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An Alternative Method to Screen for Pepper Spray Residue*

ABSTRACT: A method was developed to screen for pepper spray residue using instruments and methods other than those techniques commonly employed to analyze chemical residue (i.e., gas chromatography mass spectrometry-GCMS or liquid chromatography mass spectrometry-LCMS). The method employed gas chromatography (GC), thin layer chromatography (TLC), and diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) to screen for dried pepper spray stains. Pepper sprays from nine different manufacturers were investigated. Capsaicin and dihydrocapsaicin were identified and unique IR reflectance spectra are presented. An additional five compounds were presumptively found. Results showed that a particular stain could be characterized as a pepper-based stain.

KEYWORDS: forensic science, pepper spray products, capsaicin, dihydrocapsaicin, thin layer chromatography, diffuse reflectance infrared Fourier transform spectroscopy, gas chromatography

Many techniques have been reported for the analysis of pepper sprays, including TLC, ultraviolet spectroscopy, Fourier transform infrared spectroscopy (FTIR), ambient temperature ion mobility spectrometry, GCMS, high performance liquid chromatography (HPLC), and LCMS (1–12). Much of the literature to date describes the identification of capsaicin or the capsaicinoids using instruments such as GCMS, HPLC, or LCMS (1–8). However, many criminalists have limited access, if any, to these instruments for this type of analysis; even if the instrumentation were available in the laboratory, it may be dedicated to other high volume laboratory needs such as fire debris or drug analysis. Therefore, an alternative method is desirable to screen for pepper spray residue.

Methods

Samples

Pepper sprays ranging from 0.5% to 18% oleoresin capsicum (OC) were obtained from nine manufacturers. Stains were made on previously unstained cotton swatches. All stains were made outdoors, with the nozzle of the canister nearly touching the cotton swatch. The duration of each discharge was kept constant at approximately 1 s. After application, stains were stored at room temperature in the laboratory and allowed a minimum of 96 h to dry.

Analysis

Stains were extracted then separated into major components using TLC. Each isolated compound was identified (or tentatively identified) using DRIFTS. The component was then injected into the GC to determine its retention time. The original formulation was then injected into the GC and the retention times of the known components were matched to the peaks in the pepper spray's chromatogram.

RP8GF and RP18GF 10 cm×10 cm HPTLC plates, RP18GF 20×20 cm TLC plates, Silica GF TLC plates, and an HPTLC chamber were purchased from Analtech Inc. A Spectra Tech HATR base with a Gemini Reflectance Head was used in a Nicolet Impact 410 FTIR. IR data was analyzed using OMNIC software. A Varian Star 3400CX GC equipped with an electronic plotter was used with a 30 m AT-1 poly methylsiloxane, 0.25 mm i.d., 0.25 μm film (Alltech Inc.). The injection was splitless for 0.75 min after injection. The temperature was held at 130°C for 5 min, ramped at 8°C/min to 320°C with no final hold time. The injection and detector ports were held at 345°C. The flow rate was ~34 cm/sec (manually set) at 130°C (He).

One quarter of each stain was extracted with approximately 1 mL of dichloromethane for 30 min before analysis. A small sample of the extract was manually applied to solvent cleaned TLC plates. The preparative plate was then developed twice using hexanes: ethyl ether (80:30). Bands were located using 254 nm UV light or a spray reagent consisting of bromocresol green in 0.2% methanolic KOH. When the spray reagent was used, only the left and right side of the plate were sprayed leaving the center portion of the plate (approximately 50% of the plate) unsprayed so that the analyte could be extracted without interference from the spray reagent. Compounds were scraped off the plate and extracted from the silica gel with 4 mL of distilled spectral-grade dichloromethane: methanol (3:1). After decantation and filtration, extracts were evaporated to dryness under vacuum at ambient temperatures. The analyte was resolubilized in a small amount of dichloromethane and re-spotted onto reverse phase TLC or HPTLC plates. Fatty

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TABLE 1—*Results summary.*

Compound	TLC	Drifts	Average* R_T (min)
Myristic acid	$R_f = 0.49^{\dagger}$	Fatty acid class	13.59
Methyl palmitate	$R_f = 0.93^{\dagger}$ $R_f = 0.06^{\ddagger}$	Ester class	15.38
Palmitic acid	$R_f = 0.49^{\dagger}$ $R_f = 0.12^{\ddagger}$	Fatty acid class	16.46
Methyl stearate	$R_f = 0.93^{\dagger}$ $R_f = 0.02^{\ddagger}$	Ester class	17.59
Stearic acid	$R_f = 0.49^{\dagger}$ $R_f = 0.06^{\ddagger}$	Fatty acid class	18.53
Capsaicin	$R_f = 0.04^{\dagger}$ $R_f = 0.75^{\S}$	Fig. 2(*b*)	23.06
Dihydrocapsaicin	$R_f = 0.04^{\dagger}$ $R_f = 0.69^{\S}$	Fig. 2(*d*)	23.34

