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Technical Notes

Screening of Nerve Agent Degradation Products by MALDI-TOFMS

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A novel method for the rapid screening of degradation products derived from nerve agents by matrix-assisted laser desorption ionization time-of-flight mass spectrometry is described. Five standard products were selected as model compounds, including isopropyl methylphosphonic acid (IMPA), pinacolyl methylphosphonic acid (PMPA), ethyl methylphosphonic acid (EMPA), isobutyl methylphosphonic acid (*i*-BuMPA), and cyclohexyl methylphosphonic acid (CHMPA), which are degradation products of Sarin (GB), Soman (GD), VX, Russian VX (RVX), and GF, respectively. For comparison, CHCA (α-cyano-4-hydroxycinnamic acid) and DCCA (7-(diethylamino)coumarin-3-carboxylic acid) were used as the MALDI-matrix when the third harmonic generation (355 nm) of a Nd:YAG laser and a hydrogen Raman laser (multifrequency laser) were used, respectively. The method permitted the five nerve agent degradation products to be screened rapidly and successfully, suggesting that it has the potential for use as a routine monitoring tool.

Sarin terrorism by Aum Shinrikyo first occurred in Matsumoto City on June 27, 1994. In the next year, in five coordinated attacks, the conspirators released Sarin aerosol on several lines of the Tokyo subway (March 20), killing and injuring many people. Degradation product analysis of the Tokyo subway incident was summarized and published. In fact, in addition to Sarin (GB), a number of nerve agents (known as chemical warfare agents), including G-type agents such as Soman (GD) and GF, and V-type agents such as Russian VX (RVX) and VX, the chemical structures of which are shown in Figure 1, are all potentially very dangerous. The "G" agents tend to be nonpersistent, whereas the "V" agents are persistent. At room temperature Sarin (GB) is a comparatively volatile liquid and therefore is nonpersistent; Soman (GD) also has significant volatility. VX is a relatively nonvolatile liquid and therefore is persistent. The above compounds comprise a group of highly toxic organic esters of phosphoric acid derivatives and readily undergo degradation when they come into contact with moisture; the degradation pathway is also shown in Figure 1. The degradations products of Soma, GF, Sarin, RVX, and VX are pinacolyl methylphosphonic acid (PMPA), cyclohexyl methylphosphonic acid (CHMPA), isopropyl methylphosphonic acid (IMPA), isobutyl methylphosphonic acid (*i*-BuMPA), and ethyl methylphosphonic acid (EMPA), respectively. After they are produced, these alkyl methylphosphonic acids then undergo a slow hydrolysis to methylphosphonic acid (MPA). A number of analytical methods have been developed for their identification, including capillary electrophoresis (CE),[1−4] an electrochemical sensor,[5,6] liquid chromatography−electrospray ionization−mass spectrometry (LC-ESI-MS),[7] gas chromatography/mass spectrometry (GC/MS),[8−14] CE microchip,[15,16] and electrospray ion mobility spectrometry.[17−21] Each of the above methods has unique advantages and disadvantages with respect to sensitivity, precision, and simplicity of use. However, all of these methods are difficult to use as a rapid screening tool for the on-line detection of a nerve agent, as

