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Detection of Pepper Spray Residues on Fabrics Using Liquid Chromatography-Mass Spectrometry

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ABSTRACT: The analysis of trace evidence for the presence of biological and nonbiological residues is an integral part of many criminal and civil investigations and the use of pepper spray self-defense weaponry by the general public, criminals, and law enforcement agents is increasing. Therefore, the possibility that pepper spray residues may be present as forensic evidence at crime scenes or from civil disturbances becomes more likely. We have investigated the effects of storage and washing on the detection of pepper spray residues (i.e., capsaicinoids) on cotton, cotton-polyester blend, wool, and nylon fabrics. The concentrations of the capsaicinoid analogues on the fabrics decreased between 5 to 60% during six months of storage when compared with samples of each fabric type that were prepared and analyzed at the onset of the stability study (Time 0). The rate of disappearance of the capsaicinoids was analogue specific. Degradation of the capsaicinoids was independent of fabric type and temperature of storage. We also investigated the effects of washing the fabrics on the detection of capsaicinoids. Fabrics were washed with water, 1% detergent, 1% bleach, or 5% Spray and Wash™. Water was the least effective method of removing the capsaicinoids from the fabric and bleach the most effective. Retention of the capsaicinoids on the fabrics following washing was affected by fabric type as well as the chemical properties of the individual capsaicinoid analogues. The uses and limitations of capsaicinoid residue evidence as an indicator of exposure to pepper sprays or use of pepper sprays are discussed.

KEYWORDS: forensic science, pepper spray products, capsaicinoids, fabric, forensic markers, liquid chromatography-mass spectrometry

In 1996, it was estimated that nearly 15 million Americans carried pepper spray self-defense weapons (1). Increasingly, these products are being used to deter aggressive behavior and attacks (1,2) and to aid in perpetrating assaults (3). Pepper spray products are also used as the standard less-than-lethal (LTL) weapons for law enforcement personnel (2). LTL weapons temporarily incapacitate the victim or attacker by causing intense pain, temporary blindness, and choking (4–8). In most instances, few long-term side effects result from exposure to pepper sprays (9,10). However, exposed victims may require emergency medical treatment for respiratory difficulties, skin irritation, and damage to the eyes (1,11–14).

In forensic investigations, law enforcement personnel often rely on trace evidence including blood (15), urine (16,17) body fluid stains (18), chemical markers (19,20, and references within), finger or lip prints (21–23), hair and fiber (24,25), DNA (26,27), and fabric (28) to support individual accounts of the events and to determine whether a particular suspect or victim was involved. Highly publicized events where victims of pepper spray use sought legal recourse include the Seattle, Washington World Trade Organization demonstrations in November and December of 1999 (29) and demonstrations in Humboldt County, CA where logging protesters were allegedly tortured with pepper spray by local authorities (October 1997) (30,31).

As the popularity of LTL self-defense weaponry increases, it becomes increasingly likely that the presence of pepper spray residues may be available objective chemical evidence of involvement in a crime or altercation. Therefore, the detection of capsaicinoids may be valuable evidence in identifying suspects and corroborating accounts of events where LTL weapons were allegedly used. A potential difficulty in detecting forensic evidence like capsaicinoids on easily altered materials, such as clothing, is the possibility that the clothing may be cleaned or washed in an attempt to remove the chemical residue (15,18). In addition, the time between exposure and testing and the storage conditions of the evidence may also affect the detection of certain forensic markers (16).

Several scenarios can be envisioned where storage, time, and garment treatment may be factors in detection of capsaicinoid residues: (1) The victim of an assault used a pepper spray in self-defense. In an attempt to remove the spray residue, the assailant washed or discarded his/her clothing and the clothing was discovered weeks to months after the attack. (2) An individual was involved in civil disturbance where pepper spray use resulted in accidental exposure and injury. The individual's clothing may provide evidence of exposure. (Or 3) A suspect was sprayed by a law enforcement agent, but not apprehended. The suspect discarded or washed his/her clothing prior to apprehension.

The primary purpose of this study was to determine if residues of pepper sprays could be detected on clothing following scenarios, such as those described above. Specifically, we wanted to determine the effects of fabric type, storage temperature, duration of storage, and washing on the detection of capsaicinoids from clothing samples.

Materials and Methods

Chemicals—A pepper spray product was purchased from Guardian Personal Security Products, Inc. (Phoenix, AZ). This product was selected because its capsaicinoid concentrations

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were representative of typical commercial products (32,33). The contents of the peppery spray canister were collected as previously described (32,33). Capsaicin (97% and 60%), nonivamide (99%), and dihydrocapsaicin (90%) were purchased from Sigma Chemical Corp. (St. Louis, MO). Nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin were purified from the 60% capsaicin purchased from Sigma using HPLC (32). Octanoyl vanillamide (the internal standard for quantification of the capsaicinoids) was synthesized as previously described (34). Methanol was purchased from Fisher Scientific (Fairlawn, NJ). *n*-Butyl Chloride was purchased from J.T. Baker (Phillipsburg, NJ). Purified water (specific resistance = 18.2 M Ω *cm) was obtained from a Millipore Milli-Q Plus water purification system (Millipore Corp., Burlington, MA). Samples of clothing consisting of 100% cotton, 100% wool, 60%:40% cotton:polyester blend, and 100% nylon were donated by laboratory staff. Laundry detergent (Purex™), bleach, and Spray and Wash™ were purchased from a local grocery store.