* Average over 47 injections—R_T error was set to ± 0.10 min
† Preparative Plate developed twice with hexanes: either (80:30).
‡ RP18 TLC developed with 92.5% methanol.
§ RP8 HPTLC developed with 0.05M $AgNO_3/H_3BO_3$ in 80% methanol.

acids and fatty esters were separated on RP18 GF plates developed with 92.5% methanol. Capsaicinoids were separated on RP8 GF HPTLC plates developed with 0.05M A_gNO_3 and 0.05M H_3BO_3 in 80% methanol. Twenty cm plates were developed 16 cm from the origin and HPTLC plates were developed 7.5 cm from the origin. Compounds were then relocated and extracted using the procedure previously described.

Once extracted, residue was reconstituted with approximately 1 mL of distilled dichloromethane, to which ~40 mg KBr powder was added. A KBr-compound mixture was formed by evaporating the solvent at ambient temperature and analyzed using DRIFTS. Samples were scanned 256 times at 4 cm^{-1} resolution (no correction or filling). Spectra were background subtracted using a clean KBr powder background. All spectra are displayed in Kubelka-Munk format. After DRIFTS, the analyte was resolubilized in dichloromethane and injected into the GC to determine its retention time in comparison to the chromatogram of the entire pepper spray.

All chemicals were purchased from Fisher Scientific.

Results

Results are summarized in Table 1. An example of a common pepper spray chromatogram is presented in Fig. 1. Retention times were not plotted on the chromatogram but were printed in summary format immediately following the end of the chromatogram. Time markings underneath the chromatogram in Fig. 1 indicate 5-min intervals. Retention times were considered a match as long they were within 0.1 min of the average. The 0.1-min retention time window accounted for any variation as a result of manual flow control settings, manual injections, extract concentration, and general variation from day to day.

Two compounds were positively identified and five other compounds were tentatively identified. Using the preparative plate, classes of compounds appeared to travel in distinct and separate bands. Each "class" band from the preparative plate was analyzed separately. The three main classes of compounds included the capsaicinoids, fatty acids, and fatty esters. Analyzing the capsaicinoid band, capsaicin and dihydrocapsaicin were easily resolved by RP-HPTLC and were conclusively identified through DRIFTS (Fig. 2). Capsaicin and dihydrocapsaicin were identified through comparisons to previously published IR peak assignments and the two published spectra of crude capsaicinoids (2,3,13). Tentative peak assignments for both capsaicin and dihydrocapsaicin include 3270 cm^{-1} (broadened OH and NH stretch), 2930 cm^{-1}, 2960 cm^{-1}, 2870 cm^{-1} (CH stretch), 1640 cm^{-1}, 1600 cm^{-1} (C═O, C═N stretch), 1550 cm^{-1} (amide bend), 1515 cm^{-1} (phenyl II), 1460 cm^{-1}, 1430 cm^{-1}, 1365 cm^{-1}, 1275 cm^{-1}, 1235 cm^{-1}, 1215 cm^{-1} 1155 cm^{-1}, 1125 cm^{-1}, 1035 cm^{-1} (various including: CH bend, OH bend, CO stretch, C-N stretch, C-C stretch), strong 970 cm^{-1} (trans, H-C═C bend, capsaicin only), 800 cm^{-1} (NH bend, CH bend). Peak values are consistent with previously reported values, although the publication that reported peak values did not publish a plotted spectrum for comparison and reported a transmission spectrum, not a reflectance spectrum (13). Capsaicin can be distinguished from dihydrocapsaicin by a strong peak at 970 cm^{-1}, probably corresponding to the additional trans carbon-carbon double bond present in capsaicin.

