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Stability and Detectability of Lachrymators and their Degradation Products in Evidence Samples*

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ABSTRACT: The detectability and stability of lachrymators [2-chloroacetophenone (CN), *o*-chlorobenzylidene malononitrile (CS) and synthetic capsaicin (nonivamide)] were investigated using dichloromethane extraction followed by gas chromatography-mass spectrometry. Ultrasonication at 40°C slightly improved the extraction yields of nonivamide to almost quantitative yield, compared to low yields (30 to 40%) of CN and CS. In terms of the stability of low spiked concentrations (6 to 7 μg), from the surface of glass and stainless steel (60 to 100 cm square), CN rapidly disappeared, CS disappeared gradually, and nonivamide was almost quantitatively recovered. The three lachrymators in absorbent cotton (0.3 g) gradually disappeared. From water (20 mL), nonivamide gradually disappeared and was undetectable after eleven days. CN decreased gradually, and instead, acetophenone appeared. CS disappeared rapidly, and *o*-chlorobenzaldehyde, *o*-chlorobenzyl alcohol, and *o*-chlorobenzoic acid were produced. The stability of high spiked concentrations of CN and CS in water (0.5 to 0.6 mg per 20 mL) was also investigated using liquid chromatography. CN was quantitatively recovered. The concentrations of CS decreased to about an 8% recovery level within 2 h. Concomitantly, *o*-chlorobenzaldehyde appeared at a concentration of about 80% recovery.

KEYWORDS: forensic science, lachrymators, evidence samples, stability, solvent extraction, gas chromatography-mass spectrometry, high performance liquid chromatography

Lachrymators are usually classified as nonlethal chemical warfare agents (1–3). Lachrymators exert their effect by causing intense sensory irritation such as ocular erythema, local inflammation, and respiratory distress (1,2). They have been widely used by law enforcement agents as a means of nonlethal control of crowds and riots. Lachrymators are also used in tear gas sprays, which are widely marketed for the purpose of personal protection on a worldwide scale. In Japan, the purchase and use of tear gas sprays is not regulated. Recently, crimes involving robbery, pickpocketing, and rape using tear gas sprays have increased. In addition, a number of criminal cases occurred wherein people were attacked via the use of dispersed offensive odors in public places, and some of these cases have been attributed to tear gas sprays.

2-Chloroacetophenone (CN), *o*-chlorobenzylidene malononitrile (CS), and oleoresin capsicum (OC) are representative active ingredients in tear gas sprays (4). OC is an oily hot pepper extract and many compounds in OC are responsible for pungency (5). The most active ingredients in OC are capsaicinoids with a basic structure of vanillylamide: capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin. Capsaicin is the most predominant and active compound among the capsaicinoids. A considerable number of studies have been performed in terms of its usefulness as a medicine (6) and as a food additive (5). Commercial tear gas sprays contain single or plural active ingredients, the total concentrations of which are less than 10% of the total spray formulation (4).

Criminal investigation teams should often be involved in the identification of tear gas sprays that have been used in crimes. It is usually impossible to identify the causative substances by on-site detection of an air sample because of their low volatility. Instead, off-site detection of an on-site evidence sample is more realistic. In Japan, on-site evidence samples, such as spray containers, water, and wipe samples taken from the on-site surfaces with absorbent cotton, and victims' samples such as clothes and wipe samples taken from their skin and clothing, are collected and sent to forensic science laboratories. Numerous types of analytical methods for lachrymators have been developed: thin layer chromatography (7,8), gas chromatography (GC) (9–11), high performance liquid chromatography (HPLC) (12–14), capillary electrophoresis (15,16), gas chromatography—mass spectrometry (MS) (17–20), LC-MS (21), nuclear magnetic resonance spectroscopy (22), and ion mobility spectrometry (23). It is, however, very difficult to identify such materials in offensive odor cases. There have been also only limited reports on the analysis and detection of lachrymators from evidence samples (17,19,24–26), and these reports do not provide sufficient information on the stability and the detectability of lachrymators.

In this paper, we selected CN, CS, and nonivamide (synthetic capsaicin) as lachrymators tested (Fig. 1). Considering the real on-site evidence samples collected in offensive odor incidents, we selected "absorbent cotton" as wiped samples taken off the surfaces and "water" as the aqueous liquid, and reported on the study of the extraction efficiency of three lachrymators from these surfaces by solvent extraction followed by GC-MS analysis. Considering that tear gas is often sprayed in public places, we selected "glass" surfaces representative of windows and victims' spectacles, "stainless steel" for metals, and "absorbent cotton" for victims' clothes, have investigated the stability of lachrymators on these materials, and also the detectability of the degradation products.

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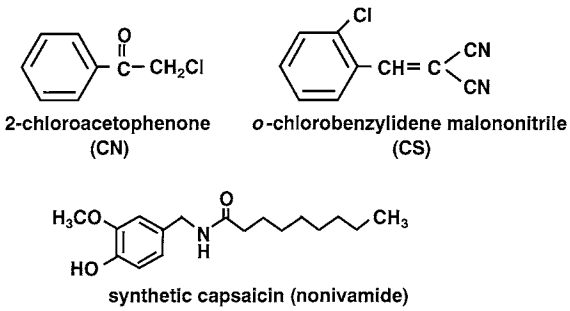

FIG. 1—*Chemical structure of lachrymators.*

Materials and Methods

Reagents

CN, *o*-chlorobenzaldehyde and *o*-chlorobenzoic acid were purchased from Kanto Chemicals (Tokyo, Japan). *o*-Chlorobenzyl alcohol was obtained from Sigma Chemical (St. Louis, Missouri). Nonivamide, acetophenone, and the other reagents used were obtained from Wako Pure Chemical Industries (Osaka, Japan). All reagents were of analytical grade. CS was synthesized by the condensation of *o*-chlorobenzaldehyde with malononitrile (27), recrystallized from cyclohexane (m.p. 94 to 95°C), and structurally confirmed by ^{1}H-NMR and ^{13}C-NMR in $CDCl_3$ (22).