Storage and Stability of the Fabric Samples—Cotton, wool, blended, and nylon fabric samples (1 cm²) ($n = 3$) were treated with 50 μ L pepper spray (~0.5 mg capsaicinoids) and permitted to dry overnight at room temperature. The samples were placed in 16 \times 100 culture tubes and sealed. Three storage conditions were evaluated: room temperature in the dark, 4°C in the dark, and -20°C in the dark. Samples were stored using these conditions for up to six months. To ensure uniform storage conditions, storage temperatures were continually monitored using a calibrated Dickson KTX chart recorder (Dickson Instruments, Addison, IL). At 3- and 6-month time intervals, samples of each fabric type that were stored under each condition were removed, fortified with internal standard, extracted, and analyzed for capsaicinoids using LC/MS. Stability was assessed by comparing the percentage of each analogue remaining versus samples of each fabric type that were prepared the day of analysis at the onset of the study (Time 0).

Washing of Fabric Samples—Cotton, wool, blended, and nylon fabric samples (1 cm²) were fortified with 25 μ L pepper spray (~0.25 mg capsaicinoids) and permitted to dry overnight at room temperature. To simulate potential washing scenarios, we added 4 mL of one of the following solutions to each fabric sample ($n = 3$): dH₂O, 1% (v/v) laundry detergent in dH₂O, 1% (v/v) bleach in dH₂O, or 5% (v/v) Spray and Wash™ in dH₂O. The samples were “washed” at room temperature by agitation using a mechanical shaker for 15 min (~22°C). Following “washing” the fabric samples were removed from the solutions using forceps and placed into a pipette tip. The residual liquid was removed from the fabric by centrifugation at 2500 rpm for 5 min. The fabric samples were then extracted and analyzed by LC/MS. Results were calculated as the percentage of each capsaicinoid analogue remaining on the fabric versus unwashed samples ($n = 3$) that were prepared and extracted in parallel to the washed samples.

Analysis of the Capsaicinoids by LC/MS—Analysis of the capsaicinoids (i.e., capsaicin, dihydrocapsaicin, nonivamide, nordihydrocapsaicin, homocapsaicin, homodihydrocapsaicin) in the pepper spray product and on the fabric samples was performed using a Hewlett Packard Series 1100 LC-MSD (Agilent Technologies, Palo Alto, CA), as previously described (32,33). Briefly, samples were chromatographed using a MetaSil Basic reversed-phase C₂-C₈ HPLC column (100 \times 3.0 mm; 3 μ m) equilibrated with

57.5% methanol:43.5% distilled water containing 0.1% (v/v) formic acid at 0.25 mL/min and 40°C. The capsaicinoids were eluted using a gradient of methanol and 0.1% formic acid, as previously described (32,33). The mass spectrometer was interfaced with an electrospray ionization source and was operated in selected-ion monitoring mode for positive ions of capsaicin (m/z 306), dihydrocapsaicin (m/z 308), nonivamide (m/z 294), nordihydrocapsaicin (m/z 294), homocapsaicin (m/z 320), and homodihydrocapsaicin (m/z 322). Calibration standards for quantification of the capsaicinoid analogues were prepared from 1 to 2500 ng using stock solutions containing all the capsaicinoid analogues, as previously described (32,33). Fabric samples (1 cm²) were prepared for analysis by adding internal standard (500 ng octanoyl vanillamide; methanolic stock prepared fresh) and extracting with 2 mL *n*-butyl chloride (in 13 \times 100 cm screw-capped tubes). The capsaicinoids were extracted from the fabric by agitation on a mechanical shaker for 20 min at room temperature. After extraction, the fabric was removed with forceps and placed into a plastic pipette tip. The tip was placed into the mouth of the extraction tube and the tubes were centrifuged for 2 min at 2500 rpm to recover the remaining solvent from the fabric. The extracts were evaporated to dryness at 40°C under a stream of air. The dried residues were reconstituted in 4 mL 70% methanol:dH₂O, transferred to autosampler vials, and 2.5 μ L of sample was injected into the HPLC.

