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Desorption Electrospray Tandem MS (DESI-MSMS) Analysis of Methyl Centralite and Ethyl Centralite as Gunshot Residues on Skin and Other Surfaces

ABSTRACT: A direct and sensitive method for the detection of methyl centralite (MC) and ethyl centralite (EC) as gunshot residues (GSRs) has been developed. This method uses desorption electrospray ionization (DESI)-tandem mass spectrometry and directly desorbs and detects analytes from surfaces without any sampling process. Typical transitions for MC and EC, m/z 241 to m/z 134 and m/z 269 to m/z 148, respectively, were used to improve the assay sensitivity. It has been shown that MC and EC can be detected on various surfaces, with detection limits of 5–70 pg/cm². Interferences, detection time after shooting and the number of times hands were washed after shooting were also evaluated. None of the materials interfered with the results and the detection window for organic GSRs was up to 12 h and hands could be washed at least six times. Further samples were analyzed to confirm the reliability of this method, and showed that it could discriminate shooters from nonshooters. This method should be of significance in forensic science, especially in analyzing GSRs, because of its simplicity, high throughput, and the direct detection of MC and EC on suspects' hands, clothes, and hair.

KEYWORDS: forensic science, gunshot residues, methyl centralite, ethyl centralite, direct analysis, DESI mass spectrometry, DESI-MSMS, crime scene investigation

Firearm-related events, such as terrorist attacks, murder, armed robbery, and suicide, have been considered as dangers to society and the ability to investigate them is critical to forensic science. In this field, detection and identification of gunshot residues (GSRs) are useful in the investigation of firearm-related events and provide important evidence in trials resulting from the incident (1). As the test results can be used as evidence in court, accurate analysis of GSRs is of value to forensic scientists. The residues are principally composed of burnt and unburnt particles from the propulsive charge, as well as components from the primer, the bullet, the cartridge case and the firearm itself (2). Generally, although chemical compositions of GSRs are complex, there are two major components, inorganic particles, usually metals such as barium, lead, and antimony, and organic compounds, which mainly originate from the primer and propellant.

At present, the methodologies based on the analysis of inorganic particles have been widely accepted and used in most forensic laboratories (3,4). First, the detection of inorganic elements and their combinations might not be "unique" enough, because metal particles might possibly be introduced from other pathways, such as fireworks, various parts of cars, and paints (5). Second, it has been accepted that contamination might exist during hand sampling and transportation process (6), this is difficult to estimate and can be difficult to control. Finally, the methodology based on metal analysis is not easy to use *in situ*, and therefore is inappropriate for direct screening at the scene.

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Identification and characterization of organic GSR, which mainly results from the incomplete combustion of smokeless powder (7), has some inherent advantages to forensic scientists. The most commonly determined organic GSRs are propellant, nitrocellulose (NC) and nitroglycerin (NG), and the stabilizers include ethyl centralite (EC) and methyl centralite (MC) as well as diphenylamine (DPA) and its nitration products. However, NC which also occurs in lacquers, varnishes, and celluloid films, and NG which is also used in pharmaceutical preparations, might arise from other sources. The stabilizer DPA is widely used in rubber products and in the food-stuff industry; however, EC and MC rarely exist in the normal environment and were considered as highly credible organic GSR (8).

Obviously, the detection of organic GSR does not depend on the sizes of particles, and characteristic organic gunpowder components, which have not been found in the general population (1), can provide valuable evidence about firearm-related events. Many analytical methods have been reported, such as capillary electrophoresis (CE) (9), TLC and HPLC (10), ion mobility spectrometry (IMS), GC-MS and GC-TEA (11), ESI-tandem mass spectrometry (12), and time-of-flight secondary ion mass spectrometry (TOF-SIMS) (13). However, one obstacle to the present methods for detecting organic GSRs is the sensitivity requirement, because the amounts of organic GSRs remaining on the shooter's hands several hours after the incident might be very small (normally nanograms) (1). Another constraint is often the need for a tedious sample recovery and pretreatment process.