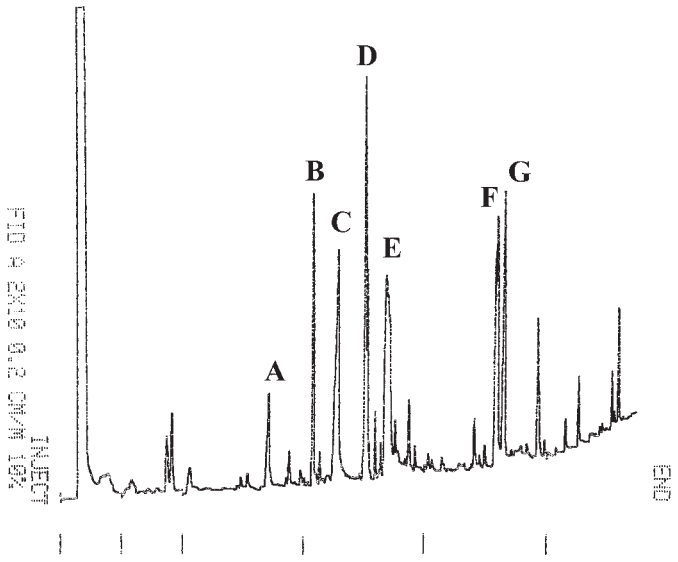

FIG. 1—*Sample pepper spray chromatogram. Retention times: 13.63 (A–Myristic Acid); 15.41 (B–Methyl Palmitate); 16.54 (C–Palmitic Acid); 17.63 (D–Methyl Stearate); 18.57 (E–Stearic Acid); 23.08 (F–Capsaicin), 23.36 (G–Dihydrocapsaicin).*

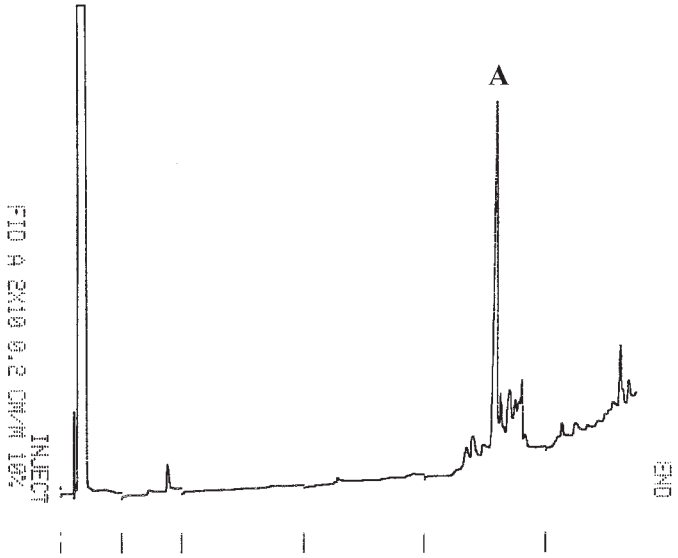

FIG. 2—*(a) Chromatogram of isolated capsaicin to demonstrate purity. Retention time: 23.00 (A).*