Results

LTL Product Analysis—The total capsaicinoid concentration of the pepper spray product, as determined by LC/MS, was 10 μ g/ μ L. The relative concentrations of the capsaicinoid analogues were 47% capsaicin, 43% dihydrocapsaicin, 4.8% nordihydrocapsaicin, 2.1% homodihydrocapsaicin, 1.8% nonivamide, and 1.3% homocapsaicin. The relative capsaicinoid analogue concentrations were consistent with previously reported values for extracts of fresh peppers and other OC-containing pepper spray products (5,32,33, 35,36).

Analysis of Capsaicinoids on Fabrics by LC/MS—Quantification of the capsaicinoid analogues extracted from the stored and washed fabric samples was achieved using LC/MS. A typical reconstructed ion chromatogram obtained from the analysis of a cotton fabric control sample is shown in Fig. 1. Each capsaicinoid that was identified in the pepper spray product was detected in the extract of the fabric.

Storage and Stability of the Capsaicinoids on Fabrics—The effects of storage temperature and duration on the concentrations of the capsaicinoids are shown in Table 1. Results are shown by storage condition and fabric type. The stability of the capsaicinoids is expressed as the percentage of capsaicinoid analogue remaining compared to that detected on samples of each fabric type at the onset of the study (Time 0). The decrease in the capsaicinoid concentration over time was independent of fabric type and temperature. All capsaicinoids exhibited a time-dependent decrease in concentration. However, the rates of decomposition varied; nordihydrocapsaicin exhibited the greatest stability and homodihydrocapsaicin the least. Nordihydrocapsaicin, nonivamide, and capsaicin concentrations decreased between 5 to 15% after six months of storage at all temperatures. Dihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin were markedly more labile. Their concentrations decreased 18 to 60% during six-month of storage at all temperatures.

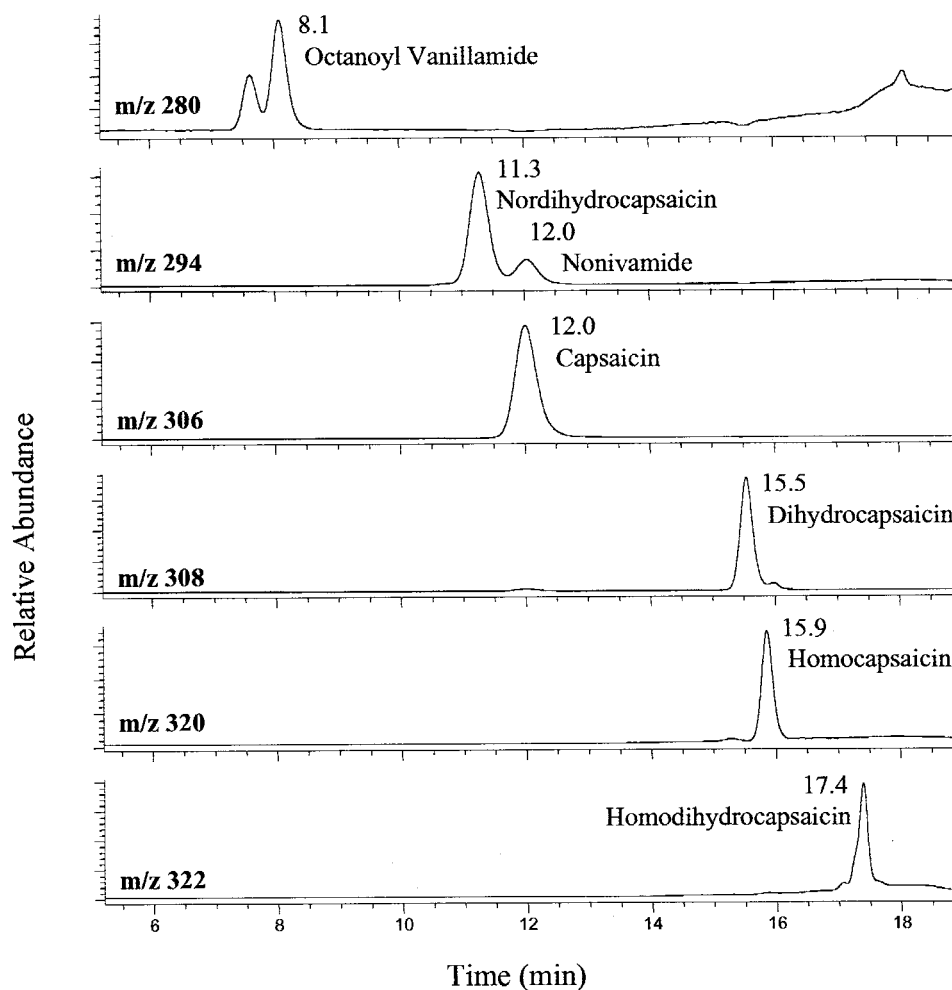


FIG. 1—Reconstructed-ion mass chromatogram from the analysis of the capsaicinoid analogues extracted from fortified samples of cotton fabric.

