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Development and validation of AccuTOF-DART™ as a screening method for analysis of bank security device and pepper spray components

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ABSTRACT

Analysis of bank security devices, containing 1-methylaminoanthraquinone (MAAQ) and o-chlorobenzylidenemalononitrile (CS), and pepper sprays, containing capsaicin, is a lengthy process with no specific screening technique to aid in identifying samples of interest. Direct Analysis in Real Time (DART™) ionization coupled with an Accurate Time of Flight (AccuTOF) mass detector is a fast, ambient ionization source that could significantly reduce time spent on these cases and increase the specificity of the screening process. A new method for screening clothing for bank dye and pepper spray, using AccuTOF-DART™ analysis, has been developed. Detection of MAAQ, CS, and capsaicin was achieved via extraction of each compound onto cardstock paper, which was then sampled in the AccuTOF-DART™. All results were verified using gas chromatography coupled with electron impact mass spectrometry.

1. Introduction

Bank dye security packs are commonly used to deter bank robberies. They normally consist of a red dye, 1-methylaminoanthraquinone (MAAQ); an oxidizer, potassium chlorate; confectioners' sugar; and often a tear gas, o-chlorobenzylidenemalononitrile (CS). The device also contains a remote activation system which causes the device to explode moments after being removed from the bank. The money is rendered useless when coated with the red dye, and the robber is similarly stained and somewhat incapacitated by the tear gas [1–6]. The combination of MAAQ and CS is unique to these security packs, often making the identification of these chemicals essential to the case [3,6].

Pepper spray can be used by either the general public as a defense spray or by police officers as a less-than-lethal (LTL) control tactic for subduing riots or stopping assailants [7–10]. The main active ingredient in most pepper sprays is oleoresin capsicum, which is the oily extract of hot peppers [7–8,10–12]. Although oleoresin capsicum is composed of several capsaicinoids, capsaicin is the most abundant and thus the most appealing for analytical purposes [7–8,10–12]. Although capsaicin is not unique to pepper spray, its presence on clothing can aid in identifying a suspect of an assault that was sprayed with pepper spray or can corroborate a policeman's testimony that LTL force was used [9].

Both bank dye and pepper spray have current validated methods of analysis at Virginia's Department of Forensic Science (DFS) laboratories. The method involves a visual screen for stains aided by UV scanning. For bank dye cases with no stain visible, such as on dark clothing, evidence is sampled with methanol-soaked swabs to locate red-stained areas that could contain MAAQ. Swabbing is done in several places. Because many pepper sprays are colorless, the methanol swab technique is not employed for such cases. There is no method for screening other than a visual analysis for stains. Areas of interest from each sample are then extracted and analyzed using gas chromatography/mass spectrometry (GC/MS) as the means of identification. Because of the lack of a specific screening method, the samples then have to be "verified" using another analytical technique such as GC that employs a different stationary phase [13].

Although GC/MS coupled with a second GC stationary phase is a very accurate scheme of analysis, it can be time consuming. Extraction is necessary before proceeding to any analytical tests, and the chromatography itself is lengthy. In cases where no stain is visible, with no other method to screen for stains, the compounds of interest can be difficult to locate. Employing a more specific screening technique prior to analysis by GC/MS would save time by eliminating negative samples early on, quickly locating areas of interest on the sample, and eliminating the need of using an additional analytical technique for confirmation by GC/MS.

Direct Analysis in Real Time (DART™) is an ambient ionization source in which a heated stream of helium gas is used to ionize the sample. The pressure from the gas stream coupled with a vacuum

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system aid in directing the ions into a charged orifice that leads to the Accurate Time of Flight (AccuTOF) detector. The AccuTOF is capable of detecting ions with a resolving power greater than 6000. This resolving power allows for mass accuracy to the milliDalton (mDa) range. Due to the exact mass capabilities of the AccuTOF, there is little chance of two unrelated compounds overlapping, so there is no need for chromatography prior to ionization. Because of the speed of analysis and its sensitivity, the AccuTOF-DART™ is an ideal technique for screening forensic samples [14–20].

To date, there has been no published research using AccuTOF-DART™ to analyze bank dyes or pepper sprays on clothing. A method for analyzing these types of substances using the AccuTOF-DART™ has been developed and validated. Validation included determination of the lower limit of detection (LLOD), selectivity, reproducibility, and robustness of the technique.