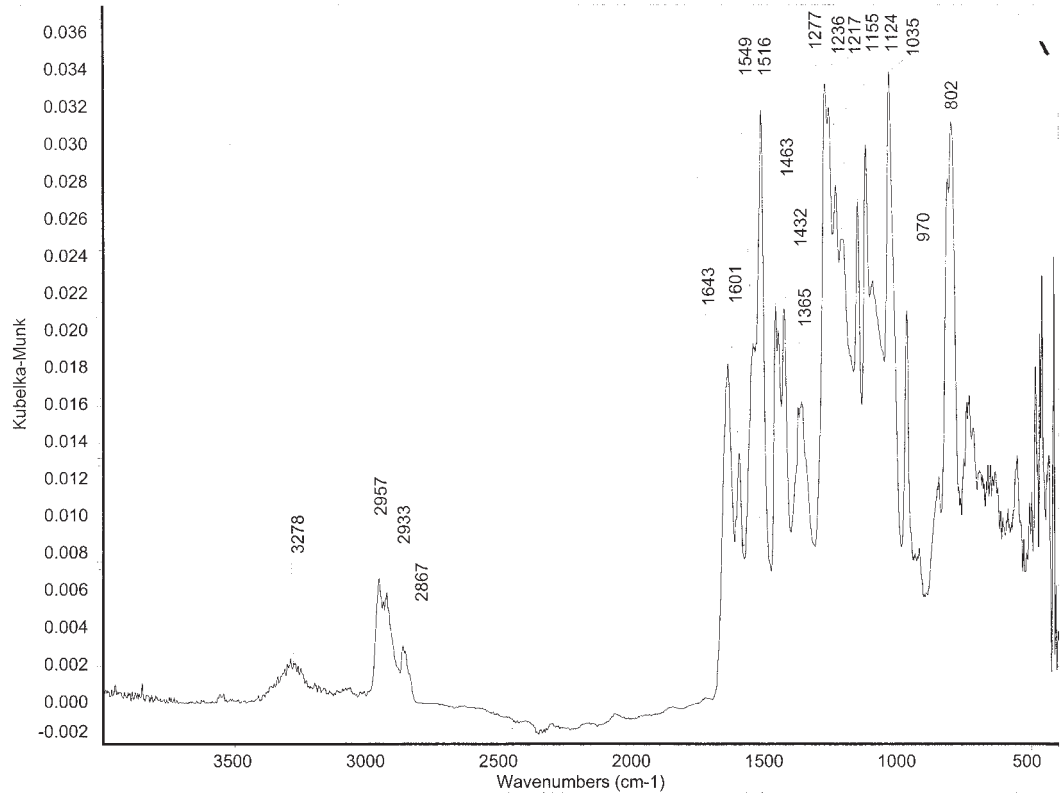

FIG. 2—*(b) IR reflectance spectrum of capsaicin.*

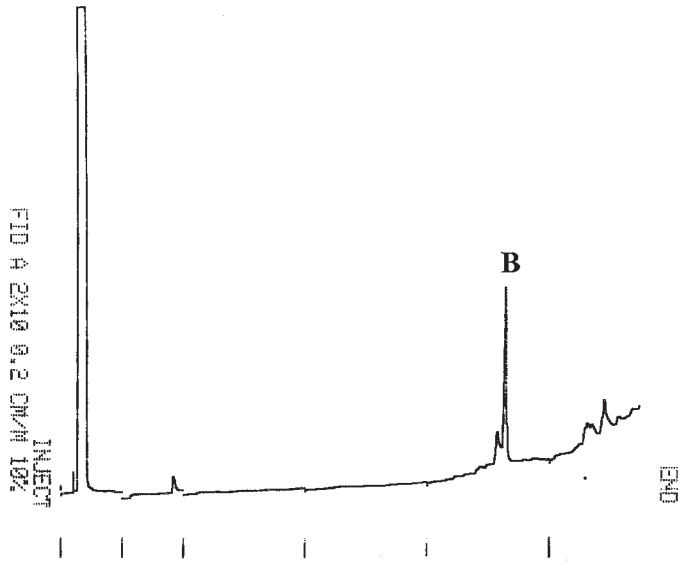

FIG. 2—*(c) Chromatogram of isolated dihydrocapsaicin to demonstrate purity. Retention time: 23.28 (B).*

The other classes of compounds, in addition to the capsaicinoids, were expected to be fatty acids and their esters (1). Tentative IR reflectance identification of the two class bands from the preparative plate was based, in part, on similarities to standards of stearic acid for the fatty acids and methyl stearate for the fatty esters. GC analysis of the fatty acid band showed three major peaks with retention times similar to standards of stearic acid (C18), palmitic acid (C16), and myristic acid (C14) and GC analysis of the fatty ester band showed two major compounds with retention times similar to standards of methyl palmitate and methyl stearate. Unfortunately, reflectance spectra for each fatty acid and fatty ester could not be obtained. Stearic acid, palmitic acid, methyl stearate, and methyl palmitate could be located on the RPTLC (Rf values were similar to standards), but they could not be extracted in large enough quantities to provide an acceptable spectrum for identification. Myristic acid could not be located on the RPTLC plate so tentative identification was partially based on its retention time (similar to a standard).

Capsaicin, dihydrocapsaicin, fatty acids, and fatty esters were isolated from stains of each manufacturer with two exceptions. Methyl palmitate and methyl stearate were not detected in the chromatogram of an 18% OC spray and no compounds could be isolated from the 0.5% OC stains. In the case of the 0.5% OC stain, a more sensitive method, such as GCMS or LCMS, would be recommended since conclusions using our method could be based on retention times only.

Discussion

The Rf values of capsaicin and dihydrocapsaicin using RP8 plates with the same solvent system were different from those previously reported (14). The previously reported Rf values were 0.50 and 0.32 for capsaicin and dihydrocapsaicin respectively (14). Regardless of the discrepancies between our data and previously published data, the developing solvent used in this study was found to be effective at separating dihydrocapsaicin and capsaicin.