TABLE 1—Effects of storage temperature and duration on the detection of capsaicinoids on various fabrics.

Capsaicinoid Analogue and Duration of Storage	Percentage of Capsaicinoid Analogue Remaining and Storage Temperature											
	Wool			Cotton			Cotton-Poly Blend			Nylon		
	+20°C	4°C	−20°C	+20°C	4°C	−20°C	+20°C	4°C	−20°C	+20°C	4°C	−20°C
<i>Three Months</i>												
Nordihydrocapsaicin	100	98	98	95*	97	95*	96*	97	96	99	98	100
Nonivamide	89*	94	96	90	93	91*	97*	94*	91	94	95	95
Capsaicin	87*	93	99	88	94	93*	93*	95	93	95*	95	97
Dihydrocapsaicin	70*	80*	91	74*	83*	82*	82*	82*	82*	83*	83*	85*
Homocapsaicin	87*	94*	91	85	87*	93*	93	92*	88*	90*	93*	98
Homodihydrocapsaicin	70*	63	78	69*	63*	76*	72*	73	68*	76	80*	72
<i>Six Months</i>												
Nordihydrocapsaicin	92	97	95	91	98*	92*	94	98*	95*	97	91*	96
Nonivamide	77*	82*	83	78*	86	88*	80	83*	80*	82	82*	82
Capsaicin	81*	88*	89	82*	91*	87*	85*	89*	86*	88*	89*	88*
Dihydrocapsaicin	69	80*	83	70*	82*	80*	76*	80*	77*	80	80*	80*
Homocapsaicin	62	76*	73*	66*	71*	77*	70*	76*	71*	73*	79*	77*
Homodihydrocapsaicin	38*	41*	51*	41*	42*	50*	43*	47	44*	46*	52*	46*

*Represents values that differed significantly from Time = 0 samples ($P < 0.025$). Significance was determined using the one-tailed Student's *t*-test at the 95% confidence interval.

TABLE 2—Effects of various washing protocols on the detection of the capsaicinoid analogues on different fabrics.

Capsaicinoid Analogue	Percentage of Capsaicinoid Analogue Remaining and Wash Protocol															
	Wool				Cotton				Cotton-Poly Blend				Nylon			
	H ₂ O	Soap (1%)	Bleach (1%)	Spray & Wash	H ₂ O	Soap (1%)	Bleach (1%)	Spray & Wash	H ₂ O	Soap (1%)	Bleach (1%)	Spray & Wash	H ₂ O	Soap (1%)	Bleach (1%)	Spray & Wash
NDHC*	43	19	8	19	55	40	0.5	28	46	30	0.8	29	15	3.3	0.2	1.3
Nonivamide	51	24	10	23	66	48	1.2	36	55	36	1.6	35	20	4.3	0.7	1.7
Capsaicin	48	24	9	22	60	45	0.2	34	52	35	0.8	35	20	4.4	0.2	1.7
DHC*	61	27	12	26	78	55	0.5	47	68	43	1.5	44	37	9.7	0.3	3.1
HC*	61	26	11	27	81	55	0	50	66	44	1.7	55	34	7.5	0	13
HDHC*	70	25	11	26	96	59	0	56	81	42	2.0	54	45	7.2	0	5.2

* Abbreviations for nordihydrocapsaicin (NDHC), dihydrocapsaicin (DHC), homocapsaicin (HC), and homodihydrocapsaicin (HDHC).

Effects of Washing on the Detection of Capsaicinoids on Fabrics—Washing the fabric samples with dH₂O, 1% laundry detergent, 1% bleach, or 5% Spray Wash™ decreased the concentrations of the capsaicinoids. However, the effects of washing varied (Table 2 and Figs. 2A–D). These data are presented as the percentage of capsaicinoid analogue remaining on the fabric after washing compared with unwashed samples prepared in parallel. From 2 to 90% of the fortified capsaicinoids remained on the washed fabric samples. This was dependent upon the type of fabric and the wash solution. Washing with dH₂O was the least effective method of removing the capsaicinoids from the fabrics. Spray and Wash™ (5%) and detergent (1%) were more effective than water at removing the capsaicinoids from the fabrics. Less than 5% of the fortified capsaicinoid analogues were recovered from fabrics treated with bleach.

Retention of the capsaicinoids on the washed fabrics was dependent upon fabric type (Table 2 and Figs. 2A–D). In general, 100% cotton was the most effective fabric for retaining the capsaicinoids followed by 60:40 cotton:polyester blend, wool, and nylon. Typically, 50 to 90% of the capsaicinoids were retained on the cotton fabric (except bleach washed fabric). Nylon exhibited the poorest retention properties with only 1 to 40% of the individual capsaicinoids remaining following washing.

