-NO-DPA was detected in combination with 4-NO$_2$-DPA and 2-NO$_2$-DPA in samples collected from the firing of Dynamit Nobel Sintox, Federal Ballisticlean, Fiocchi 7.65 mm Browning and Fiocchi .22 Long. Based on the degradation pathway of DPA, the detection of 4-NO$_2$-DPA or 2-NO$_2$-DPA with the absence of N-NO-DPA could arise if DPA as well as N-NO-DPA were both converted into nitroderivatives. The combination of 4-NO$_2$-DPA or 2-NO$_2$-DPA with DPA in trials using Fiocchi Gas Check or Fiocchi-ZP can only be explained by contaminations of the firearm, which was not cleaned between successive firing sequences. Indeed, the quantitative analysis of propellant powders showed that Fiocchi type ammunitions are specifically stabilized by EC, which also constitutes the single stabilizer for the Speer & Lawman ammunitions. Consequently, the firing of those ammunitions cannot logically release DPA or its nitroderivatives. Dynamit Nobel Sintox and Federal Ballisticlean ammunitions are stabilized by DPA together with EC. These compounds were detected in combination with derivatives of DPA. Finally, it should be stressed that neither AK II nor MC was observed in the tested samples.

Conclusions

The aim of this work was to develop methods for the analysis of organic components of propellant powder residues and to assess their application in chemical ballistics. It is motivated by the increasing need to overcome the inherent problems with the analysis based on the characterization of the metallic ingredients of the primer, more particularly when the use of lead-free ammunitions or ammunition without "heavy metals" is involved. In this framework, protocols were set up for sample collection and preparation, simultaneously with the development of a selective and highly sensitive LC-MS/MS method for the analysis of propellant powder stabilizers residues collected from the hands of the shooter. The results confirm that EC and DPA are usually used as stabilizing

TABLE 4—*Influence of weapons and ammunitions.*

			Detected compounds									
			N-NO-DPA		4-NO$_2$-DPA		DPA		EC		2-NO$_2$-DPA	
Firearm	Ammunition	Caliber	ROD	RSD (%)	ROD	RSD (%)	ROD	RSD (%)	ROD	RSD (%)	ROD	RSD (%)
FN GP 35	DN Sintox	9 mm Para	48	106	26	129	120	175	31	126	18	177
	Fiocchi-ZP	9 mm Para	< LOD	–	< LOD	–	11	36	76	52	3 (< LOQ)	42
	Fiocchi Gas check	9 mm Para	< LOD	–	10	70	11	112	31	267	8 (< LOQ)	63
	Speer & Lawman	9 mm Para	< LOD	–	< LOD	-	5 (< LOQ)	275	115	80	< LOD	–
	Federal Ballisticlean	9 mm Para	61	57	27	100	289	56	< LOD	115	15	71
MABD	Fiocchi	7.65 mm Browning	80	105	7(< LOQ)	52	86	104	< LOD	–	1(< LOQ)	45
FN 1922	DN	7.65 mm Browning	1 (< LOQ)	45	< LOD	–	4 (< LOQ)	82	< LOD	–	< LOD	–
	MFS	7.65 mm Browning	6(< LOQ)	122	< LOD	–	42	92	< LOD	–	< LOD	–
FN 1910	RWS	7.65 mm Browning	2 (< LOQ)	45	< LOD	–	210	46	< < LOD	–	< LOD	–
	S & B	7.65 mm Browning	1 (< LOQ)	45	< LOD		4 (< LOQ)	82	< LOD	–	< LOD	–
Bernardelli Sport	Remington	.22 Long	1 (< LOQ)	109	< LOD		2 (< LOQ)	41	< LOD	–	< LOD	–
	CCI	.22 Long	< LOD	–	< LOD	–	5 (< LOQ)	32	< LOD	–	< LOD	–
	Winchester	.22 Long	15	119	< < LOD		1 (< LOQ)		< LOD		1 (< LOQ)	
	Fiocchi	.22 Long	5 (< LOQ)	156	2 (< LOQ)	96	5 (< LOQ)	52	< LOD	–	2(< LOQ)	160
	RWS	.22 Long	1(< LOQ)	160	< LOD	–	3 (< LOQ)	45	< LOD	–	< LOD	–

A ROD value constitutes a ratio of a concentration (nmol/L) to the corresponding limit of detection; it is presented without a decimal significant figure because of the high level of the uncertainty linked to the concentration; LOD = 3 × (S/N) and LOQ = 10 × LOD.