The study also generated IR reflectance spectra for capsaicin and dihydrocapsaicin. The spectra of purified capsaicin and purified dihydrocapsaicin are the first known published IR reflectance spectra of both compounds. However, not all the compounds in each

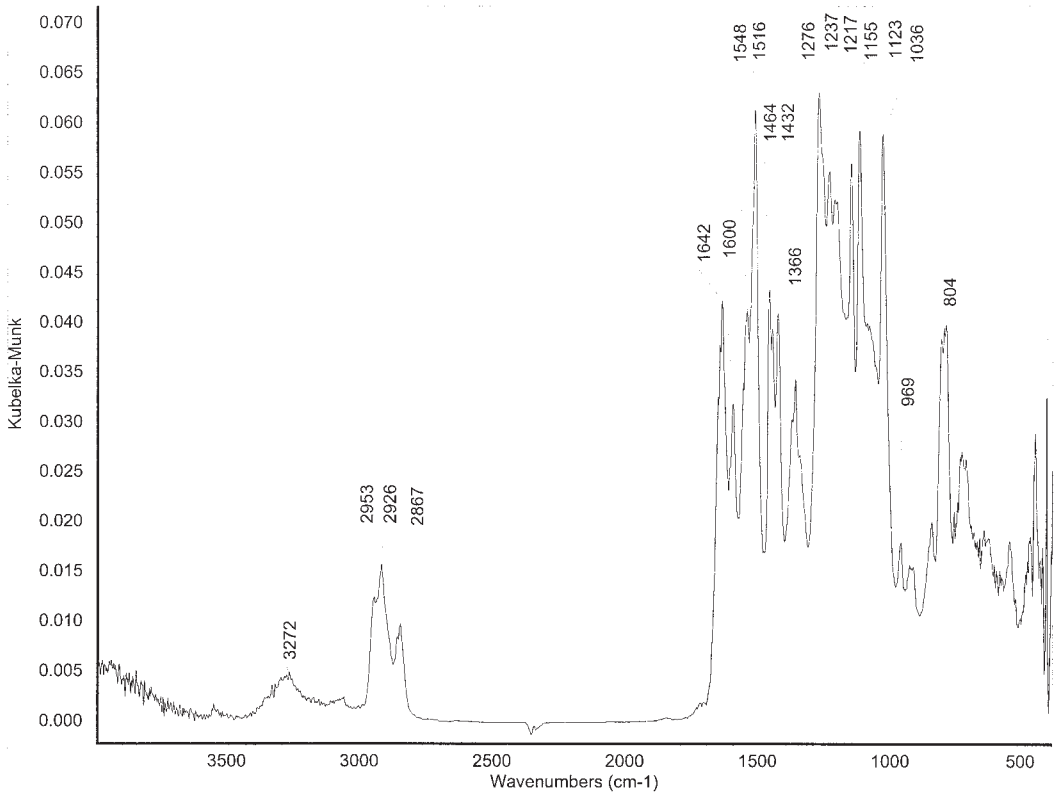

FIG. 2—*(d) IR reflectance spectrum of dihydrocapsaicin.*

chromatogram were identified, or could have been identified by this method. Only major compounds were identified, including capsaicin and dihydrocapsaicin, which are indicative of a pepper-based product such as pepper spray. Compounds, such as nonivamide, nordihydrocapsaicin, homodihydrocapsaicin, homocapsaicin, unsaturated fatty acids and esters, extended chain fatty esters, citrates, and other large molecules such as lycopene were not identified. However, it is unclear how identifying these additional compounds would make an analyst any more certain a stain was from pepper spray to the exclusion of other pepper-based products.

Quantitation of some of the compounds in a pepper spray may provide an additional level of certainty regarding the identification of a pepper spray, as has been reported in recent publications (6–7). However, instability of the various components in a pepper spray stain as a result of time or from exposure to heat, washing, chemical treatment, sunlight, and severe weather severely restrict any interpretations that may be conducted regarding the identification of a pepper spray stain (5,7). Some pepper spray manufacturers add markers, such as UV dyes, to their products. Future research to differentiate pepper spray stains from other pepper-based stains should focus on components unique to pepper sprays, such as UV markers, certain inorganic salts, and low volatility solvents (such as ethylene glycol), amongst others. While some quantitative works on inorganics and the capsaicinoids have been published (1,6–7), more extensive studies comparing pepper sprays and other pepper-based products are needed.

Conclusions

The goal of this study was to demonstrate that pepper spray residue could be analyzed without the use of a mass spectrometer. The research was successful in proving that valid alternatives to GCMS and LCMS are available to screen for pepper spray residue. This alternative approach confirmed the findings of previously published research (1,6–7) that a pepper spray formulation can include myristic acid, palmitic acid, stearic acid, methyl palmitate, methyl stearate, capsaicin, and dihydrocapsaicin. While the method presented here was not able to identify all of the compounds in each pepper spray chromatogram, it was able to identify some of the major components. Using this method, an analyst who has difficulty gaining access to a mass spectrometer still has the potential to gain valuable information pertaining to a suspected pepper spray stain.

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