Retention of the capsaicinoids on the washed fabrics appeared also to be dependent on the chemical properties of the capsaicinoids. For example, approximately 50% of the nordihydrocapsaicin (most hydrophilic) was removed from the cotton fabric by washing with dH₂O, while only 5% of homodihydrocapsaicin (most hydrophobic) was removed (Fig. 2A). This trend was apparent with all fabrics; however, it was less pronounced when the fabrics were washed with nonpolar solutions such as 1% detergent or 5% Spray and Wash™.

Discussion

A typical canister of pepper spray can deliver between 1 to 5 mL of dilute oleoresin capsicum per second. The rate of delivery varies depending upon the canister pressure, spray characteristics, temperature, and prior use of the weapon. However, based on our previous research, a “normal” canister or LTL self-defense weapon will deliver from 1 to 50 mg capsaicinoids/second, depending on the individual properties of the weapon. This delivery rate varies depending upon the concentration of the capsaicinoids and aerosol characteristics of the product (32,33). In this study, we treated the fabric with 0.25 to 0.5 mg/cm² fabric (25 to 50 μL) and were able to detect the capsaicinoids for up to six months and after various washing procedures. Detection of the capsaicinoids was feasible

because of the sensitivity of the LC/MS assay, the inherent chemical stability of the capsaicinoids, and their resistance to removal by washing. Thus, detection of pepper spray residues on clothing can likely be achieved even after significant time has passed since the exposure and after conscientious or accidental attempts to remove the capsaicinoids by washing.

Several precautions should be observed when using chemical residue evidence to determine if there was an exposure to capsaicinoids. One problem emanates from the possibility that the evidence was contaminated (15,16,18). For example, exogenous fingerprints, DNA, hair, chemicals, etc. can unknowingly be introduced into a crime scene. Because capsaicinoids are naturally occurring compounds, they may be found on human skin as a result of contact with spicy foods (5,35,36). They may also be present following the use of medicinal plasters for treatment of chronic pain or arthritis (5). Fortunately, the quantity of capsaicinoids delivered by a typical pepper spray weapon is likely to be considerably larger than that deposited by inadvertent exposure. Therefore, the presence of large concentrations of capsaicinoids on clothing is likely the result of direct exposure to a pepper spray.

Another potential complication associated with using trace chemical evidence to determine exposure to pepper spray is that the pepper spray residue may be removed or rendered undetectable by decontamination attempts or chemical treatments. Evidence may also be discovered, collected, stored, and processed after the incident (15,16,18). Unfortunately, the duration and temperature of the storage may result in degradation of forensic markers (i.e., certain chemicals and biological samples). We have demonstrated that the capsaicinoids exhibit substantial stability as well as persistence on fabrics following most washing protocols.

It has been demonstrated that oleoresin capsicum, the concentrated extract of peppers that is used to prepare pepper spray products, consists of a diverse combination of chemicals (35,36). Therefore, pepper sprays contain a complex mixture of capsaicinoids and other chemicals that varies from product to product or even among manufacturer lots (32,33). A detailed analysis of the individual capsaicinoids in a clothing residue may circumvent some of the complications outlined above and thus provide more conclusive evidence as to the origin or source of the capsaicinoids (i.e., from skin creams, foods, or even individual pepper spray products.). For example, some pepper sprays are formulated using only nonivamide as the active ingredient. Because the concentration of nonivamide in natural sources of capsaicinoids is quite low (32), a large concentration of nonivamide in a residue may be indicative of exposure to a pepper spray. A detailed chemical analysis may also be useful in cases when a pepper spray canister is

