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The Utilization of MS-MS Method in Detection of GSRs

ABSTRACT: This paper describes a novel method to detect gunshot residues (GSRs). The components contained in the double-based smokeless powder were separated by using a HPLC system with UV detection at 223 nm. The result indicated that there are mainly two kinds of organic ingredients in the double-base smokeless powder used. These two organics do not include nitrocellulose because it has no evidential value in the HPLC-UV trace of the smokeless powder sample. By matching retention times of these two compounds with that of the five standards occurring in smokeless powder, the compounds are tentatively identified as methyl centralite (MC) and nitroglycerin (NG). These two compounds were finally identified by using MS spectrum. This MS-MS method was developed to detect MC since MC is less commonly found in the environment than NG. In Chinese ammunition, MC is widely used as a stabilizer instead of EC. Sixteen samples were used to evaluate the reliability of the method. The mean ($\pm$SD) MC level in the eight samples is 520.1 ± 271.9 pg/μL and that in the blanks is 31.0 ± 20.8 pg/μL. The result ($P = 0.0007$) shows that there is a significant difference between the two serial data. Sampling hands over a 24 h period showed that GSR could be detected even after 8 h with a 75% decrease in the level of MC. The result of the washing test shows that it is still possible to apply the proposed method to detect GSRs on a shooter's hands even if their hands have been washed three times. Another test shows that if the shooters wear gloves while firing, MC on the gloves can be easily detected using the proposed method.

KEYWORDS: forensic science, gunshot residues, methyl centralite, tandem mass spectrometry

When a gun is discharged, many kinds of materials are expelled onto the shooter's hands, hair, clothes, etc. These materials, including inorganic materials and organic materials, are called gunshot residues (GSRs). The detection of GSRs has become fairly important in the forensic field because the results can be used as judicial evidence in court.

Many studies have been done in the past fifty years to try and establish sensitive, selective, reliable methods to detect GSRs. Primary works were focused on the detection of inorganic GSRs by using neutron activation analysis (NAA) (1), anodic stripping voltammetry (ASV) (2), inductively coupled plasma-mass spectrometry (ICP-MS) (3,4), atomic absorption spectrophotometry (AAS) (5,6), scanning electron microscopy/energy dispersive X-ray analysis (SEM/EDX) (7–9), etc. The last two methods have been highly developed. The inorganic species (such as Pb, Sb and Ba) (10) are common to the environment and are not unique to gunshot residue. Considering that organic GSRs may be more characteristic than inorganic residues, forensic scientists have been trying to develop methods for the detection of organic GSRs in recent years (11–15). Among the compounds studied in organic residues, methyl centralite (MC) or ethyl centralite (EC), which are common stabilizers used in smokeless powder, were considered two of the most characteristic compounds in GSRs (16). The structures of MC and EC are shown below. However, since the amount of the stabilizer in residues was reported as being extremely low, developing methods for sensitive detection of this compound in GSRs was a challenge to analytical scientists.

The structure of MC is:
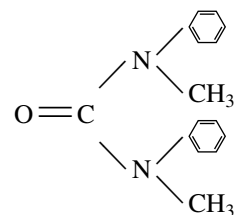
The structure of EC is:
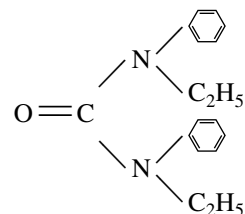
Efforts have been made to detect EC in GSRs by a few researchers. Candy et al. developed a very sensitive method for EC based on TLC (14) and HPLC (15) with fluorescent detection. As the procedures, including preseparation and precolumn derivatization, had to be carried out for the necessary fluorescent detection, the method is relatively complicated and time-consuming. Our preliminary test showed that the MC in GSRs could be detected potentially with a very simple procedure based on the MS-MS technique (17). In this paper, a simple method is developed for the analysis of actual GSRs samples and the sensitivity, reliability, and tolerance of the developed method is evaluated for forensic use.

Experimental

Apparatus

HPLC was carried out with a Waters chromatograph system. Separations were performed using a Waters Model 600 pump on an ODS column (250 mm by 5 μm). Samples were eluted with methanol-water (7:3, v/v) at a flow-rate of 0.9 mL per min at the temperature of 40°C. Samples were detected with a Waters Model 486 detector at a 223 nm wavelength.