Recently, desorption electrospray ionization (DESI) (14) coupled to mass spectrometry was demonstrated as a method for direct analysis of solid surfaces, therefore avoiding sample pretreatment (15). In previous research, a broad range of analytes can be detected and analyzed by DESI, including explosives (16),

chemical warfare agents (17), and biomolecules (18). Coupled with MS, DESI has the potential to supply structural information in real time on surfaces, such as skin (17), clothes (18), and biological specimens (16–20). Noticing that *in situ* analysis by DESI-MS could be easily performed on human hand and hair and is non-destructive, it might be an ideal technology for GSR analysis. Miniature mass spectrometers have been extensively researched in recent years, and DESI has been successfully coupled to a portable mass spectrometer (21), which potentially makes it an ideal choice for direct analysis and screening at the crime scene.

In this study, we established a DESI-MSMS method for detecting MC and EC from GSRs left on various types of solid surfaces, including the human hand. To detect these organic GSRs in the subnanogram range, we utilized tandem mass spectrometry and optimized the acquisition parameters of DESI-MSMS. The effects of interferences, how many times the hands were washed after shooting, and the detection time after shooting were also estimated to confirm the applicability of this method. The reliability of this methodology was proven by a test of 10 actual GSR samples and 10 nonshooter samples.

Experimental Section

Chemicals

HPLC-grade solvents were commercially available and used without further purification. Ultrapure water was produced by a Milli-Q water purification system (Millipore, Milford, MA). Methyl centralite (1,3-dimethyl-1,3-diphenyl-urea) and ethyl centralite (1,3-ethyl-1,3-diphenyl-urea) were purchased from Sigma-Aldrich (St. Louis, MO).

DESI Source and Mass Spectrometry

A home-made DESI source was used in this work. The spray emitter was produced according to the previous literature (22). The spray emitter was a 0.5-cm-long fused silica capillary (0.15 mm o.d., 0.1 mm i.d.). The i.d. of the coaxial nebulizing tube was 0.25 mm. The emitter was positioned *c.* 5 mm from the ion sampling tube of the mass spectrometer and 3 mm higher than the sample. The angles: ion sampling tube—sample plate—nebulizing tube, were, respectively, *c.* 10° and 60°. The sample plate was positioned on a movable 3-D stage (Saifan, Beijing, China), and was operated manually. Helium nebulizing flow rate was estimated at 500 mL/min. The spray voltage was typically set at 4 kV and provided by a high voltage source (Hengbo, Tianjin, China). Spraying solvent was delivered to the needle by the syringe pump integrated in the mass spectrometer and controlled by the mass spectrometer software, at a flow rate of 3–10 $\mu\text{L}/\text{min}$.

DESI-MSMS experiments were performed using, as a spray solvent, water/methanol solution (1:1). Analytes solubilized in methanol or water was deposited onto the chosen surface for the experiments. Positive ions formed were sampled using a Thermo Finnigan LTQ mass spectrometer (San Jose, CA). The doping area on the surface was set at *c.* 1 cm^2 .

Ammunition, Shooting, and Sampling

This was a blind test to evaluate the reliability of our DESI method. Firing was carried out in an indoor shooting range in the Chinese People's Armed Police Force Academy, using ten 7.26 mm semiautomatic 77# pistols. Ten volunteers from the Chinese People's Armed Police Force Academy shot in this

experiment, respectively. Because of DESI's ability of direct analysis, there are no special sampling processes in this experiment. Moreover, we also performed sampling experiment by using low-density polyethylene (LDPE) film and medical cotton, respectively, to evaluate the applicability of DESI on different conditions.

Safety Consideration

The DESI source contains a high voltage component, which might be potentially harmful to the analyst. It is therefore important that the appropriate shields and interlocks be used to avoid accidental contact with this component.

Results and Discussion

The experimental results showed that MC and EC could produce their corresponding ions on various surfaces during the positive DESI process, and that these are detectable by MSMS. Figure 1 displays the positive DESI-MS spectrum of MC and EC from a human hand, respectively. The peak of m/z 241 in Fig. 1A is the corresponding protonated ion of MC (MW 240) and the peak at m/z 134 is a typical fragment formed during the DESI process, which arose from the loss of a neutral methylaniline molecule.