2. Materials and methods

2.1. Material

All solvents were HPLC grade with methanol from Fisher Scientific (Fair Lawn, NJ), dichloromethane from Burdick and Jackson (Muskegon, MI) and hexane from EMD Chemicals Inc. (Gibbstown, NJ). 1-(Methylamino)-anthraquinone was purchased from Aldrich Chemical Company, Inc (Milwaukee, WI) at 98% purity. O-chlorobenzylidenemalononitrile was purchased from Alfred Bader Library of Rare Chemicals (Milwaukee, WI). 8-Methyl-n-vanillyl-6-nonenamide (capsaicin) was purchased from Sigma Chemical Co (St. Louis, MO) at approximately 98% purity.

Fabric samples were obtained from several different sources. Four t-shirts and a pair of denim jeans were obtained from employees at DFS. The t-shirts consisted of two 100% cotton white shirts (Hanes® and Faded Glory®), one 100% cotton purple shirt (Fruit of the Loom®) that contained dried sweat, and one 50/50 cotton/polyester red shirt (Russell Athletic®). The denim jeans (l.e.i.®) were labeled as 92% cotton, 7% nylon, and 1% spandex. Array® white cardstock was manufactured by Riverside Paper Company (Appleton, WI). Glass capillary tubes used for liquid sampling in the AccuTOF-DART™ were obtained from Kimble Glass Company (Vineland, NJ).

Human blood was obtained from the Toxicology Laboratory at DFS. A small fragment of a red brake light cover was obtained from the Trace Evidence Laboratory at DFS. A variety of red FD&C dyes (Allied Chemical and Dye Corporation, New York, NY) were obtained from the standards collection of the Controlled Substances Laboratory at DFS. A variety of hot sauces were donated by employees at DFS. Two pepper sprays, Punch II M-3 (AERKO International, Ft. Lauderdale, FL) and MK 6 (Federal Laboratories Inc, Casper, WY), were obtained from the standards collection of the Trace Evidence Laboratory at DFS. A third pepper spray, MACE® (MSI, Bennington, VT), was obtained from an employee at DFS.

2.2. AccuTOF-DART™ parameters

The DART™ source employed helium as the ionization gas at a flow of 2.5 L/min. The discharge needle was set at 4000 V, discharge electrode set at 150 V, and grid electrode set at 250 V. Ion detection was achieved with the AccuTOF™ ion guide peak voltage set at 600 V, the reflectron voltage at 990 V, orifice 2 voltages at 5 V, ring lens voltage at 10 V, and an orifice 1 temperature of 80 °C. The mass range monitored for MAAQ and CS was 100–400 Da, while the mass range monitored for capsaicin was 50–650 Da. Instrument calibration for capsaicin was performed within each data file using polyethylene glycol (PEG) 600 (Chem. Service, West Chester, PA). Mass drift was compensated by using a solution of cocaine (cocaine HCl from USP, Rockville, MD) as a lock mass standard. Instrument calibration for MAAQ and CS was performed within each data file using a mixture of acetanilide (Matheson Coleman and Bell, Norwood, OH), anthraquinone (DFS Laboratory Chemical Collection), and dicyclohexylphthalate (Sigma–Aldrich, St. Louis, MO). Daily calibration was performed by sampling methyl stearate (Eastman Organic Chemicals, Rochester, NY) after calibrating with PEG 600 and the cocaine lock mass standard. Calibrations were deemed acceptable if the measured mass of methyl stearate was within a range of ±3 mDa of the calculated ($[M + H]^+$ of 299.2950 Da) mass.

The voltages applied to orifice 1 and the temperature of the DART™ gas stream were optimized for each compound. A range of different voltages between 20 V and 90 V were analyzed for each compound to determine voltages that produced mainly protonated molecular ions and voltages that produced significant fragmentation.

After the ideal voltages were chosen for each method, the gas temperature was optimized to produce the most volatilization of each compound while producing the least amount of background ions from the substrate. Cotton swabs were chosen as the substrate to be tested because they would likely produce ions similar to cotton fabric swatches and were very easy to sample. Temperatures ranging from 200 °C to 400 °C were tested for each compound at 50 °C increments. Average total ion chromatogram (TIC) intensity and average mass peak intensity over three separate wandings were calculated for each compound as well as for blank cotton swabs. Additionally, the intensity of the burn on the swab was visually noted at each temperature. A final temperature was selected that produced a compromise between highest compound intensity, lowest swab intensity, and least amount of observed burn on the swab.