A PE SCIEX API-3000 tandem triple-quadrupole mass spectrometer equipped with a turbo ion spray source was used. When testing the possible components of the smokeless powder, the tandem MS was coupled with HPLC to utilize LC-MS-MS mode to identify the materials separated by HPLC. The extracted GSR sample was analyzed under the multiple reaction monitoring (MRM) mode of the tandem MS. In the MRM mode, the first quadrupole selected a precursor ion and the second quadrupole selected a product ion. The existence of MC was identified by both the precursor ion and product ion. The MRM mode provided additional selectivity and eliminated most interferences. In this step, HPLC separation was not required. A column before the sample injection (a six-way valve) was installed in order to keep the flow rate stable and therefore keep the ion current stable.

Materials and Sample Preparation

The standard samples of methyl centralite (MC), nitroglycerin (NG), trinitrotoluene, 2,4-dinitrotoluene, and diphenylamine (DPA) were donated by the Department of Chemical Engineering, Beijing Institute of Technology of China. The smokeless powder tested in the experiment is a double-base smokeless powder found in revolver propellants; it was obtained from the Weapons Identification Lab, Chinese Armed Police Institute, Langfang, P.R. China.

Nitroglycerin (NG), trinitrotoluene, 2,4-dinitrotoluene, diphenylamine (DPA), and methyl centralite (MC) (widely used in Chinese ammunition instead of EC) are the five organic components that ordinarily occur in Chinese smokeless powder. A standard mixture was prepared by dissolving 1.0 g of each in 40.0 mL acetone. The smokeless powder sample was prepared by dissolving 1.0 g double-based smokeless powder in 40.0 mL acetone.

The gloves used in the experiment were purchased from the Beijing Textile Mill, Beijing, China. This glove is the most widely used in China where people normally use them to keep their hands warm.

Analysis Procedure

MC and NG in the smokeless powder sample were separated by HPLC. Separations were performed using a Waters Model 600 pump on an ODS column (250 mm by 5 μm). Samples were eluted with methanol-water (7:3) at a flow-rate of 0.9 mL per min at a temperature of 40°C. The LC-MS-MS spectra were collected in the m/z range of 50–300. It should be noted that in former separation procedures, methanol-water was used as the mobile phase to make better separation. When utilizing LC-MS-MS method, the mobile phase is changed to pure methanol to promote the efficiency of ionization.

A cotton wool swab soaked with methanol was used to extract GSRs on the shooter's hand. The diameter of the swab was 1 cm and the amount of methanol used was 0.3 mL. The back of the shooter's hand, especially the thumb and the index finger, was investigated. The sample was squeezed from the cotton swab using a medical syringe and diluted to 1.00 mL. A blank solution was obtained using the same procedures from the hand of a nonshooter.

The quantitative and qualitative analyses of MC in the GSR sample were carried out by using a multiple reaction monitoring (MRM) mode of tandem MS. According to our primary work (17), the molecular ion m/z 241 was the precursor ion and m/z 134 was the product ion. The parameters of tandem MS were optimized for MRM transition pairs to enhance the sensitivity of the detection. The parameters were listed in Table 1. Under the optimum conditions, 20 μL of the sample solution were injected into the instrument through a six-way valve.

Results and Discussion

Possible Components of Smokeless Powder

In this procedure, tests were carried out to investigate the main organic components in the double-base smokeless powder other than nitrocellulose. We used acetone to prepare a standard mixture of the organic components normally found in smokeless powder. After appropriate dilution, the standard mixture was separated by a reverse-phase HPLC with UV detector at the wavelength of 223 nm. Figure 1a shows the separation result under the elution conditions noted above. The standard mixture was separated in less than 25 min. By comparison with authentic samples, it indicates that peaks 1–6 respectively represent acetone, NG, trinitrotoluene, 2,4-dinitrotoluene, DPA, and MC. We used 1.0 g double-based smokeless powder sample dissolved in 40.0 mL acetone as an actual powder sample. Under the same conditions as above, the powder sample was separated as well. Figure 1b shows that there are mainly two kinds of organic compounds in double-base smokeless powder other than the solvent peak of acetone. By comparing their retention times with those of standards (shown in Fig. 1a), the two materials should be nitroglycerin (NG) and methyl centralite (MC).