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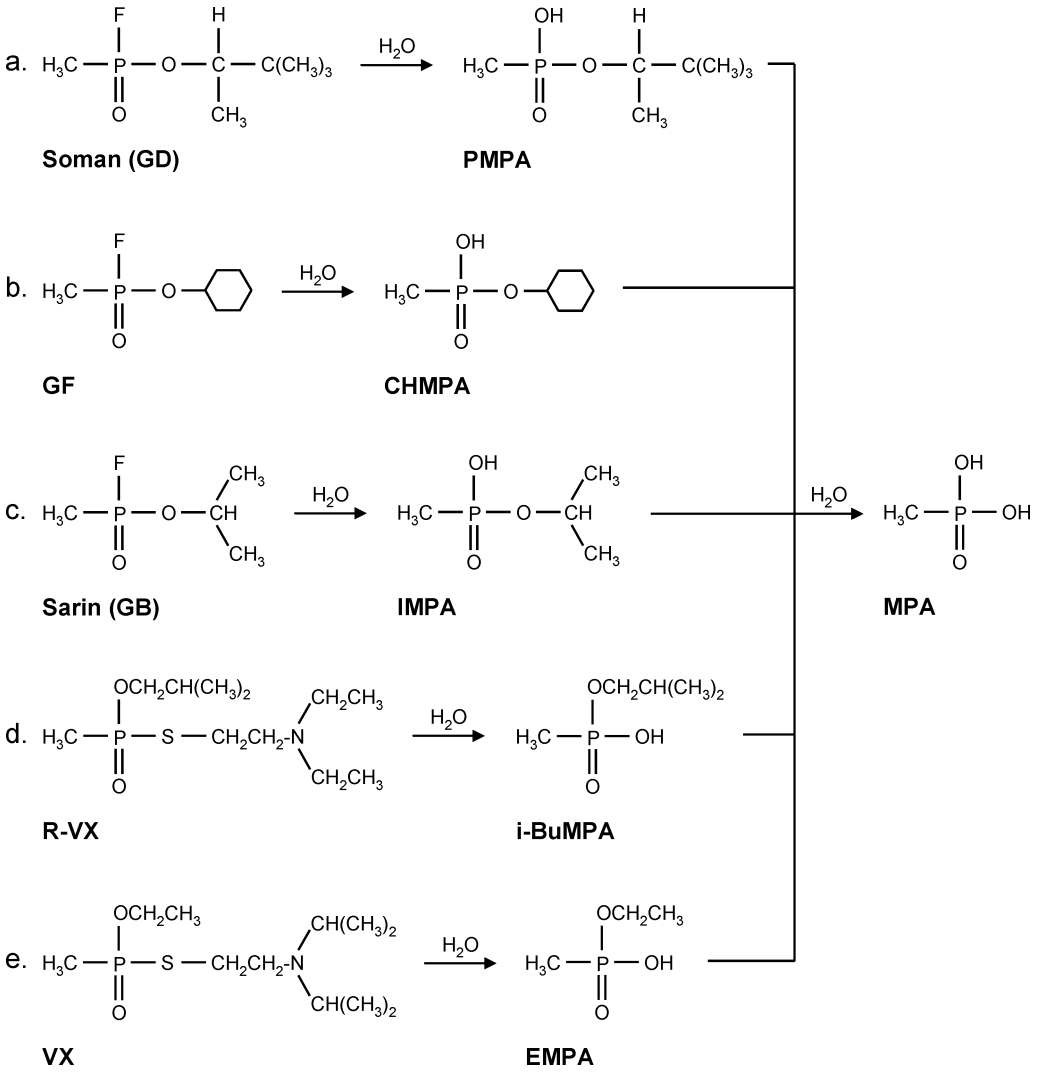

Figure 1. Chemical structures of Soman (GD), GF, Sarin (GB), RVX, and VX and their degradation products including pinacolyl methylphosphonic acid (PMPA), cyclohexyl methylphosphonic acid (CHMPA), isopropyl methylphosphonic acid (IMPA), isobutyl methylphosphonic acid (*i*-BuMPA), and ethyl methylphosphonic acid (EMPA), respectively. The final product of these is methylphosphonic acid (MPA).

well as degradation products thereof, especially if it is required for routine monitoring. In other words, to protect against acts of domestic terrorism in which a nerve gas is used, a rapid and accurate analytical technique for their detection would be highly desirable.

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) is a soft-ionization method and has become a very popular and powerful tool for the analysis of biomolecules. Recently, the analysis of low-mass (m/z < 500) molecules using this method has also been reported.[22−29] In this study, we now report, for the first time, on a method for the rapid screening of nerve agent degradation products based on MALDI-TOFMS. Optimal conditions, such as the source of the laser and the types of matrix for use, were investigated. Actual groundwater samples from the university campus were examined, after being spiked with six standards of the nerve agent degradation products using MALDI-TOFMS, and the findings are reported herein.