Because the chemical structure of CS indicates that it should produce negative ions more effectively than positive ions, MAAQ and CS were analyzed in both positive and negative ionization mode to determine the most efficient ionization mode for the combined analysis of these two compounds. The overall abundances were compared, as well as the LLOD of each compound. Due to capsaicin's chemical structure, it was only analyzed in positive ionization mode.

2.3. GC/MS parameters

The GC/MS system consisted of a Hewlett–Packard 6890 gas chromatograph coupled with a Hewlett–Packard 5973 mass spectrometer. A pulsed splitless injection was used with the injection volume being 2 μL and the injector temperature set at 290 °C. The pulse pressure was 40 psi and was applied for 0.6 min. The stationary phase consisted of an HP-1 MS column (30 m × 0.25 mm i.d., 0.25 μm in thickness) and the carrier gas used was helium. For MAAQ and CS analysis, the GC oven was programmed at 90 °C for 2 min followed by a 20 °C/min ramp to 170 °C and a 30 °C/min ramp to 290 °C, with a final hold time of 3 min and a constant carrier gas flow of 1.2 mL/min. For capsaicin analysis, the GC oven was programmed at 125 °C for 1 min followed by a 30 °C/min ramp to 280 °C with a final hold time of 3.83 min and a constant carrier gas flow of 1.8 mL/min. The mass detector was set to scan between 14 Da and 400 Da, with a scan rate of 3.74 scans/s for the pepper spray method and 1.95 scans/s for the bank dye method.

2.4. Lower limit of detection determination

The lower limit of detection (LLOD) was determined for each compound on the AccuTOF-DART™. Serial dilutions of each compound were prepared in methanol and analyzed. Due to unwanted cross-reactions between the CS at high concentrations with methanol in the heated gas stream, the CS and MAAQ were prepared in dichloromethane. The LLOD was determined by finding the lowest concentration of each compound that would produce a protonated molecular ion within ±5 mDa of the expected mass of the protonated molecule at the lower orifice 1 voltage. This criterion was used to determine the LLOD for each technique tested throughout the study.

The LLOD was then determined for each compound on the GC/MS. Each compound was analyzed on the appropriate GC/MS program at a concentration of 1 mg/mL in order to determine the retention time (R_t) and a mass spectrum for each compound. Serial dilutions of each compound were prepared and analyzed on the appropriate GC/MS program. The LLOD was determined by checking that the GC R_t was within 0.03 min of the expected time and analyzing the mass spectrum to confirm that all of the major ions were present with visible ^{13}C isotope peaks. The lowest concentration of each compound that met these criteria was determined to be the LLOD.

2.5. Extraction solvent

Three different solvents – methanol, dichloromethane, and hexane – were tested to determine the most efficient extraction solvent for each compound. Swatches of cotton, denim, 50/50 polyester/cotton, and sweat-stained cotton were spiked with either 25 μg MAAQ and 25 μg CS or 25 μg capsaicin. One MAAQ/CS stain and one capsaicin stain were prepared on each fabric type for each of the three solvents tested. Each stain was cut out and inserted into an autosampler vial. One milliliter of the appropriate solvent was added to each vial. Vials were agitated for 5 s and the liquid extract was transferred to a new autosampler vial. Each extract was run on the appropriate GC/MS program. The abundance from the TIC of all three fabric types was averaged for each compound to determine the solvent with the best response.

2.6. AccuTOF-DART™ sampling technique

Stains were prepared by spiking fabric swatches with 25 μL of solution containing the compound(s) of interest in methanol and/or dichloromethane at an appropriate concentration that would deposit the desired amount of the compound(s) onto the fabric swatch. The particular concentrations varied depending on the compound being analyzed and the type of fabric being prepared. Several techniques were tested for sampling the stains on the AccuTOF-DART™, which included direct sampling, swabbing, liquid extraction, and extracting onto cardstock.

The direct sampling technique involved cutting the fabric through the middle of the stained area and inserting the stained edge directly into the DART™ sampling gap. No additional sample preparation was necessary for this technique.

The swabbing technique involved wetting a cotton swab with an appropriate solvent – hexane for MAAQ and CS or methanol for capsaicin – and rolling the swab over the stained portion of fabric to transfer the stain to the swab. The stained portion of the swab was then inserted directly into the DART™ sampling gap for analysis.

Liquid extraction involved cutting out the stained portion of fabric and inserting it into an autosampler vial. Approximately 1 mL of the appropriate solvent was