To identify those two components in the smokeless powder sample as truly NG and MC, the method of LC-MS-MS was utilized.

TABLE 1—*Experimental conditions for the tandem MS.*

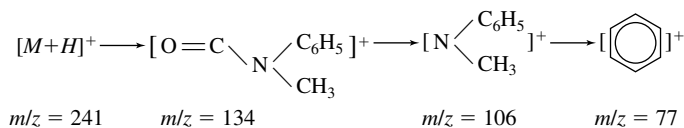| HPLC Conditions	
C_{18} column	2.1 mm × 50 mm
Mobile phase	Methanol
Mobile rate	150 μL/min
Sampling volume	20 μL
Mass Spectrometer Conditions	
Instrument	PE Sciex API 3000
Ionization mode	ESI, Positive ion mode
Scanning mode	MRM
Precursor ion	m/z 241
Product ion	m/z 134
Scanning rate	1000 ms
State Table Parameters	
Parameters	Value:
NEB	5
CUR	9
CAD	6
IS	4000
OR	43
RNG	130
RO1	−11
RO2	−34
RO3	−36
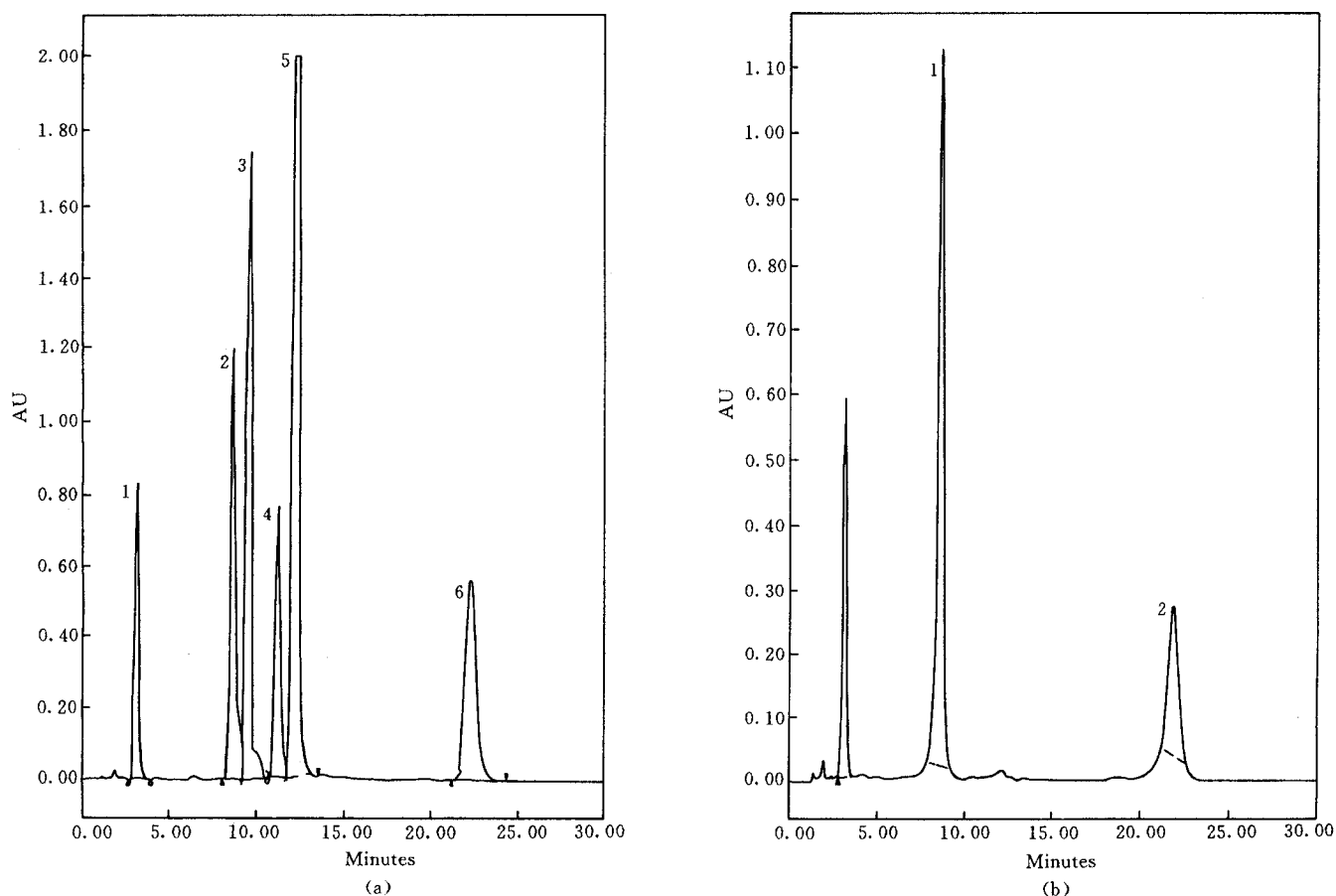
FIG. 1—*HPLC separation of the standard mixture and the powder sample.* (a) *HPLC separation of the standard mixture: 1. acetone (3.2 min) 2. NG (8.8 min) 3. Trinitrotoluene (9.5 min) 4. 2,4-dinitrotoluene (11.2 min) 5. DPA (12.0 min) 6. MC (22.3 min)* (b) *HPLC separation of the powder sample: solvent peak (3.1 min), peak 1 (8.5 min), peak 2 (21.6 min).*