EXPERIMENTAL SECTION

Reagents. All chemicals used were of analytical grade. α-Cyano-4-hydroxycinnamic acid (CHCA; MW, 189.17) was purchased from Aldrich (St. Louis, MO). 7-Diethylaminocoumarin-3-carboxylic acid (DCCA; MW, 261.27), ferulic acid, 3-hydroxypicolinic acid (3-HPA), 2,6-dihydroxyacetophenone (2,6-DHAP), and 2,4,6-trihydroxyacetophenone (2,4,6-THAP) were obtained from Fluka (Buchs, Switzerland). TFA (trifluoroacetic acid) and phosphoric acid were purchased from Acros (New Jersey). 2,5-dihydroxybenzoic acid (2,5-DHB) was obtained from Sigma-Aldrich (St. Louis, MO). Methylphosphonic acid (MPA; MW,

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96.02), ethyl methylphosphonic acid (EMPA; MW, 124.08), isopropyl methylphosphonic acid (IMPA; MW, 138.10), cyclohexyl methylphosphonic acid (CHMPA; MW, 178.17), and pinacolyl methylphosphonic acid (PMPA; MW, 180.18) were obtained from Radian International (Austin, TX) (Catalog Nos. ERM-038, ERE-024, ERI-015, ERC-034, and ERP-083, respectively (1 mg/mL in methanol)). Isobutyl methylphosphonic acid (*i*-BuMPA) was generously donated by the Military Police Command, Forensic Science Center, Taiwan.

Sample Preparation. *Matrixes.* A 10 mg amount of CHCA was dissolved in a 1.0 mL aliquot of a water/acetonitrile (v/v, 50/50) solution that contained 0.1% trifluoroacetic acid. In the case of DCCA, 1.0 mg of DCCA was dissolved in a 1.0 mL aliquot of water/acetonitrile, which also contained 0.1% trifluoroacetic acid.

Standards. A drop of aqueous (1.0 μL) matrix compound solution was mixed with the analyte solution (1000 ppm in methanol) and dried, resulting in a solid deposit of analyte-doped matrix crystals that was introduced into the TOFMS for analysis.

Actual Samples. Groundwater samples were obtained from the university grounds and used directly without any pretreatment. A 1.0 mL aliquot of groundwater was evaporated to dryness in a vacuum centrifugal evaporator. The resulting residue was dissolved in 10 μL of deionized water and then agitated for 5 min on a Minishaker (Model MS1, IKA Works Asia Sdn. Bhd., Malaysia). To 1.0 μL of sample solution, 1.0 μL of matrix solution was added and the solution transferred to the MALDI plate for the subsequent analysis, as describe above.

MALDI-TOFMS Apparatus and Lasers. The linear type of time-of-flight mass spectrometer, which was a modified Wiley–McLaren design (R. M. Jordan Co., Grass Valley, CA), and the data acquisition system used were similar to that described previously[30,31] and are abbreviated herein. The third harmonic generation (THG, 355 nm) radiation generated from a Nd:YAG laser (Spectraphysics GCR-170, Mountain View, CA) was used as the fundamental beam. A Raman shifter (50 cm in length, 1 in. in diameter, two arms equipped with a 1 cm thick quartz window) was used for the generation of the multifrequency laser when high-pressure hydrogen (5 atm) was used as the Raman medium. The detectable lines can cover the region from 242.6 to 865.9 nm with different intensities.[32] CHCA and DCCA were selected as the MALDI matrix when THG (355 nm) of Nd:YAG and multifrequency lasers were used, respectively. The laser power was also well-controlled in the range from 20 to 200 μJ, depending on the specific situation, which permitted good quality mass spectra to be obtained. Mass calibration was conducted using fragments produced from a CHCA standard.

RESULTS AND DISCUSSION

Figure 2 shows the mass spectra of the five nerve agent degradation products obtained by the MALDI-TOFMS method using CHCA as the matrix (laser: the THG of Nd:YAG laser, 355 nm); spectra a–e correspond to the PMPA, CHMPA, *i*-BuMPA, IMPA, and EMPA standards (concentration, $\sim 1.0 \times 10^{-7}$ g/mm^2 on the MALDI plate), respectively. As can be seen from spectra a–e, parent ions (marked $[M + H]^+$) can be observed in all cases.