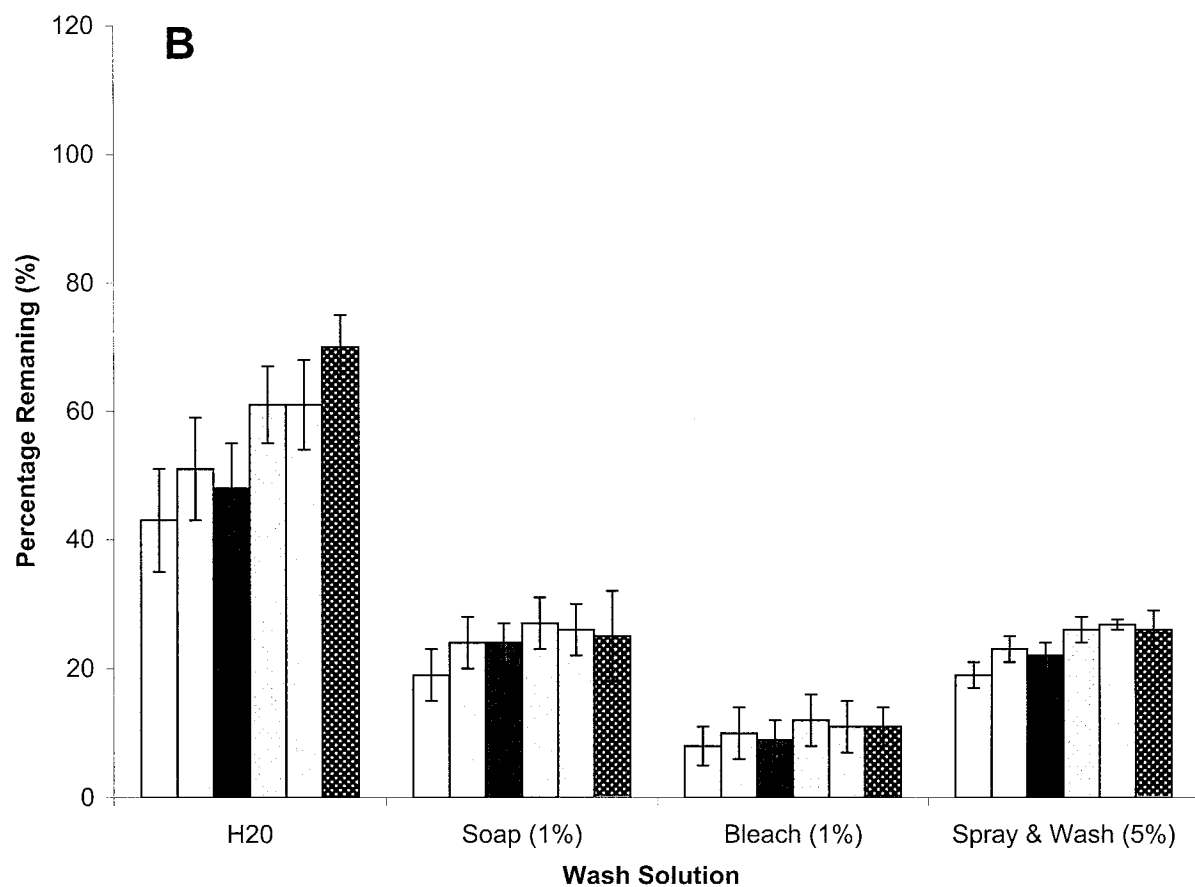
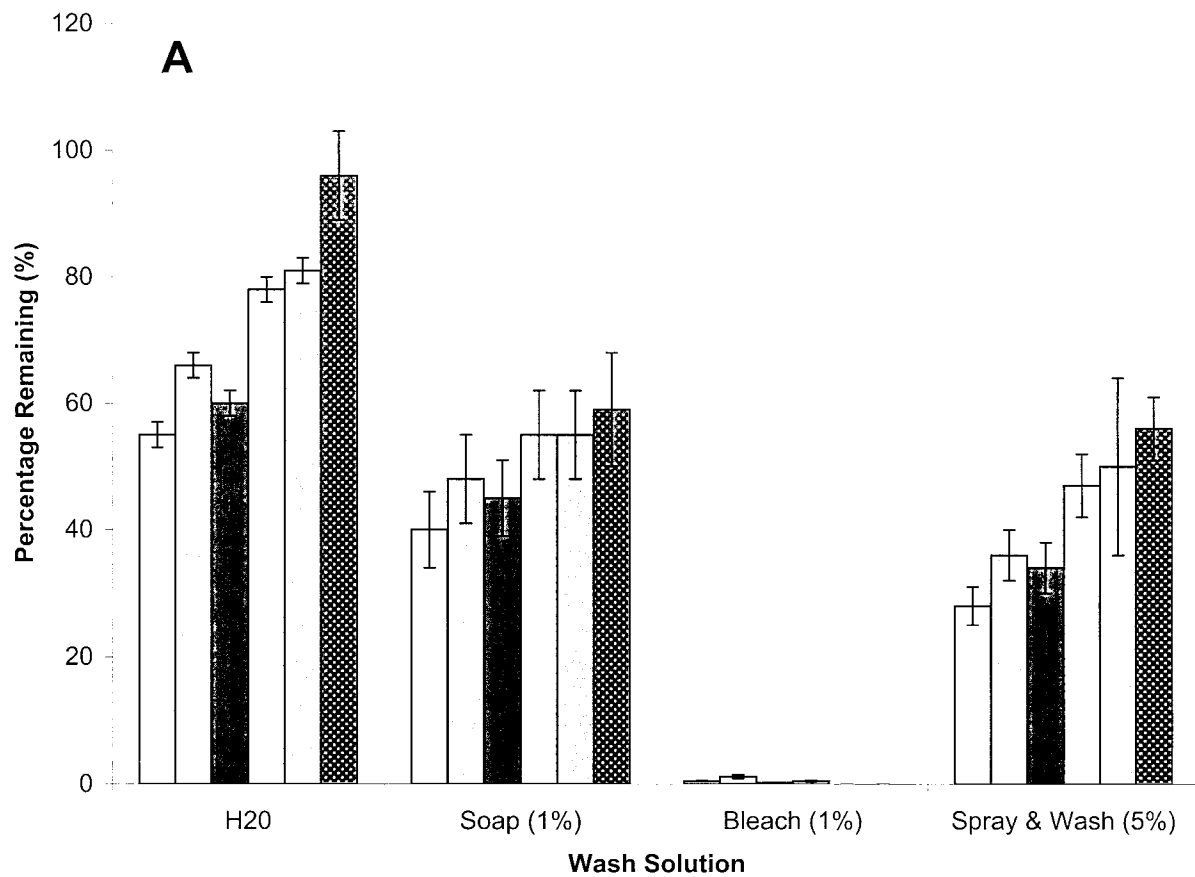