CN, nonivamide, CS, *o*-chlorobenzaldehyde, *o*-chlorobenzoic acid, *o*-chlorobenzyl alcohol, and acetophenone were dissolved in acetonitrile (1000 ppm, w/v) respectively, and stored at 4°C as stock solutions. A working solution was prepared by diluting the stock solution with acetonitrile.

Extraction of Lachrymators

A 100 μL volume of an acetonitrile solution containing 6 to 7 μg of CN, CS, or nonivamide was added to 0.3 g of "absorbent cotton" and 20 mL of "water" in a glass tube, and were then allowed to stand at 20°C for 2 h without closing. The lachrymators were extracted from the absorbent cotton with two portions of dichloromethane (CH_2Cl_2, 20 mL) by ultrasonication (US-1, Iuchi, Tokyo, Japan) for 10 min at 40°C. From the water, the lachrymators were extracted with two portions of CH_2Cl_2 (20 mL) by shaking for 10 min at 20°C. The collected organic phase was dehydrated over anhydrous sodium sulfate and filtered through No. 2 filter paper (Advantec, Tokyo, Japan). A 100 μL volume of an acetone solution containing 44.4 μg/mL of anthracene [internal standard (I.S.)] was added to the extracted organic phase. The mixed solution was concentrated under reduced pressure at 30°C, and analyzed by GC-MS.

Gas Chromatography-Mass Spectrometry

GC-MS was performed basically according to a previous described method (28) with minor modifications. The GC-MS system consisted of an HP 6890 series gas chromatograph combined with an HP 5973 quadrupole mass selective detector (Yokogawa Analytical Systems, Tokyo, Japan). The stationary phase was a capillary column HP-5 MS (30 m × 0.25 mm i.d., 0.25 μm in thickness, Yokogawa Analytical Systems). The carrier-gas (helium) flow-rate and the splitter ratio were adjusted at 1.0 mL/min and 20, respectively. The injection port, transfer line and ion source were maintained at 200, 250, and 230°C, respectively. Electron ionization (ionization energy 69.9 eV, ionization current 34.6 μA) was used as the ionization mode. The oven temperature was controlled by a program [starting at 100°C (1 min hold), increasing to 290°C at 20°C per min (5 min hold)]. The acquisition mass range was 50 to 550, and the scan rate was 0.8 scans per sec. A 1 μL volume of sample was applied to the GC-MS system. Acquisition was started just after the sample injection. The extracted ion chromatograms were obtained at m/z 105 for CN and acetophenone, m/z 153 for CS, m/z 137 for nonivamide, m/z 139 for *o*-chlorobenzaldehyde and *o*-chlorobenzoic acid, m/z 142 for *o*-chlorobenzyl alcohol, and m/z 178 for I.S., respectively. The analyte concentrations were obtained from the peak area ratios of the analytes to I.S., using the standard calibration curve which was made by plotting the peak area ratios against the designated concentration of the analytes.

High Performance Liquid Chromatography

HPLC system consisted of a 600S controller, 626 pump, and 996 photodiode array detector (Waters Corporation, Milford, Massachusetts). The stationary phase was a J'sphere ODS-H80 column (150 × 4.6 mm I.D., Yamamura Chemical, Kyoto, Japan) which was maintained at 40°C. The flow rate was 1.2 mL/min. For the analysis of CN and acetophenone, the mobile phase was a mixed solution (35:65, v/v) of acetonitrile and 1.3% (v/v) acetic acid in water. For the analysis of CS, *o*-chlorobenzaldehyde, *o*-chlorobenzyl alcohol, and *o*-chlorobenzoic acid, the mobile phase was controlled by a solvent linear gradient program [mixture of acetonitrile and 1.3% (v/v) acetic acid in water from 25:75 to 65:35 in 15 min]. A 10 μL volume of sample was applied to the HPLC systems. The signals were monitored at 260 nm for CN and acetophenone, 252 nm for *o*-chlorobenzaldehyde, 267 nm for *o*-chlorobenzyl alcohol, 280 nm for *o*-chlorobenzoic acid, and 300 nm for CS, respectively. The analyte concentration in samples was obtained from the peak areas of the analytes, using the standard calibration curve, which was made by plotting the peak areas against the designated concentration of the analytes.

Experiment for Stability of Lachrymators

A 100 μL volume of an acetonitrile solution containing 6 to 7 μg of CN, CS, or nonivamide was spiked on the previously cleaned surface of five glass plates (60 cm^2) and five stainless steel plates (100 cm^2), and to water (20 mL) in six glass tubes and absorbent cotton (0.3 g) in five glass tubes.

The spiked surfaces of the glass and stainless steel plates were covered with aluminum foil and allowed to stand at 20°C. After appropriate periods of time (2 h, 1, 3, 7, and 15 days), the spiked surface were wiped off with 0.3 g of absorbent cotton dampened with CH_2Cl_2, and the lachrymators were extracted with two portions of CH_2Cl_2 (20 mL) from the wiped cotton. The organic layer was concentrated and analyzed by GC-MS.

The absorbent cotton samples spiked with the lachrymators were allowed to stand at 20°C in closed tubes. After appropriate periods of time (2 h, 1, 3, 7, and 15 days), the lachrymators were extracted with two portions of CH_2Cl_2 (20 mL) from the cotton samples in the tubes and analyzed by GC-MS.