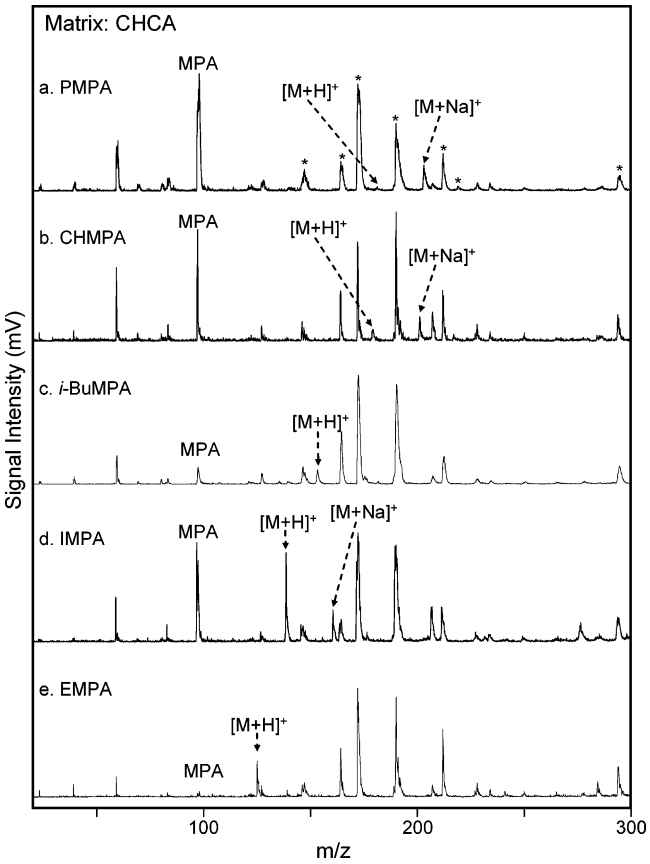

Figure 2. Mass spectra of the five standards of nerve agent degradation products obtained by the MALDI-TOFMS method using CHCA as the matrix (laser: the THG of Nd:YAG laser, 355 nm). Spectra a–e correspond to PMPA, CHMPA, *i*-BuMPA, IMPA, and EMPA standards, respectively. The peaks, marked with asterisks (*) in spectrum a, correspond to matrix-related ions of CHCA.

A large peak, marked as MPA, corresponds to the final product (methylphosphonic acid). The peaks, marked with asterisks (*) in spectrum a, correspond to matrix-related ions of CHCA, and their m/z values are 146.04, 164.05, 172.04, 190.05, 212.08, 228.01, and 294.07 (possible ion forms: $[M + H-CN-H_2O]^+$, $[M + H-CN]^+$, $[M + H-H_2O]^+$, $[M + H]^+$, $[M + Na]^+$, $[M + K]^+$, and $[2M + H-CO_2-C_2H_3N]^+$, respectively). Careful note should be made of these matrix-related peaks to avoid erroneous assignments. The peaks of the parent-ion $[M + H]^+$ and the final product ($[MPA + H]^+$; m/z, 97.02), can serve as distinguishing characteristics. However, in the cases of PMPA and CHMPA, the parent ions $[M + H]^+$ are extremely weak, whereas MPA peaks are strong (in spectra a and b). The reason for this is because CHMPA and PMPA readily decompose to MPA under acidic conditions, such as when CHCA and TFA are used (pH $\sim$ 2). We tested different types of matrixes, including 2,5-DHB, 3-HPA, 2,6-DHAP, and 2,4,6-THAP, respectively, but better results were not obtained. Thus, in the case CHMPA and PMPA, instead of the $[M + H]^+$ peak, the $[M + Na]^+$ is more useful for identification. Figure 3A shows a mass spectrum of a mixture of the five standards obtained by MALDI-TOFMS using CHCA as the matrix; the source is the THG of Nd:YAG (355 nm, 6 Hz). As shown in the figure, each individual standard and the final product can clearly be observed. The inset shows the UV-absorbance spectrum and chemical structure of CHCA, providing evidence that the use

