

TECHNICAL NOTE

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An Assessment of Four Solvents for the Recovery of 2-Chlorobenzylidenemalononitrile and Capsaicins from "CS" and "Pepper" Type Lachrymator Sprays, and an Examination of Their Persistence on Cotton Fabric

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ABSTRACT: The rising incidence of assaults involving lachrymator sprays has led to an increase in items being submitted to this laboratory for the analysis of the associated chemical residues. The following work was undertaken to identify an efficient solvent with which to extract the compounds of interest from cotton fabric. The persistence and subsequent recovery of such compounds was also examined following protracted exposure to wind and rain.

Ethyl acetate was established as the most efficient solvent of those examined for the extraction of 2-chlorobenzylidenemalononitrile and a range of capsaicins from "CS Gas" and "Pepper" sprays respectively. Controlled experiments undertaken showed that capsaicins were recoverable after 72 h of exposure to the "elements" and 2-chlorobenzylidenemalononitrile was still recoverable after one week.

KEYWORDS: forensic science, lachrymators, recovery, persistence, gas chromatography, capsaicin, dihydrocapsaicin, 2-chlorobenzylidenemalononitrile

Over recent years the number of cases submitted to this laboratory involving lachrymator canisters and suspected offenses involving lachrymators has markedly increased. The most common chemical defense lachrymators we have encountered are "CS Gas" and "Pepper" sprays. These sprays have been the focus of significant media attention recently as a result of the controversial introduction of "CS Gas" by some United Kingdom Police Forces for use as a deterrent/immobilizer (1). They have been encountered during instances where they were used as a form of self defense against attack, or where they were used as a weapon in illegal assaults. Items submitted for forensic examination involving these agents typically consist of either a spray canister of a suspected lachrymator and/or

contaminated clothing from a victim or an assailant. The identification of these agents from partially full canisters is relatively well documented and causes little difficulty. The preparation of a basic extract of "Pepper" spray solution can sometimes aid identification of the components (2). However, this laboratory has experienced difficulties when attempting to recover the active ingredients of "CS Gas" or "Pepper" sprays from clothing. It was unclear whether this problem could be attributed to inappropriate sampling methods which were failing to recover the compounds of interest, the lack of persistence of the compounds on the items being examined, or the genuine absence of the compounds in the first instance. As a result, experimental work was undertaken to identify a suitable solvent for the extraction of 2-chlorobenzylidenemalononitrile, capsaicin, and dihydrocapsaicin. With a few exceptions, the majority of the literature on this subject dates back to the 1970s and early 1980s. A large proportion of which concentrates on the uses and physiological effects of the various sprays (3), rather than their recovery and subsequent analysis. Most of the information relating to their analysis centers on the use of infrared techniques and gas chromatography using packed columns (4–6). These techniques have since been superseded by capillary columns and mass spectrometry (6,7).

The recent publication of a study looking at the persistence and recovery of "CS Gas," "CN" and "Pepper" sprays under a range of conditions and on a variety of fabrics provided data more relevant to modern techniques (8). The work detailed herein concentrates primarily on the determination of the most effective solvent for the recovery of the compounds of interest. A brief persistence study is also detailed.

Experimental

Gas Chromatography/Mass Spectrometry (GC/MS)

Electron Ionization—Analyses were performed using a Hewlett Packard 5890 Series II gas chromatograph with a Hewlett Packard 7673 autosampler, coupled to a Hewlett Packard 5972 Series quadrupole mass selective detector. The instrumental operating conditions were as follows: 1 μ L of sample was injected into the instrument operating in split mode (30:1); carrier gas, helium, head pressure 10 psi.

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Oven Conditions—The compounds of interest were resolved using the following conditions. Initial oven temperature, 100°C for 1 min, ramp rate 10°C/min to 220°C held for 5 min; injection temperature, 250°C; transfer line temperature, 280°C; source temperature, 200°C; quadrupole temperature, 100°C; source manifold pressure, 2.7×10^{-4} Torr; analyzer manifold pressure, 4.3×10^{-6} Torr; foreline pressure, 1.4×10^{-1} Torr.

Mass spectrometric scanning control parameters were as follows; start mass 40; end mass 400; scan time 0.9 s; interscan time 0.1 s.

GC column, Hewlett Packard HP5; phase type 5% phenyl-95% dimethylpolysiloxane, film thickness 0.25 μm , length 30 m, internal diameter 0.25 mm.

Data Handling—Data were collected using a Hewlett Packard Vectra multimedia VL2 computer with Hewlett Packard MS ChemStation software.

Gas Chromatography/Flame Ionization Detection (GC/FID)

Analyses were performed using a Hewlett Packard 5890 Series II gas chromatograph fitted with a flame ionization detector. The instrumental operating conditions were as follows: 2 μL of sample was injected into the instrument operating in split mode (50:1); carrier gas, helium, head pressure 12 psi.