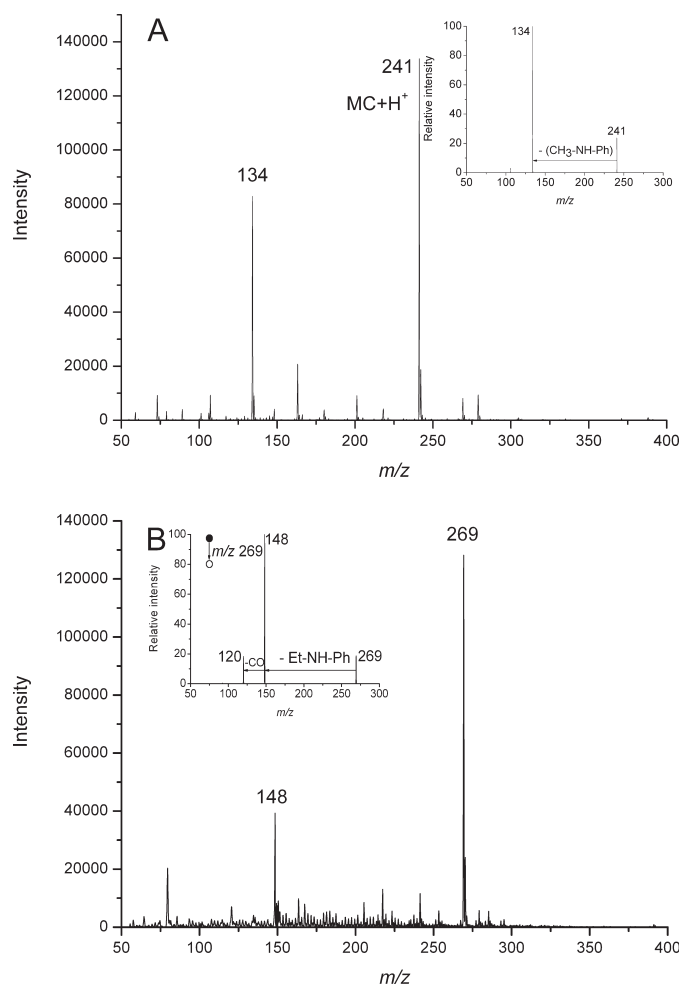


FIG. 1—Positive DESI-MS spectra of methyl centralite and ethyl centralite. (A) Positive DESI-MS spectrum of 24 ng methyl centralite on the area *c.* 1 cm^2 from a human hand. (B) Positive DESI-MS spectrum of 26 ng ethyl centralite on the area *c.* 1 cm^2 from a human hand.

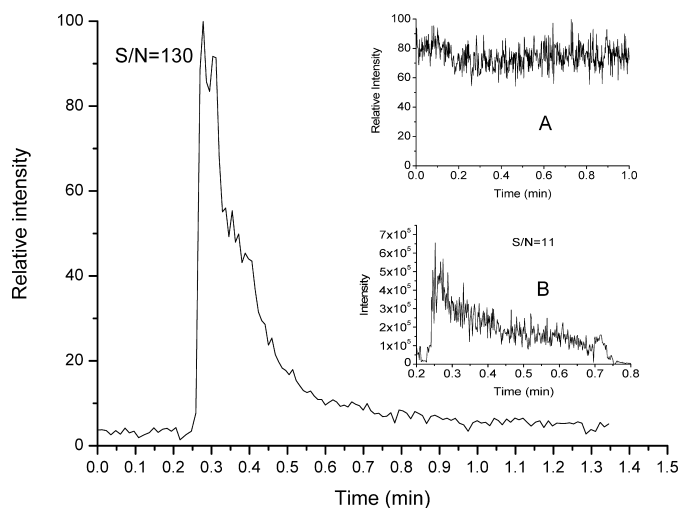


FIG. 2—Selected-ion (m/z 134) monitoring chromatography produced by selected DESI-MSMS process, SRM chromatograph of blank skin (inset A), and SRM chromatograph produced by simple DESI process (inset B).

These structure assignments could be confirmed by MS/MS experiments and existing ESI-MS spectra (23). The inset in Fig. 1A displayed a CID MS spectrum of the parent ion of m/z 241 and the product is also the ion of m/z 134. Further tandem mass experiments were performed and the results were consistent with previous work in our research group (12). These results are reproducible with a typical RSD of 4.9%. Figure 1B is the mass spectrum of EC (MW 268) from a human hand to which EC had been added. In this figure, the ions at m/z 269 and m/z 148 correspond to the protonated EC molecule and the fragment from losing a neutral ethylaniline molecule, respectively. Tandem MS experiment was also performed and shown in the inset of Fig. 1B.