Figure 2 shows the result, where A (negative ion MS spectrum) is the MS spectrum of peak 1 in Fig. 1*b* and B (positive ion MS spectrum) is the spectrum of peak 2. For A, molecular ion $(M-H)^-$ at m/z 226 and typical fragmentation ions: $(M-HNO_3)^-$ at m/z 163, $(NO_3)^-$ at m/z 62 can be seen. All these characteristic ions attest that the component must be NG. For B, the characteristic ions of molecular ion m/z 241 and fragmentation ions at m/z 134, m/z 106, m/z 77, and m/z 51 confirm that the organic is MC. The fragmentation mechanism is listed as follows:

$$[M+H]^+ \longrightarrow [O=C \diagdown {}_{N}^{C_6H_5}]^+ \longrightarrow [N \diagup^{C_6H_5}_{\diagdown CH_3}]^+ \longrightarrow [\bigcirc]^+$$

$m/z = 241 \qquad m/z = 134 \qquad m/z = 106 \qquad m/z = 77$

The results above clearly identify the main organic components of double-based smokeless powder used in the present experiment as nitroglycerin and methyl centralite. It seems that to detect GSRs, we may attempt to detect the presence of both NG and MC (used instead of EC in Chinese ammunition). While nitroglycerin is commonly found in the environment, particularly in pharmaceutical preparations, MC is not common and is rarely used except in smokeless powder (16). Therefore, MC can be considered characteristic of smokeless powder, particularly when detected in conjunction with NG.