The spiked water samples were allowed to stand at 20°C in closed tubes. After appropriate periods of time (2 h, 1, 4, 7, 11, and 15 days), the lachrymators and degradation products (acetophenone for CN, and *o*-chlorobenzaldehyde and *o*-chlorobenzyl alcohol for CS) were extracted with two portions of CH_2Cl_2 (20 mL) from the water samples in the tubes and analyzed by GC-MS. For the determination of *o*-chlorobenzoic acid, the extracted aqueous

phase was adjusted to pH 1.5-2.0 with 1.0 M hydrochloric acid solution and extracted with two portions of CH_2Cl_2 (20 mL). The organic phase was concentrated and analyzed by GC-MS. The recovery yields of the degradation products of lachrymators were expressed as molar percentages, compared to the spiked original lachrymators.

In a second stability experiment, a 100 μL volume of an acetonitrile solution containing 0.5 to 0.6 mg of CN or CS was spiked to 20 mL of water. The spiked water samples were allowed to stand at 20°C in a closed container. Ten μL aliquots were periodically (2 h, 1, 3, 7, 11, 15 days) sampled and injected to HPLC. The recovery yields of the degradation products were expressed as the molar percentage compared to the original spiked lachrymators.

Results and Discussion

Analysis of Lachrymators and their Degradation Products by Gas Chromatograph-Mass Spectrometry

As shown in Fig. 2, CN, CS, nonivamide and their degradation compounds were clearly separated from one another by GC-MS analysis. Their mass spectra are shown in Fig. 3. Molecular ions (M^+) were clearly observed for all compounds except CN. For CN and acetophenone, the peaks of m/z 105, which correspond to the ions [M-49 $(CH_2Cl)]^+$ and [M-15 (methyl)]$^+$, respectively, were identified as base peaks. For CS, the peak at m/z 153, which corresponds to the ion [M-35 (Cl)]$^+$ was identified as a base peak. For *o*-chlorobenzaldehyde and *o*-chlorobenzoic acid, the peaks of m/z 139, which correspond to the ions M^+ and [M-17 (OH)]$^+$, respectively, were identified as base peaks. For *o*-chlorobenzyl alcohol, the peak of m/z 77, which corresponds to the ion [M-35 (Cl)-31 $(CH_2OH)]^+$ was identified as a base peak. For nonivamide, the peak of m/z 137, which corresponds to the ion [M-156 {NH-CO-$(CH_2)_6CH_3$}]$^+$, was identified as a base peak.

The detection limits for GC in the split mode (splitter ratio of 20) are listed in Table 1. The repeatability ($n = 5$) for the determination of lachrymators and related compounds (each 20 ng), in terms of injection volume (1 μL) ranged from 0.66% (CS) to 10% (*o*-chlorobenzoic acid) expressed as the relative standard deviation (R.S.D.). Nonivamide and *o*-chlorobenzoic acid showed low detection levels.

Extraction Yield of Lachrymators from Forensic Samples

Absorbent cotton and water were fortified with CN, CS, or nonivamide (about 20 μg/g for absorbent cotton and 0.30 μg/mL for water) and the efficiency of the extraction methods and the extraction yields of lachrymators were examined. If the concentration of lachrymators in tear gas spray is assumed to be 1%, the amounts of lachrymators spiked in this experiment correspond to 1 mL of the sprayed contents dispersed in about 450 g of cotton of 29 L of water.

The efficiency of organic solvents (CH_2Cl_2, *n*-hexane, diethylether, ethylacetate) in the extraction of CN, CS, and nonivamide from water and absorbent cotton was examined. The extraction yields of CS were very low and not so different among the solvents examined. The extraction yields of CN from cotton were also low (around 20%) and not so different. Those from water were high (over 70%) and not so different among the solvents. However, the yields of nonivamide from water and cotton by CH_2Cl_2 were almost quantitative, compared to the low yields ($< 50\%$) by the other solvents. Therefore, we selected CH_2Cl_2 as the extraction solvent. As shown in Fig. 4*A*, the yields of nonivamide from the water sample were nearly quantitative by the extraction with shaking (300 strokes/min, for 10 min) at 20°C. The yield of CN was also fairly high. On the other hand, less than 3% of CS was recoverable. Instead, large amounts of *o*-chlorobenzaldehyde were detected. CS is easily hydrolyzed to *o*-chlorobenzaldehyde and the half life of CS in water at neutral pH (25°C) is reported to be 15 min (1). In this experiment, spiked water samples were allowed to stand at 20°C for 2 h prior to solvent extraction.

TABLE 1—*Detection limits for lachrymators and their degradation products by gas chromatography-mass spectrometry.*

Compounds	MW	Extracted Ion	Detection Limits,* ng	R.S.D.,† %
2-chloroacetophenone	154	105	0.19	2.0
Acetophenone	120	105	0.081	2.3
o-chlorobenzylidene malononitrile	188	153	0.10	0.66
o-chlorobenzaldehyde	139	139	0.037	2.0
o-chlorobenzyl alcohol	142	142	0.39	3.2
o-chlorobenzoic acid	156	139	10	10
Nonivamide	293	137	0.41	7.7

* Detection limits are defined as the level of analyte that gives a signal-to-noise ratio of 3:1 and are represented as ng in injection volume (1 μL).

† R.S.D. (relative standard deviation) are defined as the repeatability ($n = 5$) in the determination of 20 ng of each compound in injection volume (1 μL) by GC-MS.