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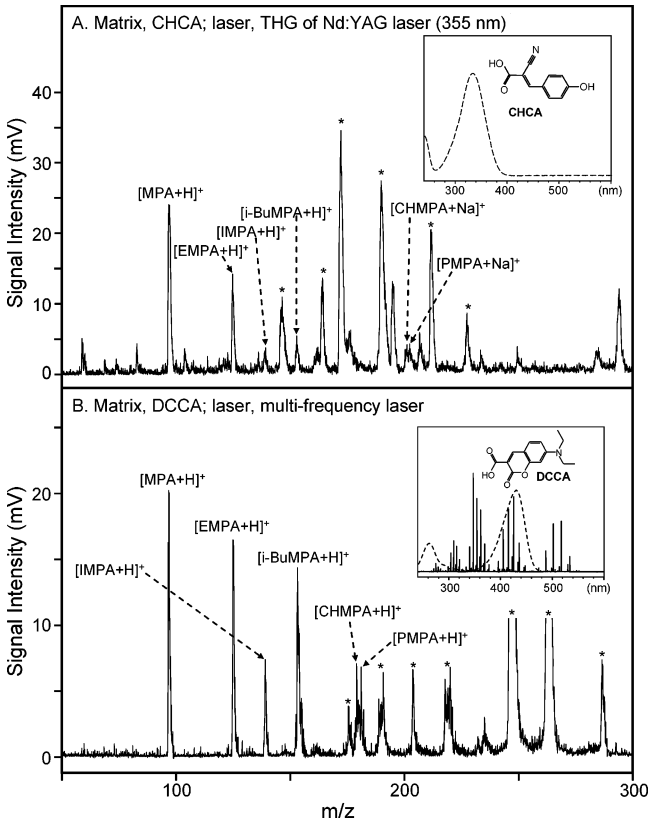

Figure 3. (A) Mass spectrum of a mixture of the five standards obtained by MALDI-TOFMS using CHCA as the matrix (source: the THG of Nd:YAG (355 nm, 6 Hz). (B) Mass spectrum using DCCA as the matrix (source: multifrequency laser (major, 437.8 nm, 10 Hz)). The peaks, marked with asterisks (*), correspond to matrix-related ions of CHCA (A) and DCCA (B), respectively. The insets show the UV-absorbance spectra and chemical structures of CHCA and DCAA, respectively.

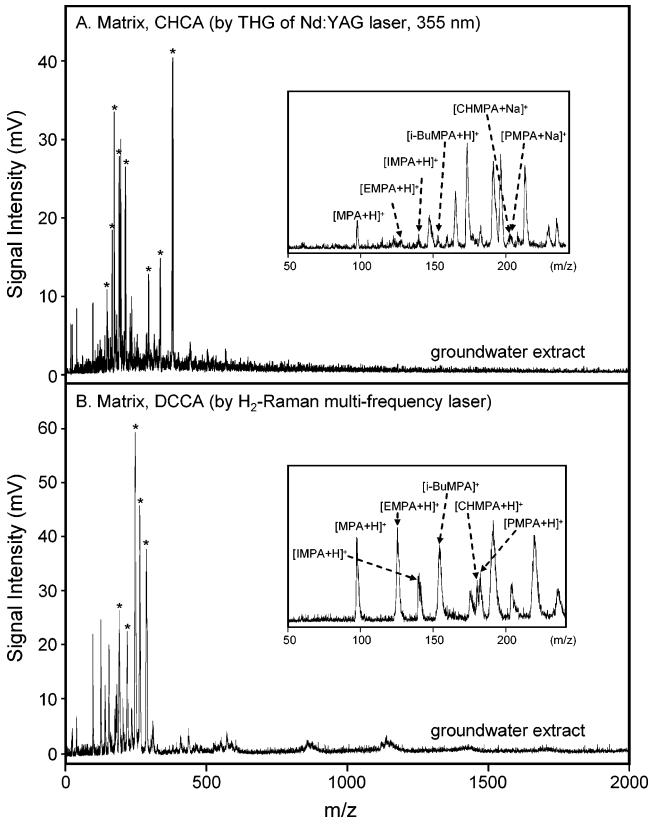

Figure 4. (A) Mass spectrum of the extract of a spiked groundwater sample, obtained by MALDI-TOFMS using CHCA as the matrix (source: the THG of Nd:YAG (355 nm, 6 Hz)). (B) Mass spectrum using DCCA as the matrix (source, multifrequency laser (major, 437.8 nm, 6 Hz)). The insets show the extended mass range from 50 to 240, respectively. The peaks, marked with asterisks (*), correspond to matrix-related ions of CHCA.