As stated above, one crucial requirement of the analytical method for detecting organic GSRs is high sensitivity. To decrease the detection limits of DESI-MS for the detection of organic GSRs, we used MSMS, not only for structure analysis, but also to enhance signal to noise ratio (S/N). Tandem mass method can significantly reduce interferences and the level of noise (23). In this work, the typical MSMS pathways of MC and EC, respectively, were selected to achieve lower detection limits. Figure 2 displays the selected-ion (m/z 134) current chromatogram of 240 pg MC from an area of 1 cm². In this MSMS experiment, when there is no MC added to the surface, such as human hand, no signal except for the base line could be detected (shown in the inset A in Fig. 2). The S/N of the base peak at 0.28 min was calculated as 130, while only a low S/N value (S/N = 15) was achieved with DESI-MS for the same amount of MC on the same surface (shown in the inset B of Fig. 2). This result clearly demonstrates that tandem mass method significantly lowers the detection limits of DESI-MSMS allowing the detection of organic GSRs at the sensitivities required for long detection windows.

Our experiments showed that both MC and EC on various types of surfaces could be efficiently ionized and detected by DESI-MSMS, partly because the two centralites are basic and capture protons during the desorption electrospray process. Utilizing tandem MS, the detection of MC and EC on several types of surfaces, including glass surface, rubber gloves, leather gloves, towel, medical gauze, absorbent cotton, and human hair, was evaluated. To determine the limits of detection (LOD) on each surface, we added MC and EC (10² pg level) to a certain area and calculated the S/N in the SRM mode. The LODs were then calculated according to

IUPAC's definition. Table 1 summarizes the approximate LODs of MC and EC by DESI-MSMS on those surfaces and the S/N at each limit of detection. We selected the MSMS transition of m/z 241–134 to obtain the LOD for MC and 269–148 to obtain the LOD for EC, respectively. The results showed that tandem MS reduced interferences from the environment, which was also previously demonstrated in another MSMS experiment by our research group (23).

The effect of other potential interference substances was also studied; MC was tested for on skin surfaces, to which milk, soft drink such as juices and sodas, and dust were added. Both DESI-MS and DESI-MSMS were conducted and all the results showed (data not shown) that MC could be detected, especially in the tandem MS mode.

To determine the detection time after shooting and hand washing, two different experiments were performed. In both experiments, DESI-MSMS SRM was used and the product ion m/z 134 was used. Two-hundred and forty nanograms of MC was added to the hands of four subjects and analyzed by DESI-MS after 1, 2, 4, 6, 8, 10, and 12 h, respectively. The results are shown in Fig. 3. The results showed that for up to 12 h, MC could still be detected on the hands. Figure 4 shows the effects of washing the hands after the addition of 240 ng MC could still be detected after washing the hands for up to six times.

TABLE 1—Methyl centralite (MC) and ethyl centralite (EC) analysis by tandem DESI mass spectrometry on several types of surfaces.

Analytes	Surface	LOD	Analytes	Surface	LOD (pg/cm ²)
MC	Human hand	8 pg/cm ²	MC	Leather gloves	8
EC		10 pg/cm ²	EC		9
MC	Human hair	14 pg*	MC	Towel	23
EC		20 pg*	EC		40
MC	Glass	5 pg/cm ²	MC	Medical gauze	48
EC		6 pg/cm ²	EC		54
MC	Rubber gloves	6 pg/cm ²	MC	Absorbent cotton	60
EC		6 pg/cm ²	EC		70

*The surface area of hair was hard to estimate, so we could only report an absolute LOD.

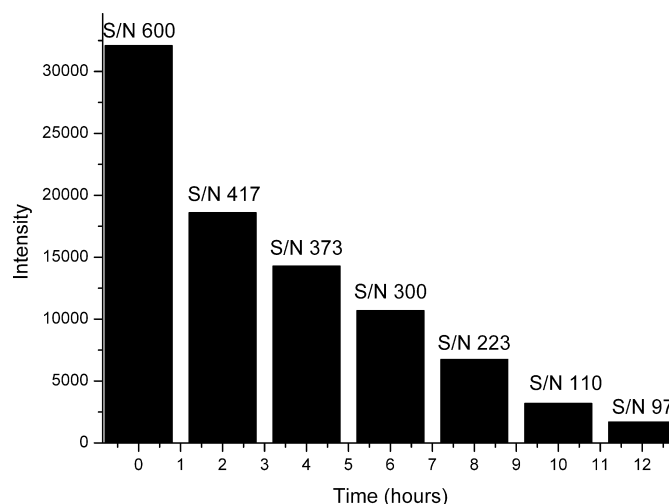


FIG. 3—The signal intensity and S/N value of selected M/MS product, m/z 134 from m/z 241 on the hands of shooters from 0 to 12 h after shooting.