DPA, diphenylamine; S/N, signal to noise ratio.

compounds of propellant powders. DPA was detected in association with its nitrated derivatives among which N-NO-DPA is the most frequently encountered, followed by 4-NO_2-DPA and 2-NO_2-DPA. When detected in combination with DPA, the nitrated derivatives could consequently be used as indicators for the presence of organic GSR. This assumption should, however, be supported by complementary investigations, including the study of the persistence of those propellant powder stabilizers on the hands of the shooter and the evaluation of their occurrence on the hands of nonshooting persons. The analyses of samples collected from the hands of a shooter demonstrate that the developed method is suitable for routine analyses of GSR but could also be used for the screening of propellant powders. The results confirm that the LC-MS/MS method constitutes an alternative technique for organic GSR analyses, in conjunction with others. However, it should be optimized for an efficient detection of organic GSR expelled from ammunitions of a caliber <9 mm Para.

Acknowledgments

We are grateful to Mrs. S. Smeets for her daily technical assistance and to her colleagues at the ''Firearms Lab'' who contributed to the firing sequences. We also thank Dr. A. Leriche, Head of Department at the NICC for her motivation and encouragement.

References

1. Kubota N. Propellants and explosives: thermochemical aspects of combustion. WILEY—Weinheim: VCH GmbH, 2002;59–97.
2. Quinchon J, Tranchant J, Nicolas M. Les poudres, propergols et explosifs (Tome 3): Les poudres pour armes. Paris: Technique et Documentation (Lavoisier), 1986;115–27.
3. Davis TL. The chemistry of powder and explosives. San Pedro: GSG & Associates Publishers, 1943;307–13.
4. Espinoza EO'N, Thornton JI. Characterization of smokeless gunpowder by means of diphenylamine stabilizer and its nitrated derivatives. Analytica Chemica Acta 1994;288:57–69.
5. Wu Z, Tong Y, Yu J, Zhang X, Yang C, Pan C, et al. The utilization of MS-MS detection of GSRs. J Forensic Sci 2001;46(3):495–501.
6. Meng H-H, Caddy B. Gunshot residue analysis—a review. J Forensic Sci 1997a;42(4):553–70.
7. Lloyd JF. Liquid chromatography of firearms propellants traces. J Energetic Mater 1986;4:239–71.
8. Curtis NJ. Isomer distribution of nitro derivatives of diphenylamine in gun propellants: nitrosamine chemistry. Propellants Explos Pyrotech 1990;15:222–30.
9. Lussier LS, Gagnon H. On the chemical reactions of the diphenylamine and its derivatives with nitrogen dioxide at normal storage temperature conditions. Propellants Explos Pyrotech 2000;25:117–25.
10. Bohn MA. Kinetic modelling of the concentrations of the stabilizer DPA and some of its consecutive products as function of time and temperature. J Therm Anal Calorimet 2001;65:103–20.
11. Kimura J. Kinetic mechanism of thermal degradation of a nitrate ester propellant. Propellants Explos Pyrotec 1988;13:8–12.
12. Schroeder WA, Wilson MK, Green C, Wilcox PE, Mills RS, Trueblood KN. Chromatographic investigation of smokeless powder: derivatives of centralite formed in double base powders during accelerating aging. Ind Eng Chem Res 1950;42(3):539–46.
13. Volk F. Determination of the lifetimes of gun propellants using thin-layer chromatography. Propellants Explos Pyrotech 1976;1:90–7.
14. Kurtis NJ, Rogasch PE. Determination of derivatives of diphenylamine in Australian gun propellants by high performance liquid chromatography. Propellants Explos Pyrotech 1987;12:158–63.