FIG. 2—The effects of washing cotton (A), wool (B), cotton-polyester blended (C), and nylon (D) fabrics with water, detergent, bleach and Spray and Wash™ on the recovery and detection of fortified capsaicinoid analogues. Nordihydrocapsaicin (open bars), nonivamide (gray bars), capsaicin (black bars), dihydrocapsaicin (white bars with gray dots), homocapsaicin (gray bars with white dots), and homodihydrocapsaicin (black bars with white dots). The data are representative of the mean \pm standard deviation of three individual samples.

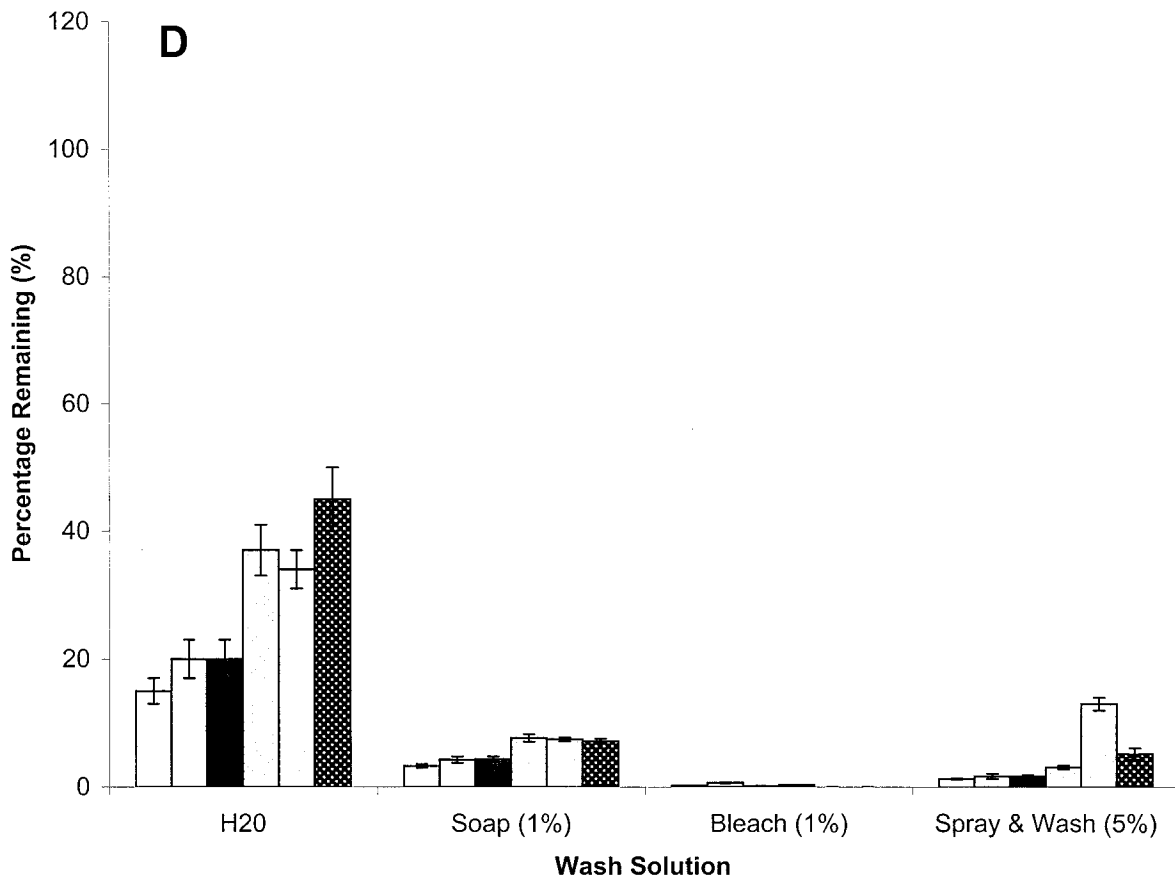
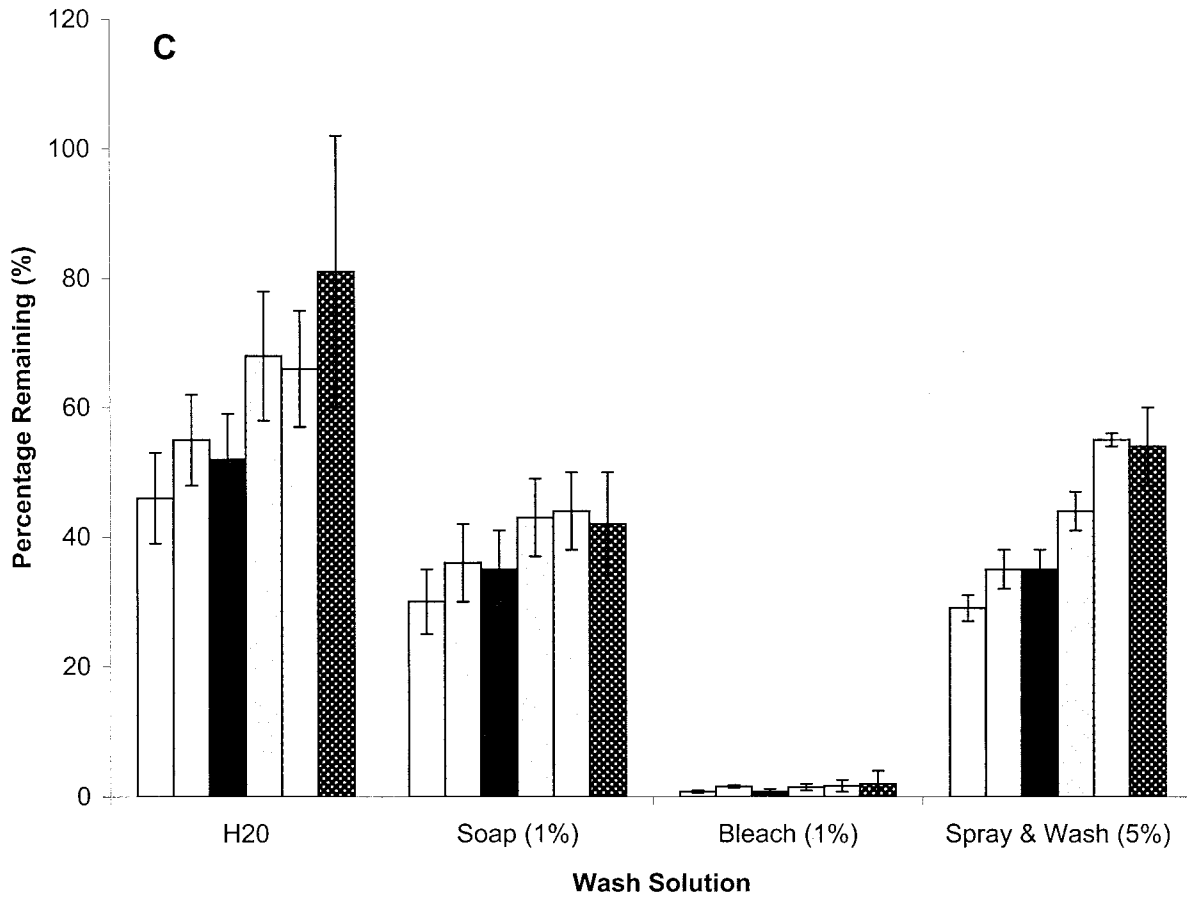