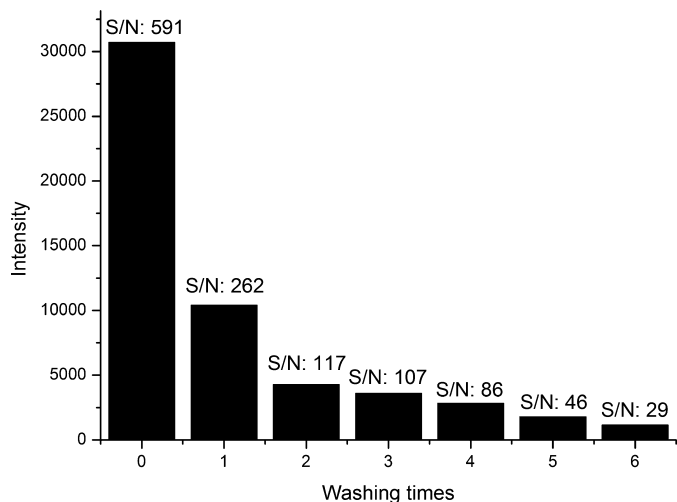


FIG. 4—The signal intensity and S/N value of selected MS/MS product, m/z 134 from m/z 241 on the hands of shooters after washing hands for up to six times.

TABLE 2—Signal intensities of the product ion, m/z 134 produced from the protonated methyl centralite by DESI-MS on 10 shooters (S) and 10 nonshooters (NS), respectively.

Number	Signal Intensity	Number	Signal Intensity
S1	4.20×10^2	NS1	14.1
S2	4.10×10^2	NS2	7.53
S3	3.77×10^2	NS3	9.62
S4	5.63×10^2	NS4	7.40
S5	8.42×10^2	NS5	2.16
S6	3.27×10^2	NS6	5.76
S7	2.82×10^2	NS7	6.62
S8	4.13×10^2	NS8	10.1
S9	3.00×10^2	NS9	9.42
S10	4.77×10^2	NS10	7.81

To further test the reliability of DESI-MSMS method for detection of organic GSRs, the hands of volunteers who had shot pistols were analyzed for MC and EC. Twenty individuals' hands were analyzed by DESI mass spectrometer, respectively. Half had shot weapons and the others were nonshooters. Results showed that the signal intensity of the selected product ion m/z 134 from the shooters' hand was *c.* 20-fold higher than from the nonshooters. Moreover, the S/N of the selected-ion current chromatogram of each shooter's hand was >30. A forensically reliable result could be obtained if the S/N was above 10 although the detection limit is defined as $S/N = 3$ by IUPAC. According to this criterion, all 10 samples from the shooters were correctly detected from the 20 samples. Table 2 summaries the signal intensities of MC detected on each hand of the 20 individuals.

Conclusion

DESI-MSMS was demonstrated to be a rapid (<5 sec) method to detect organic GSRs directly from human hand and various types of surfaces under ambient condition without any sampling and purification process. To enhance the sensitivity and specificity of this methodology, we utilized MSMS method to decrease the noise level and avoid interferences, which resulted in a detection

limit on 10 pg/cm^2 level on real sample surfaces. The tandem MS based technique can supply more characteristic information than other methods, such as chemical visualization and SEM/EDX method. It is worthy of note that in a previous study on the aging of double-base propellants, DPA and its nitration products could be directly analyzed on solid surfaces (24), which means that all types of stabilizers in smokeless powder could be detected as organic GSRs in firearm-related events. Considering that previous works have proved that DESI-MS is high throughput (25), possible to image biological tissues (20), possible to detect explosives on human skin (26) and successful to couple a portable mass spectrometer (21), DESI-MSMS could be broadly applied in forensic science.

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