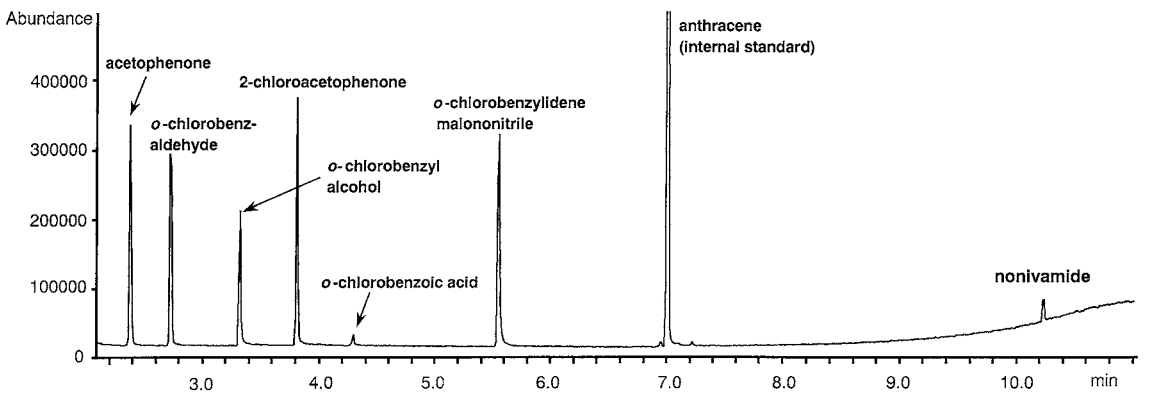

FIG. 2—*Total ion chromatogram of standard solutions of lachrymators and their degradation products [20 ng for each in injection volume (1 μL)].*

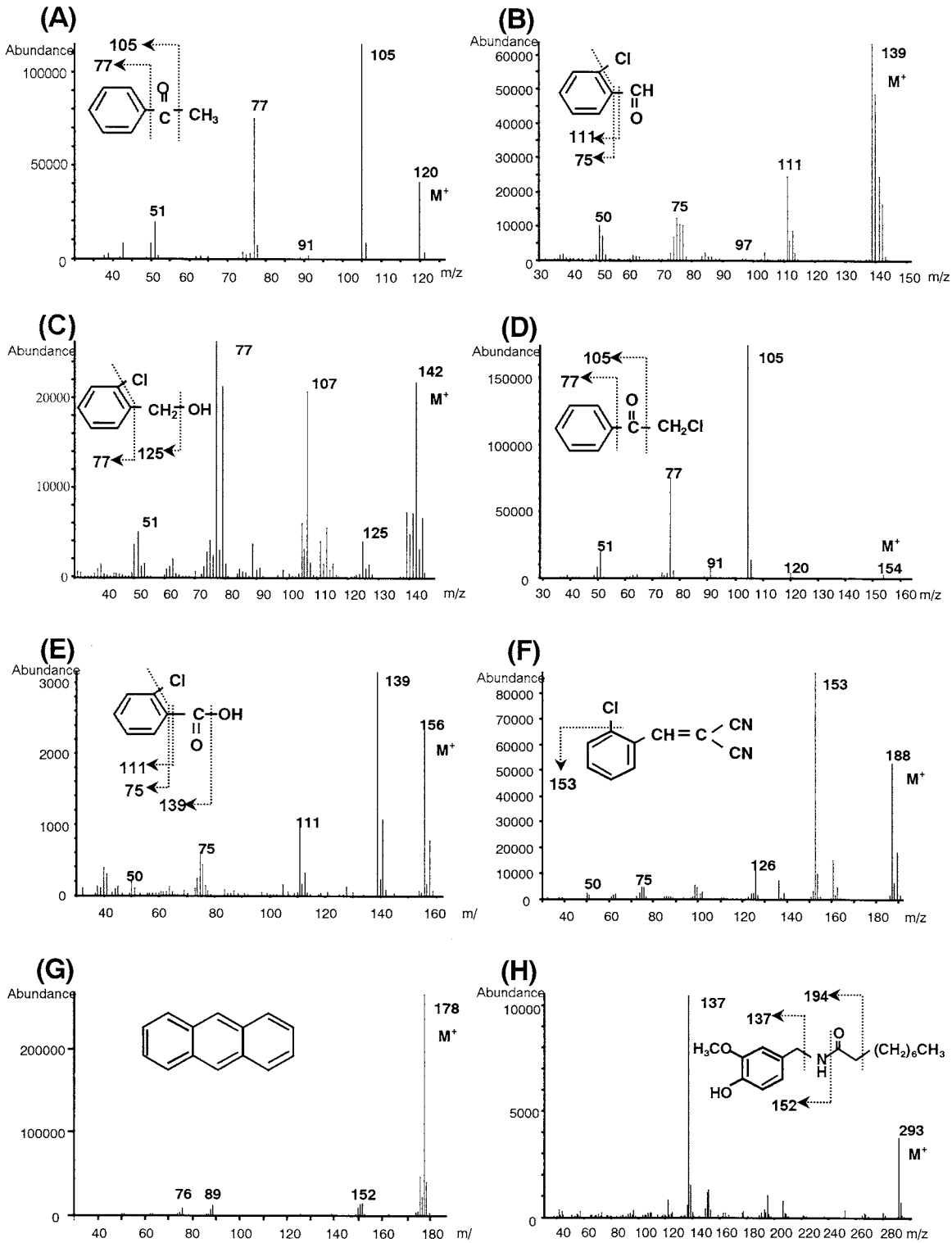

FIG. 3—*Mass spectra of lachrymators and their degradation products:* (A) *acetophenone;* (B) *o-chlorobenbzaldehyde;* (C) *o-chlorobenzyl alcohol;* (D) *CN;* (E) *o-chlorobenzoic acid;* (F) *CS;* (G) *anthracene;* (H) *nonivamide.*