FIG. 2 (continued)

found at the scene. Investigators may then be able to link the weapon to a residue. However, simply determining the relative abundances of the individual capsaicinoids in the weapon and the residue may not be adequate to conclude that the pepper spray weapon was used in the incident due to differences in degradation, removal by washing, and other uncontrolled factors. Additional supporting evidence should be collected.

Despite the limitations discussed, the use of capsaicinoids as potential forensic markers is attractive since the capsaicinoids are fairly unique and persisted on a variety of clothing types even after extended storage at different temperatures and following conscious attempts to remove them from the fabric. Greater than 85% of the original concentrations of nordihydrocapsaicin, nonivamide and capsaicin were detectable by LC/MS for up to six months after storage even at room temperature. After decontamination attempts by washing with water, detergent, and Spray and Wash™, the capsaicinoids persisted on cotton, blended, nylon, and wool cloth. Only washing with bleach effectively removed the capsaicinoids from these clothing types, presumably due to oxidative decomposition by hypochlorous acid. Since the popularity of pepper spray as LTL weapons is increasing, it is likely that use of clothing stained with pepper sprays will become an important form of forensic evidence in both criminal and civil investigations where exposure to capsaicinoids is suspected. The data presented here indicate that capsaicinoids persist under most storage and washing procedures making detection by LC/MS likely. However, as stated, capsaicinoids can be found in natural and pharmaceutical products so detection alone does not indicate that the suspect has been exposed to a LTL weapon. Additional collaborative evidence and information should be collected to link the presence of the capsaicinoids on fabric to the circumstance of the incident or crime.

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