The compounds of interest were resolved using the following temperature program; Initial oven temperature, 150°C, ramp rate 30°C/min to 300°C held for 7 min; injector temperature, 250°C; detector temperature, 250°C. GC column as for mass spectrometry.

Data Handling—Data were collected using a Spectra-Physics SP4290 Integrator.

Solvent Evaluation

Ethyl acetate, diethyl ether, methanol and *n*-hexane were assessed to determine which was the most efficient for the extraction of 2-chlorobenzylidenemalononitrile, capsaicin and dihydrocapsaicin from cotton fabric.

Determination of a Response Factor for 2-Chlorobenzylidenemalononitrile—“CS Gas” solution (100 μL) containing 2-chlorobenzylidenemalononitrile (unknown concentration) obtained directly from the canister was placed into a volumetric flask (5 mL) and made up to the mark with ethyl acetate. A 1 μL aliquot of this solution was injected into the GC/MS instrument. This analysis was repeated five times. An average response factor based on the peak area of the 2-Chlorobenzylidenemalononitrile and the concentration of the “CS Gas” solution was calculated.

Recovery Experiments for 2-Chlorobenzylidenemalononitrile—Twenty sections of cotton fabric, each approximately 3×3 cm, were spiked with 100 μL aliquots of “CS Gas” solution and left overnight in a fume cabinet to dry. Four sections of cotton fabric were left clean to act as control samples. Five doped samples and a control were then extracted in hexane (5 mL). This procedure was repeated for diethyl ether, ethyl acetate, and methanol. In each case the extraction was aided by sonicating for 5 min. The resultant extracts were reduced to dryness under a stream of nitrogen and then reconstituted in ethyl acetate (100 μL). Each solution was analyzed by GC/MS using the conditions detailed previously.

From the measurement of the peak area of the analyte detected in each analysis, and using the response factor obtained previously, the percentage recovery of the analyte achieved by each solvent was calculated.

Determination of Response Factors for Capsaicin and Dihydrocapsaicin—A standard solution (0.93 mg/mL) containing capsaicin and dihydrocapsaicin (35% w/w and 65% w/w respectively) was prepared by placing 9.3 mg of natural capsaicin into a volumetric flask (10 mL) and making up to the mark with ethyl acetate. A 10% dilution (0.093 mg/mL) of this standard was then prepared by placing 1 mL into a volumetric flask (10 mL) and making up to the mark with ethyl acetate. A 1 μL aliquot of this solution was injected into the GC/FID instrument. This analysis was repeated five times. Response factors based on the peak area and the concentration of the capsaicin and dihydrocapsaicin respectively were calculated. These response factors were used for subsequent quantitative analyses.

A range of capsaicinoids (capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin and homocapsaicin) were noted in the control canisters examined during this work. However, the recovery and persistence studies detailed herein have been based solely on the two major capsaicinoids, capsaicin, and dihydrocapsaicin.

Recovery Experiments for Capsaicin and Dihydrocapsaicin—Twenty sections of cotton fabric, each approximately 3×3 cm, were spiked with 100 μL aliquots of “Pepper” solution and left overnight in a fume cabinet to dry. Four sections of cotton fabric were left clean to act as control samples. Five of the doped samples and a control were then extracted in hexane (5 mL). This procedure was repeated for diethyl ether, ethyl acetate, and methanol. In each case the extraction was aided by sonicating for 5 mins. The resultant extracts were reduced to dryness under a stream of nitrogen and then reconstituted in ethyl acetate (100 μL). Each solution was analyzed by GC/FID using the conditions detailed previously.

From the measurement of the peak areas of capsaicin and dihydrocapsaicin detected in each analysis, and using the response factors obtained previously for each analyte, the percentage recovery of each analyte by each solvent was calculated.

Persistence Study

2-Chlorobenzylidenemalononitrile on Cotton Fabric—Five sections of cotton fabric, each approximately 3×3 cm, were spiked with 100 μL aliquots of “CS Gas” solution and, once dry, were suspended outside for a period of one week (168 h). A control sample of cotton fabric was treated similarly. During this time they were exposed to high winds and torrential rain. The samples and control were then each extracted in ethyl acetate (5 mL) and analyzed by GC/MS using the conditions detailed previously. The percentage recovery of the analyte was calculated.

Capsaicin and Dihydrocapsaicin on Cotton Fabric—Ten sections of cotton fabric, each approximately 3×3 cm, were spiked with 100 μL aliquots of “Pepper” spray and, once dry, were suspended outside. Five samples were left for a period of three days (72 h) and the remaining five samples were left for one week (168 h). A control sample of cotton fabric was treated similarly. During this time they were exposed to high winds and torrential rain. The samples and control were then extracted in ethyl acetate (5 mL) and analyzed by GC/FID using the conditions detailed previously. The percentage recovery of each analyte was calculated.