GSRs Levels in Real Samples

The MS-MS method was utilized to detect MC. As described above, we chose m/z 241 as a precursor ion and ion m/z 134 as a product ion. Under the MS-MS instrument conditions (Table 1), we analyzed actual GSRs samples. In our initial work (17), the MC levels from a shooter's hands were compared with that from a non-shooter's hands. In this experiment, 16 students volunteered for the tests. They were evenly divided into two groups. Eight persons in the first group discharged a #77 revolver for one round. Ten min later, a cotton wool swab soaked with methanol was used to dissolve the GSRs on the hand of every shooter. The sample solution was squeezed from the cotton swab using a medical syringe and diluted to 1.00 mL. Thus, eight real GSRs samples were obtained. Using the same method, eight blanks were obtained from the hands of the other eight people in the second group. The samples and blanks were injected into the tandem MS instrument. The representative analysis results of group one (sample) and group two (blank) are shown in Fig. 3. It can be seen that the sample peak is much more intense than that of the blank. The signal-to-noise ratio (S/N) of the method is 130. We felt that a reliable result could be obtained if the S/N was above 10, although the detection limit was defined as S/N = 3 by IUPAC. According to this criterion, all eight samples from the shooters were easily distinguished from the sixteen samples. The detailed detection results of the test are listed in Table 2.
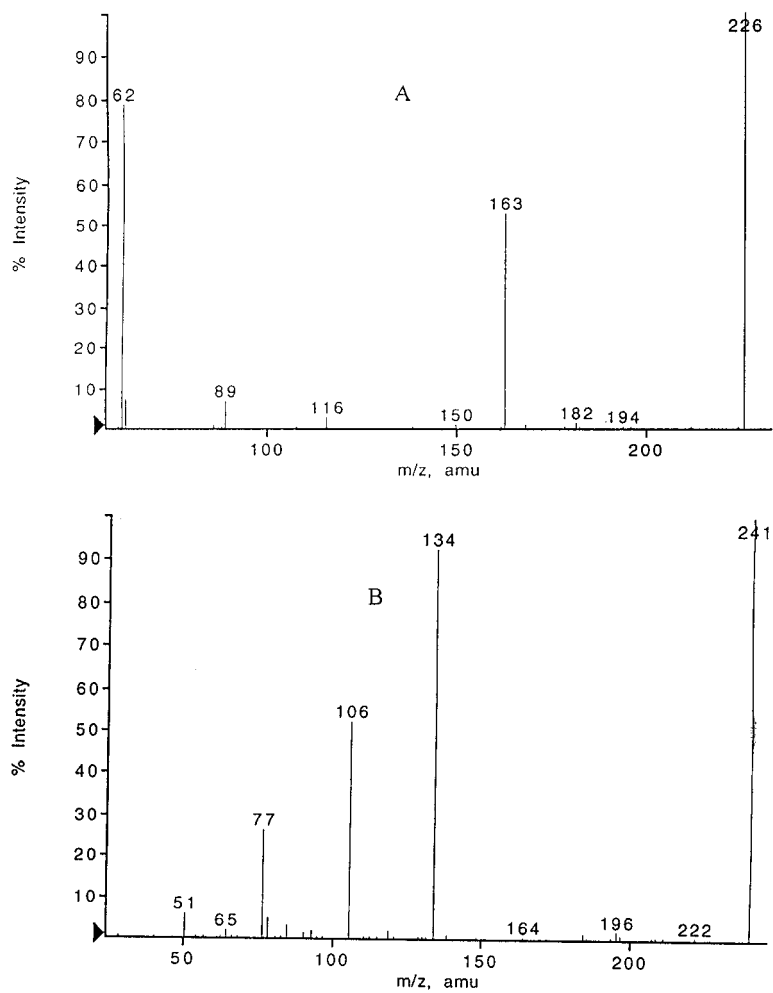
FIG. 2—*The HPLC-MS spectra of the powder sample:* (A) *MS spectrum of peak 1 in Fig 1*b (B) *MS spectrum of peak 2 in Fig 1*b.
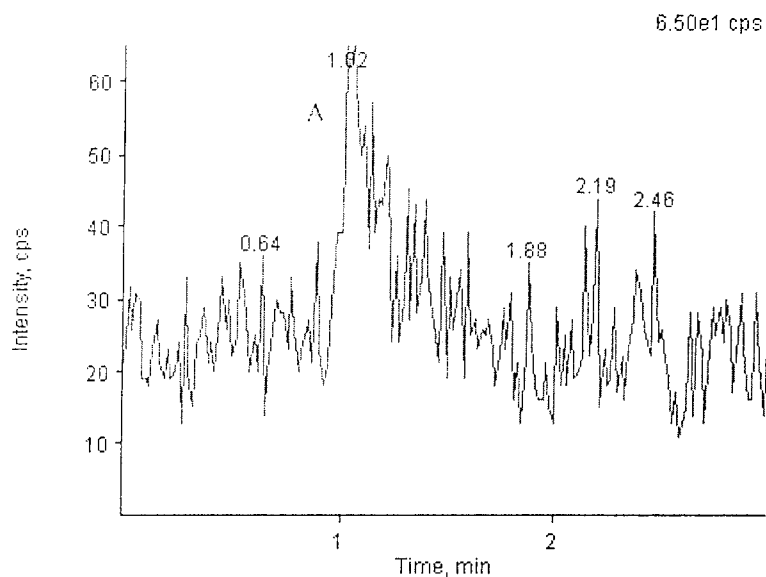