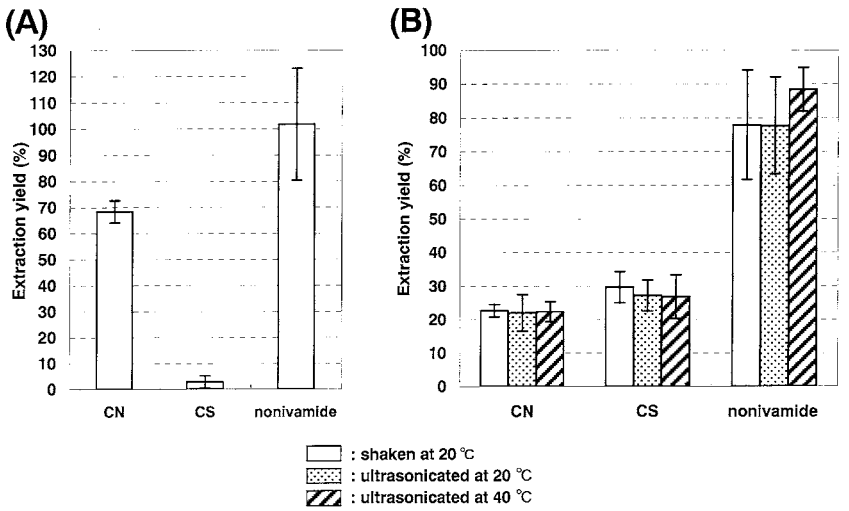

FIG. 4—*Extraction yields of lachrymators from water* (A) *and absorbent cotton,* (B). *20 mL of water and 0.3 g of absorbent cotton spiked with 100 μL of an acetonitrile solution containing 6 to 7 μg CN, CS or nonivamide were extracted with two portions of CH_2Cl_2 (20 mL). The organic layer was analyzed by GC-MS. The vertical line depicts the percentage yield of the lachrymators.*

The efficiency of ultrasonication and warming during the CH_2Cl_2 extraction from absorbent cotton samples was examined. As shown in Fig. 4*B,* yields of nonivamide were high, and were only slightly improved by both ultrasonication for 10 min and warming at 40°C. As a result, ultrasonication at 40°C for extraction of absorbent cotton was used. CN and CS were extracted in yields of around 25%, and ultrasonication and warming failed to improve the yields of these materials. The vapor pressure of CN and CS at 20°C are 5.4×10^{-3} and 3.4×10^{-5} mmHg, respectively (1), and the spiked absorbent cotton samples were allowed to stand at 20°C for 2 h in opened tubes prior to extraction. Thus, it is probable that CN and CS were partially vaporized. In addition, spreading CN and CS on the cotton surface by dilution with acetonitrile may promote the vaporization of lachrymators. Low spiked levels of lachrymators may be another reason for the observed low extraction yields.

Stability of Lachrymators—Low Spiked Level Experiment

Volatility and stability of lachrymators appear to be the main reasons for the difficulties in detecting lachrymators in cases where tear gas sprays are intentionally dispersed in public places. In this experiment, we examined the stability of lachrymators in various samples. The surface of materials (glass plates and stainless steel plates) and samples (water and absorbent cotton) were spiked with trace amount of lachrymators and allowed to stand at 20°C. After appropriate incubation, each sample was extracted, and the organic layer was analyzed by GC-MS. From the surface of glass and stainless steel plates, similar behaviors of lachrymators were observed (Fig. 5*A* and *B*). CN rapidly disappeared within one day. CS gradually disappeared, but remained detectable even after seven days. Degradation products of CN and CS were not detected from the surface of any of the materials. It is probable that CN and CS easily vaporize from the solid surface. In contrast, nonvolatile nonivamide was stable and more than 60% could be detected, even after 15 days.

Figure 5*C* shows the stability of lachrymators in the absorbent cotton and the water samples. The recovery yields of all three lachrymators from the absorbent cotton samples gradually decreased in similar manners. The initial recoveries of CN and CS from the absorbent cotton samples were higher than those found after 2 h storage at 20°C in opened tubes (Fig. 4*B*). In the experiment on the stability of lachrymators, the spiked absorbent cotton samples were kept in closed containers, which may prevent the passive diffusion of lachrymators out of the samples. Therefore, forensic scientists should pay attention to the proper storage of evidence samples. Even nonivamide, a chemically stable and nonvolatile compound, disappeared gradually.

Regarding the water samples, nonivamide disappeared gradually, and was undetectable after eleven days (Fig. 5*D*). The reason why stable nonivamide disappeared is not clarified. For CN and CS, complicated patterns of appearance and disappearance of lachrymators and their degradation products were observed (Fig. 5*E* and *F*). CS disappeared within one day, and *o*-chlorobenzaldehyde appeared in its place (Fig. 5*F*). The concentration of *o*-chlorobenzaldehyde gradually decreased, and instead, both *o*-chlorobenzoic acid and *o*-chlorobenzyl alcohol, the oxidized and reduced forms of *o*-chlorobenzaldehyde respectively, were detected after four days. When *o*-chlorobenzaldehyde and *o*-chlorobenzyl alcohol (each 6 μg) were spiked to 20 mL of water at neutral pH, the extraction yields using CH_2Cl_2 as the solvent were 80 and 77%, respectively. On the other hand, *o*-chlorobenzoic acid was not detected in water by solvent extraction at neutral pH, but was detected at acidic pH. The pKa of *o*-chlorobenzoic acid is 2.92. The extraction yield of *o*-chlorobenzoic acid at acidic pH was 96% when 6 μg was spiked to 20 mL of water. Benzaldehyde is unstable and is oxidized to benzoic acid in air (29). A portion of the *o*-chlorobenzaldehyde can be converted to *o*-chlorobenzoic acid during the solvent extraction.