TABLE 1—Recovery of 2-chlorobenzylidenemalononitrile achieved by a range of solvents following overnight drying.

Solvent	2-CBM Mean Recovery (g) (<i>n</i> = 5)	2-CBM Standard Deviation (SD)	2-CBM Coefficient of Variance COV
Ethyl acetate	3.53×10^{-5}	1.14×10^{-5}	32%
Diethyl ether	2.43×10^{-5}	7.43×10^{-6}	30%
Methanol	1.34×10^{-5}	4.26×10^{-6}	63%
<i>n</i> -Hexane	1.15×10^{-5}	6.38×10^{-7}	11%

NOTE: *n* = number of replicate analyses.
SD = standard deviation.
COV = coefficient of variation.
2-CBM = 2-chlorobenzylidenemalononitrile.

TABLE 2—Recovery of capsaicin and dihydrocapsaicin achieved by a range of solvents following overnight drying.

Solvent	Capsaicin Mean Recovery (g) (<i>n</i> = 5)	Capsaicin SD	Capsaicin COV	Dihydrocapsaicin Mean Recovery (g) (<i>n</i> = 5)	Dihydrocapsaicin SD	Dihydrocapsaicin COV
Ethyl acetate	1.20×10^{-4}	1.69×10^{-5}	14%	2.02×10^{-4}	3.35×10^{-5}	17%
Diethyl ether	1.02×10^{-4}	2.01×10^{-5}	20%	1.74×10^{-4}	3.44×10^{-5}	20%
Methanol	1.10×10^{-4}	1.08×10^{-5}	10%	1.77×10^{-4}	2.45×10^{-5}	14%
<i>n</i> -Hexane	0.43×10^{-4}	1.67×10^{-5}	39%	0.79×10^{-4}	3.21×10^{-5}	41%

Materials

Natural capsaicin standard containing capsaicin (65% w/w) and dihydrocapsaicin (35% w/w) was obtained from Fluka.

Ethyl acetate, methanol, diethyl ether and *n*-hexane were obtained from Rathburn Chemicals Ltd.

As a standard 2-chlorobenzylidenemalononitrile was unavailable commercially, a solution of KO Spray 007 CS-Gas paralysant (unknown concentration) was used. "Pepper" (Smartguard Red Pepper Defense Spray—10% oleo-resin capsicum) and "CS Gas" spray canisters were obtained from the Strathclyde Police Force. All chemicals were of analytical grade unless otherwise stated.

Results and Discussion

2-Chlorobenzylidenemalononitrile

Recovery—The recovery of 2-chlorobenzylidenemalononitrile from cotton fabric using *n*-hexane, diethyl ether, methanol and ethyl acetate is detailed in Table 1. From these results ethyl acetate is shown to be the most efficient solvent.

Persistence—The mean recovery of 2-chlorobenzylidene-malononitrile using ethyl acetate following exposure to the "elements" for a period of one week was calculated as 4.2×10^{-5} g (42%). The appreciable effect of exposure on 2-chlorobenzylidene-malononitrile detection was therefore demonstrated to be minimal over the period tested.

Capsaicin and Dihydrocapsaicin

Recovery—The recovery of capsaicin and dihydrocapsaicin from cotton fabric using *n*-hexane, diethyl ether, methanol and ethyl acetate is detailed in Table 2. Although ethyl acetate is again the most efficient solvent, each of the solvents investigated yielded a very low recovery rate.

Persistence—The mean recovery of capsaicin and dihydrocapsaicin using ethyl acetate following exposure to the "elements" for a period of 3 days (72 h) was found to be 4.30×10^{-5} g and 1.05×10^{-4} g, respectively. The analytes of interest were unable to be detected after one week (168 h) of exposure.

Conclusions

Ethyl acetate was established as the most effective solvent to achieve maximum recovery of 2-chlorobenzylidenemalononitrile (mean recovery 3.53×10^{-5} g) from "CS Gas" and capsaicin (mean recovery 1.20×10^{-4} g) and dihydrocapsaicin (mean recovery 2.02×10^{-4} g) from "Pepper" sprays.

"CS Gas" was found to persist after exposure for a period of one week. With respect to "Pepper" sprays, the relatively low recovery of the compounds of interest after 72 h of exposure (almost 50% reduction) and their total "loss" after one week, highlights the importance of appropriate packaging and timely analysis of such items in casework.

The large coefficients of variance (COV) determined in the recovery experiments for the analytes of interest in each solvent may be ascribed to: (a) The lack of homogeneity of the lachrymator solutions which may suggest poor solubility of the active substances. (b) The relatively low concentration of the active substances within the lachrymators. (c) The relatively high concentration of impurities noted in both the "CS Gas" and the "Pepper" sprays.

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