FIG. 3—*Multiple reaction monitoring MS-MS ion chromatograms of the blank and the GSRs sample on the hand:* (A) *Chromatogram of blank,* (B) *Chromatogram of GSRs sample.*
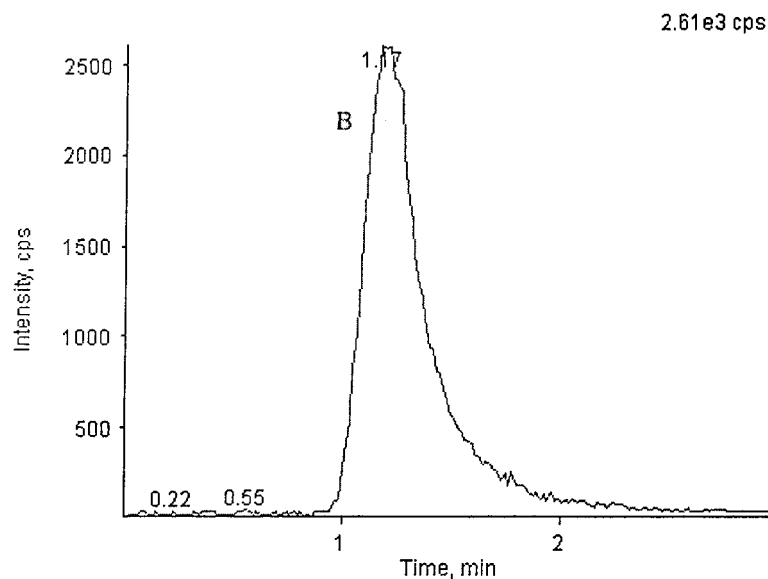
2.61e3 cps

FIG. 3—*(Continued)*.

TABLE 2—*The detail detection results of the actual GSRs test.*

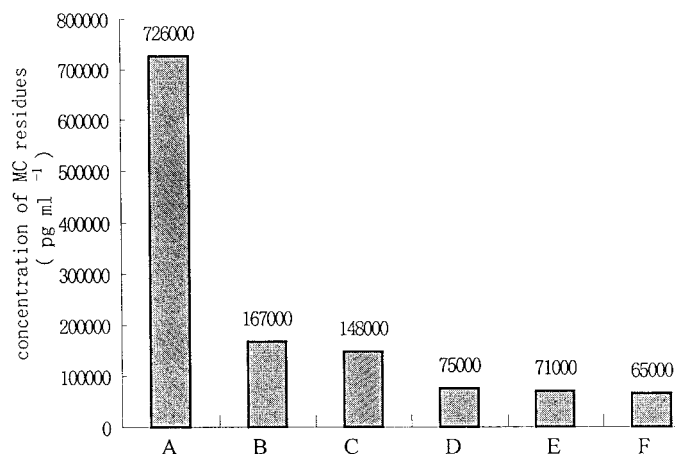| Test Subject Number	A Shooter (Yes or No)	Detection Result (Positive or Negative)	The Amount of MC Residue (pg/μL^{-1})
1	Y	P	325
2	N	N	17
3	Y	P	731
4	Y	P	613
5	Y	P	971
6	N	N	46
7	N	N	12
8	Y	P	683
9	N	N	44
10	N	N	69
11	Y	P	317
12	N	N	31
13	N	N	8
14	Y	P	362
15	N	N	21
16	Y	P	159

FIG. 4—*The effect of time on the detection results:* (A) *mean value of samples extracted from hands immediately after shooting,* (B) *mean value of samples extracted from hands 2 h after shooting,* (C) *mean value of samples extracted from hands 8 h after shooting,* (D) *mean value of samples extracted from hands 12 h after shooting,* (E) *mean value of samples extracted from hands 24 h after shooting,* (F) *mean value of the blanks.*

The 16 samples (shooter and nonshooter) were examined randomly. Table 2 shows the results of the analysis. All samples were correctly identified. The mean ($\pm$s) MC level in the eight samples is 520.1 $\pm$ 271.9 pg/μL and that in the blanks is 31.0 $\pm$ 20.8 pg/μL. The data in the two groups were tested using a Student's Test. The result ($P = 0.0007$) shows that there is a significant difference between the two sets of data. This illustrates that result by using the proposed method; persons who had discharged pistols can be distinguished from those who had not.