of an ionization laser (355 nm) is suitable. In fact, CHCA is a common matrix used in commercial instruments that are equipped with a N_2 laser emitting at 337 nm. Since the laser wavelength is fixed, a "trial and error" step for acquiring an adequate matrix is sometimes limited. As mentioned above, CHCA was not appropriate for observing parent ions of CHMPA and PMPA. Too many peaks appear within the m/z range of the five standards (from 96 to 180), and this would make subsequent peak assignment difficult. To overcome this, various types of matrixes were examined. Since different types of matrixes absorb at different wavelength of lasers, unless an OPO laser is used, it is impossible to gather all types of lasers (from the UV to the IR region) into one unit. For this, we developed a multifrequency laser system[32] and selected a weakly acidic compound, DCCA, as a model matrix (pH $\sim$ 4). The spectrum of multifrequency laser emission and the absorbance spectrum of DCCA are shown in the inset in Figure 3B. As a result, the six major peaks, including five individual standards and one final product, can be clearly observed. Herein, the major laser emission used was 437.8 nm. (If a different matrix is necessary for a trial, the wavelength can be easily switched to a different one.) The matrix-related ions of DCCA are marked with asterisks (*), and their m/z values are 174.3, 190.2, 202.2, 218.3, 246.2 ($[M + H-CH_4]^+$), 262.3 ($[M + H]^+$), and 284.3 ($[M + Na]^+$), respectively. There is almost no overlap with the parent ions of the five nerve agent degradation products, and problems associated with the use CHCA can be resolved. In the case of IMPA (degradation products of Sarin, the limit of detection for our MLDI-TOFMS system was determined to $\sim 1.0 \times 10^{-9}$ and $\sim 1.0 \times 10^{-8}$ g/mm^2 ($S/N = 3$) when CHCA and DCCA were used, respectively.

An actual sample was investigated by spiking the five standards (1.0×10^{-5} g of each) to a groundwater sample (1.0 mL), obtained from the university campus. The sample preparation method was as described in section 2.2. Parts A and B of Figure 4 show the results obtained by the MALDI-TOFMS when CHCA (source: 355 nm) and DCCA (source: multifrequency laser, major at 437.8 nm) were used as the matrix, respectively. In both cases, numerous peaks, some of which correspond to unknown components in groundwater, are detected in the m/z range of 0−2000. The insets in frames A and B show the extended m/z range from 50 to 240. As can be seen from these mass spectra, the major peaks that we expected are all observed in the spiked groundwater extracts. Although, thus far, GC/MS is the most popular and powerful technique for the analysis of volatile/semivolatile compounds and their analogues thereof, in actual experimental procedures, it is necessary to derivatize the analytes prior to their injection into the GC system. For example, in the case of GF analysis, a MTBSTFA (*n*-methyl-*n*-*tert*-butyldimethylsilyltrifluoroacetamide) derivatizing agent is reacted with GF to form a final derivative, which provides a major fragment at m/z 153, for GC/MS analysis. All of these procedures are time-consuming. In contrast to this,

our new method avoids the use of complicated derivative steps in GC/MS, appropriate matrixes were used, and the results appear within several microseconds (corresponding to the flight times of ions), when MALDI-TOFMS is used. Thus, we conclude that MALDI-TOFMS, which is clearly accurate, sensitive, and rapid, can be considered for use in rapid drug-screening and is sufficiently reliable to serve as a complementary method to GC/MS for use in this field.

CONCLUSIONS

A multifrequency laser assisted MALDI-TOFMS method can be successfully used for the rapid screening of nerve agent degradation products, including PMPA, CHMPA, IMPA, *i*-BuMPA, and EMPA, degradation products produced from Soman (GD), GF, Sarin (GB), RVX, and VX, respectively. When CHCA was used, the peaks of the final degradation product (MPA, MW: 97.02) and the individual parent ion $[M + H]^+$ serve as unique, distinguishing characteristics. In the cases of PMPA and CHMPA, instead of the $[M + H]^+$ peaks, the $[M + Na]^+$ is useful for identification. On the other hand, when DCCA was used, the peaks of the final degradation product (MPA) and the individual parent ion $[M + H]^+$ can serve as unique, distinguishing characteristics. An actual sample analysis was also successful, using spiked groundwater samples. To soil or even air samples, if the extraction step can be combined, the method developed herein has great potential for the rapid screening of chemical warfare agents and related compounds.

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