15. Druet L, Angers J. LC/MS studies of ethylcentralite-stabilized propellants. Propellants Explos Pyrotech 1988;13:88–94.
16. Jelisavac Lj, Filipovic M. Determination of diphenylamine and its mono-derivatives in single base gun propellants during aging by high performance liquid chromatography. Chromatographia 2002;55:239–41.
17. Reardom MR, MacCrehan WA, Rowe WF. Comparing the additive composition of smokeless gunpowder and its handgun-fired residues. J Forensic Sci 2000;45(6):1232–8.
18. MacCrehan WA, Patierno ER, Duewer DL, Reardom MR. Investigating the effect of changing ammunition on the composition of additives in gunshot residues (OGSR). J Forensic Sci 2001;46(1):57–62.
19. Reardom MR, MacCrehan WA. Developing a quantitative extraction technique for determining the organic additives in smokeless handgun powder. J Forensic Sci 2001;46(4):802–7.
20. Beveridge A. Explosive residue analysis in the mid-1980's – and expanding and challenging role for the forensic scientist. J Energ Mate 1986;4:29–75.
21. Yinon J, Zitrin S. The analysis of explosives. Oxford: Pergamon Press, 1981.
22. Fetterolf DD. Detection and identification of explosives by mass spectrometry. In: Yinon J, editor. Forensic applications of mass spectrometry. London: CRC Press, 1994;215–57.
23. Speers SJ, Doolan K, McQuillan , Wallace JS. Evaluation of improved methods for recovery and detection of organic and inorganic cartridge discharge residue. J Chromatography A 1994;674:319–27.
24. Zeichner A, Eldar B, Pharm M, Glattstein B, Koffman A, Tamiri T, et al. Vacuum collection of gunpowder residues from clothing worm by shooting suspects, and their analysis by GC/TEA, IMS, and GC/MS. J Forensic Sci 2003;48(5):961–72.
25. Hannum DWJE. Parameter survey of commercially available explosives detection technologies and equipment. Denver (Colorado): National Institute of Justice, U.S. Department of Justice, 1998.
26. Northrop DM, Martire DE, MacCrehan WA. Separation and identification of organic gunshot and explosive constituents by micellar electrokinetic capillary electrophoresis. Anal Chem 1991;63(10):1038–42.
27. Northrop DM. Gunshot residue analysis by micellar electrokinetic capillary electrophoresis: assessment for application to casework: part I and part II. J Forensic Sci 2001;46(3):549–72.
28. Smith KD, McCord BR, MacCrehan WA, Mount K, Rowe WF. Detection of smokeless residue on pipe bombs by micellar electrokinetic capillary electrophoresis. J Forensic Sci 1999;44(3):789–94.
29. Wu Z, Tong Y, Yang C, Yu J, Zhang X, Yang S, et al. Determination of diphenylamine stabilizer and its nitrated derivatives in smokeless gunpowder using a tandem MS method. Analyst 2001;126:480–4.
30. White P. Crime scene to court. The essential of forensic science. 2nd ed. Cambridge: The Royal Society of Chemistry, 2004.
31. Simpson NJK. Solid phase extraction. Basel: Marcel Dekker Inc, 2000;1–17.
32. Chapman JR. Practical organic mass spectrometry. 2nd ed. Chichester: John Willey & Sons Ltd, 1995;182–220.
33. Harris DC. Quantitative chemical analysis. 3rd ed. New York: WH Freeman and Company, 1999;47–67.
34. Dyson N. Chromatographic integration methods. 2nd ed. Cambridge: RSC Chromatography Monographs, 1988;35–88.

Additional information and reprint requests:
Désiré Laza, Ph.D.
INCC/NICC
Chaussée de Vilvorde 100
B-1120 Brussels
Belgium
E-mail: desire.laza@just.fgov.be