The Effect of Time and Washing

The experimental data demonstrated that the MS-MS method could reliably detect MC in hand swabs. To be useful, the method needs to be able to detect MC after significant periods of time or after hands have been washed.

Test for Investigating the Effect of Time—In this procedure, the time related test was carried out. GSRs samples from the backs of hands were examined. A total of 25 subjects were tested in groups of five. Every person fired one round from a #77 pistol. The results are listed in Fig. 4. In the figure, sample A is extracted immediately after shooting, samples B, C, D, and E were obtained two hours, eight hours, twelve hours, and 24 h after firing, respectively, and sample F is a blank. The subjects did not wash their hands between sampling.

The figure shows that the amount of MC residues on the hand of the shooters decreased dramatically over time. It can be seen that after 2 h there remained only 25% of the MC in sample B. Between 2 and 8 h there is little change in the amount of MC. The intensity

of sample D and E is about equal to that of the blank sample. Therefore, enough MC remains on a shooter's hands to be detected using this method, if 8 h or less has elapsed since firing. If more than 8 h has elapsed, GSR cannot be reliably detected. The reasons for the loss of MC in GSRs on a shooter's hand may vary. The most primary reason may be that normal daily activity simply removed the MC through transfer.

Test for Investigating the Effect of Washing—After firing a gun, a suspect may wash his hands. The next series of tests evaluates the effect of hand washing in the proposed method. Hand washing is defined as rinsing the hand under running water. Every volunteer rubbed his/her hands under water for one min. After washing, they dried their hands with paper towels. The results are shown in Fig. 5.
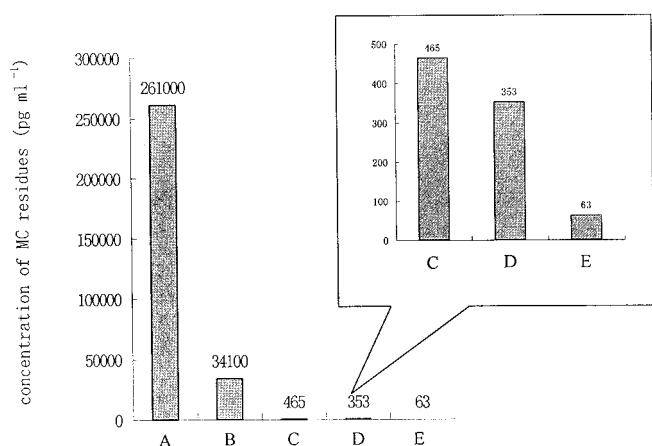

FIG. 5—*The effect of washing on the detection result.* (A) *mean value of samples extracted from hands immediately after shooting;* (B) *mean value of samples extracted from hands after the hands had been washed once with water;* (C) *mean value of samples extracted from hands after the hands had been washed twice with water;* (D) *mean value of samples extracted from hands after the hands had been washed three times with water;* (E) *mean value of the blanks.*

In Fig. 5, A is the mean result extracted from hands immediately after shooting; B represents the mean result obtained from hands that had been washed with water one time after shooting; C and D are the mean results from hands that had been washed for two times and three times with water after shooting, respectively; and E is the mean result of blank samples. Six individuals were used in the test. The test shows that the amount of MC in GSRs on the shooters' hands decreases as hand washing increases. The intensity of the signal of C is eight times that of E and the intensity of the signal of D is six times that of E. A shooter washing his/her hands greatly reduces the amount of MC. However, testing shows that MC can still be detected after three washings. No evaluation of washing with soap was done since the soap variability would make evaluation difficult.

Detection of MC in GSRs on Gloves—In firearm related crimes, the criminal may wear gloves on his/her hands while firing. Therefore, no GSR could be expected to be found on the shooting hands. In this case, their glove may be sampled. The next series of tests evaluates using the MS-MS method for recovering GSRs from gloves. Five cotton yarn shooters' gloves were examined. The thumb and the index finger were cut from each glove, soaked with 0.5 mL methanol, and then squeezed using a medical syringe. The solution obtained was diluted to 1 mL. Blanks were obtained using the same procedures from five clean gloves. The samples and blanks were injected into the instrument directly and the representative analysis result is shown in Fig. 6. It can be seen that the mean value of the sample signal is much more intense than that of the blanks. The S/N of the samples is 800. Therefore, MC on this type of glove can be reliably detected using the proposed method.