CN disappeared gradually. In contrast to CS, CN was more stable, and remained detectable even after eleven days (Fig. 5*E*). Acetophenone, a dechlorinated form of CN, was detected after four days. The issue of how acetophenone is produced from CN in water was not investigated further and remains unexplained. It has been reported that CN is slowly hydrolyzed to hydroxyacetophenone (30). This compound may be unstable in water and thus, undetectable. CN and acetophenone could not be detected at all after 15 days.

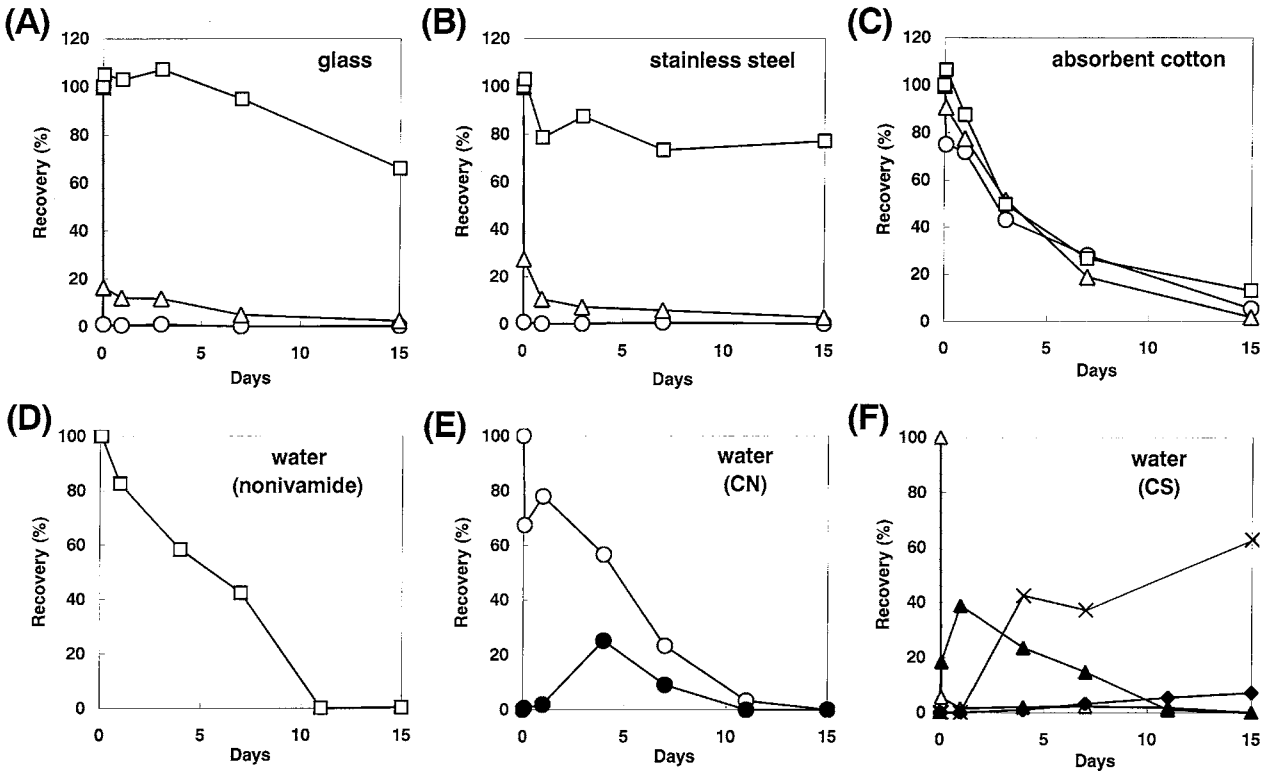

FIG. 5—*Stability of lachrymators on the surface of glass and stainless steel, and in absorbent cotton and water samples.* (A), (B) *The surface of the glass (60 cm^2) and stainless steel (100 cm^2) plates, spiked with 100 μL of an acetonitrile solution containing 6 to 7 μg CN (○), CS (△) or nonivamide (□), were wiped off with absorbent cotton dampened with CH_2Cl_2. The wiped cotton was extracted with 20 mL of CH_2Cl_2 and the organic layer was analyzed by GC-MS.* (C-F) *0.3 g of absorbent cotton and 20 mL of water, spiked with 100 μL of an acetonitrile solution containing 6 to 7 μg CN (○), CS (△) or nonivamide (□), were extracted with CH_2Cl_2, and the organic phase was analyzed by GC-MS. Degradation products of lachrymators: acetophenone (●); o-chlorobenzaldehyde (▲); o-chlorobenzyl alcohol (◆) and o-chlorobenzoic acid (×) are also shown in* (C) *and* (D)*. The horizontal line depicts the incubation periods (days) after the lachrymators were spiked. The vertical line depicts the molar recovery percentage corresponding to the spiked lachrymators.*

Determination of Lachrymators and their Degradation Products by High Performance Liquid Chromatography

Determination of the degradation products of CN and CS from water samples by GC-MS may be difficult because the unstable degradation products may be susceptible to further decomposition during analysis (from extraction to GC injection). HPLC provides a simple and direct analysis for unstable compounds in water samples. CN, CS, and their degradation compounds were clearly separated from one another (data not shown). Ultraviolet absorbance spectra of the peaks enabled the identification of the separated compounds. The detection limits under HPLC conditions are shown in Table 2. The good reproducibility was obtained in the HPLC determination of lachrymators and their degradation products.