Conclusion

The proposed method has been applied successfully to detect GSRs on shooters by detecting the existence of MC. The method offers high sensitivity with very simple procedures. Almost no interference was observed because of the application of the MRM mode of the tandem MS. The MC on the shooters' hands can be reliably
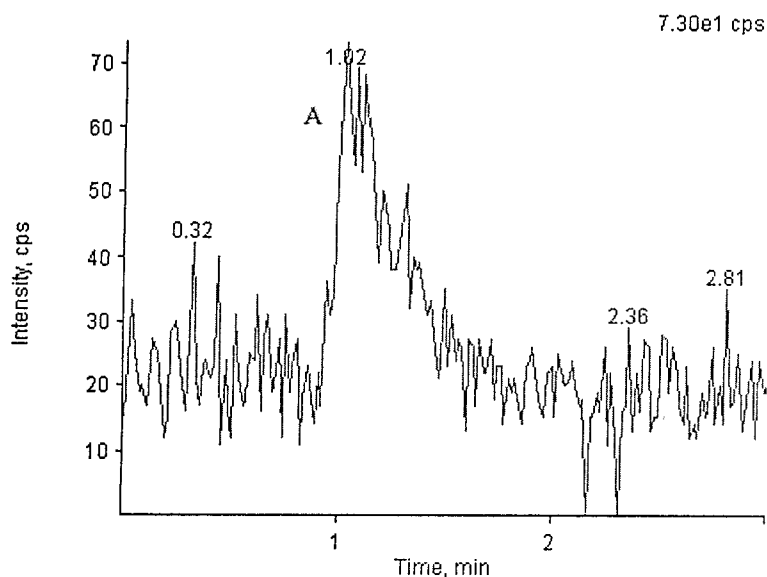

FIG. 6—*Multiple reaction monitoring MS-MS ion chromatograms of the blank and the GSRs sample on the glove.* (A) *Chromatogram of blank,* (B) *Chromatogram of GSRs sample.*
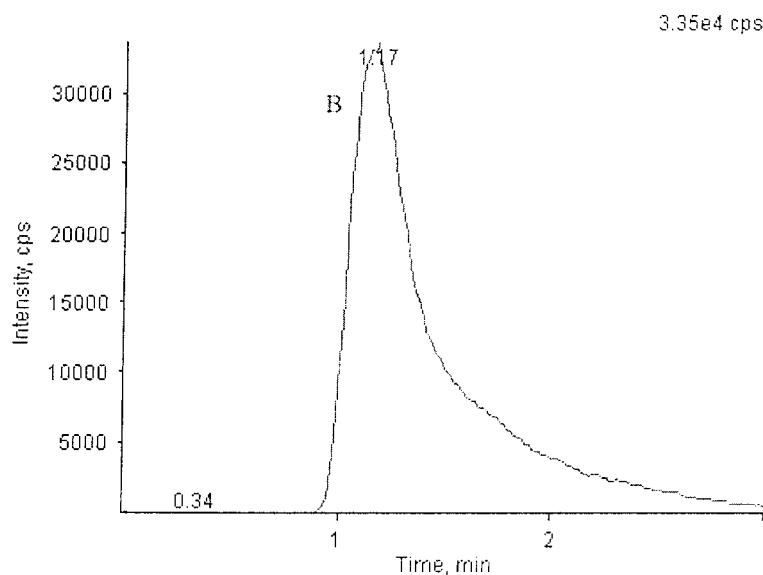
FIG. 6—*(Continued)*.

detected if the interval between firing and sampling is no more than 8 h. MC was detected on the hand after washing up to three times. Results of glove testing show that MC recovered from the shooters' gloves can be reliably detected. Some double-base smokeless powder may contain EC instead of MC. Since the structure of EC is very similar to that of MC (shown in the Introduction section), the present method is expected to detect EC with little modification.

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