TABLE 2—*Detection limits of lachrymators and their degradation products by high performance liquid chromatography.*

Compounds	Detection Wavelength, nm	Detection Limits,* ng	R.S.D.,† %
2-chloroacetophenone	260	2.3	3.2
Acetophenone	260	1.6	2.6
o-chlorobenzylidene malononitrile	300	1.5	5.4
o-chlorobenzaldehyde	252	9.0	3.8
o-chlorobenzyl alcohol	267	74	4.1
o-chlorobenzoic acid	280	16	5.1

* Detection limits are defined as the level of analyte that gives a signal-to-noise ratio of 3:1 and are represented as ng in injection volume (10 μL).

† R.S.D. (relative standard deviation) are defined as the repeatability (n = 5) in the determination of 500 ng each compound in injection volume (10 μL) by HPLC.

Stability of Lachrymators in Water—High Spiked Level Experiment

20 mL of samples of water were spiked with 100 μL of an acetonitrile solution including high levels of CN or CS (0.5 to 0.6 mg), and periodically analyzed by HPLC. The spiked amounts of these lachrymators correspond to 1.0 mL of the sprayed contents dispersed in about 350 mL of water, if the concentration of lachrymators is assumed to be 1%. The recovery yields of CN were in excess of 80%, even after 15 days (Fig. 6*A*). The concentration of CS immediately decreased to a recovery level of about 8%, and remained nearly constant for 15 days (Fig. 6*B*). Instead, *o*-chlorobenzaldehyde was detected at a nearly 80% recovery level, and the concentration also remained constant for 15 days. *o*-Chlorobenzyl alcohol and *o*-chlorobenzoic acid were not detected in the water samples. These results are inconsistent with those for the low spiked levels of lachrymators (Fig. 5*E* and *F*). The difference in the spiked amounts of lachrymators between the low level (6 to 7 μg) and the high level (0.5 to 0.6 mg) experiments should significantly influence the stability of lachrymators in water. When higher levels of CS (1 to 3 mg) were spiked to water (20 mL), the recovery of CS

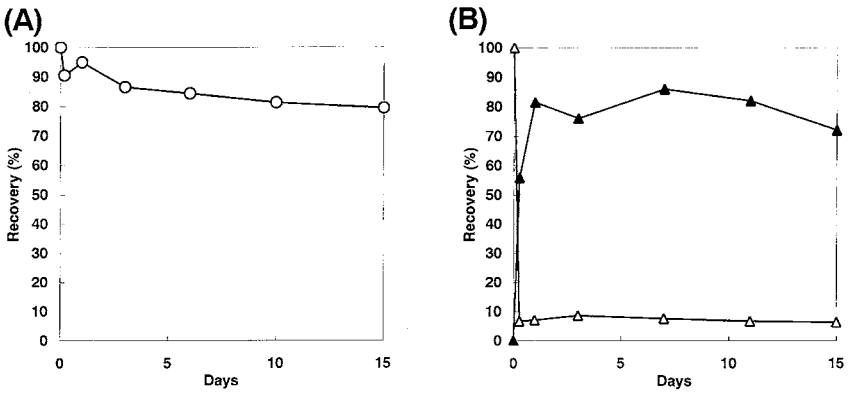

FIG. 6—*Stability of high spiked levels of CN* (A) *and CS* (B) *in water. Twenty mL of water, spiked with 100 μL of an acetonitrile solution containing 0.5 to 0.6 mg CN (○) or CS (△), were periodically analyzed by HPLC. o-chlorobenzaldehyde (▲) is also shown in* (B). *The horizontal line depicts the days of incubation after the lachrymators were spiked. The vertical line depicts the molar recovery percentage corresponding to the spiked lachrymators.*

was about 40%, and the detection level of *o*-chlorobenzaldehyde became lower (about 50%, data not shown). The solubilities of CN and CS in water at 20°C are 0.68 mg/mL and 0.038 mg/mL, respectively (1). With high spiked levels of lachrymators, especially CS, a significant fraction may not be soluble in water, but, rather, could exist as micelles or in the form of a membrane layer on the surface of the water. Small portions may be solubilized in water and subject to degradation. With low spiked levels of lachrymators, all portions may be solubilized in water and subject to degradation.

Conclusion

Our *in vitro* experiment on the stability of lachrymators demonstrates that within a few days after spiking, 2-chloroacetophenone (CN) can be detected from water or cotton, but not from the surface of glass nor stainless steel. *o*-Chlorobenzylidene malononitrile (CS) can be detected from cotton and wiped sample from the surface of glass and stainless steel, but not from water. Instead, degradation products of CS, *o*-chlorobenzaldehyde or *o*-chlorobenzoic acid can be detectable from water. Nonivamide, synthetic capsaicin, can be detected from cotton, water, and glass and stainless steel surfaces. Therefore, in crimes in which tear gas sprays are used, the surface(s) stained with tear gas should be collected as soon as possible, and then such samples should be sent in closed containers to the forensic science laboratory, where lachrymators and their degradation products can be detected by CH_2Cl_2 extraction followed by GC-MS.

References

1. Hu H. Toxicodynamics of riot-control agents (lacriminators). In: Somani SM, editor. Chemical warfare agents. San Diego: Academic Press, 1992;271–88.
2. Marrs TC, Maynard RL, Sidell FR. Chemical warfare agents—toxicology and treatment. West Sussex: John Wiley & Sons Ltd, 1996;221–9.
3. Hu H, Fine J, Epstein P, Kelsey K, Reynolds P, Walker B. Tear gas—harassing agent or toxic chemical weapon? JAMA 1989;262:660–3.
4. Lee RJ, Yolton RL, Yolton DP, Schnider C, Janin ML. Personal defense sprays: effects and management of exposure. J Am Opt Assoc 1996; 67: 548–60.
5. Govindarajan VS, Sathyanarayana MN. Capsicum—production, technology, chemistry, and quality. Part V. Impact on physiology, nutrition, and metabolism; structure, pungency, pain, and desensitization sequences. Crit Rev Food Sci Nutr 1991;29:435–74.
6. Cordell GA, Araujo OE. Capsaicin: identification, nomenclature, and pharmacotherapy. Ann Pharmacother 1993;27:330–6.
7. Ludemann WD, Stutz MH, Sass S. Qualitative thin-layer chromatography of some irritants. Anal Chem 1969;41:679–81.
8. Jane I, Wheals BB. Chromatographic characterization of lachrymatory agents in tear gas aerosols. J Chromatogr 1972;70:151–3.
9. Gag JA, Merck NF. Concise identifications of commonly encountered tear gases. J Forensic Sci 1977;22:358–64.
10. Krajewska AM, Powers JJ. Pentafluorobenzylation of capsaicinoids for gas chromatography with electron-capture detection. J Chromatogr 1988;457:279–86.
11. Gandhe BR, Malhotra RC, Gutch PK. Gas chromatographic retention indices of tear gases on capillary columns. J Chromatogr 1989;479: 165–9.
12. Raghuveeran CD, Malhotra RC. Reversed-phase high-performance liquid chromatography of some irritants. J Chromatogr 1982;240:243–6.
13. Hoffman PG, Lego MC, Galetto WG. Separation and quantitation of red pepper major heat principles by reverse-phase high-pressure liquid chromatography. J Agric Food Chem 1983;31:1326–30.
14. Law MW. High pressure liquid chromatographic determination of capsaicin in oleoresin and personal protection aerosols. J Assoc Off Anal Chem 1983;66:1304–6.
15. Khaled MY, Anderson MR, McNair HM. Micellar electrokinetic capillary chromatography of pungent compounds using simultaneous on-line ultraviolet and electrochemical detection. J Chromatogr Sci 1993; 31:259–64.
16. Lascaridou-Monnerville AL. Determination of capsaicin and dihydrocapsaicin by micellar electrokinetic capillary chromatography and its application to various species of *Capsicum,* Solanaceae. J Chromatogr A 1999;838:293–302.
17. Fung T, Jeffery W, Beveridge AD. The identification of capsaicinoids in tear-gas spray. J Forensic Sci 1982;27:812–21.
18. Ferslew KE, Orcutt RH, Hagardorn AN. Spectral differentiation and gas chromatographic/mass spectrometric analysis of the lacriminators 2-chloroacetophenone and *o*-chlorobenzylidene malononitrile. J Forensic Sci 1986;31:658–65.
19. Nowicki J. Analysis of chemical protection sprays by gas chromatography/mass spectroscopy. J Forensic Sci 1982;27:704–9.
20. Martz RM, Reutter DJ, Lasswell LD. A comparison of ionization techniques for gas chromatography/mass spectroscopy analysis of dye and lachrymator residues from exploding bank security devices. J Forensic Sci 1983;28:200–7.
21. Games DE, Alcock NJ. Analysis of pepper and capsicum oleoresins by high-performance liquid chromatography-mass spectrometry and field desorption mass spectrometry. J Chromatogr 1984;294:269–79.
22. Mesilaakso M. Analysis of the ^{1}H and $^{13}C\{^{1}H\}$ NMR spectral parameters of the tear gases α-chloroacetophenone, dibenz [*b,f*] [1.4] oxazepine, and 2-chlorobenzylidene malononitrile. Magn Reson Chem 1996;34:989–94.
23. Allinson G, McLeod CW. Characterization of lacriminators by ambient temperature ion mobility spectrometry. J Forensic Sci 1997;42:312–5.
24. Morita M, Takagi M. Discriminating method of irritant aerosol spray for self-protection. Reports Natl Res Inst Police Sci 1988;41:107–13.
25. Allinson G, Saul C, McLeod CW, Gilbert J. Identification of tear gases in suspect spray cans and cloth samples by ion mobility spectrometry. J Forensic Sci 1998;43:845–9.

26. Pepler RS, Esseiva P, Gueniat O. The study of defense gas sprays in forensic sciences: detection, identification, persistence and ballistics. Sci Justice 1998;38:203–10.
27. Corson BB, Stoughton RW. Reactions of alpha, beta unsaturated dinitriles. J Am Chem Soc 1928;50:2835–7.
28. Tsunoda N, Seto Y, Kataoka M, Ohta H. A simple and rapid analysis of irritants in tear gas sprays by capillary column gas chromatography-mass spectrometry. In: Ferrara DS, editor. Proceedings of TIAFT XXXV Annual Meeting; 1997 Aug 24–28; Padova. Italy: 1998;438–44.
29. Ohki M, Ohsawa T, Tanaka M, Chihara H. Encyclopedic dictionary of chemistry. Tokyo: Tokyo Kagaku Dojin, 1989.
30. Stewart CE, Sullivan JB. Military munitions and antipersonnel agents. In: Sullivan JB, Krieger GR, editors. Hazardous Materials Toxicology—Clinical Principles of Environmental Health. Baltimore: William & Wilkins, 1992;986–1014.

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