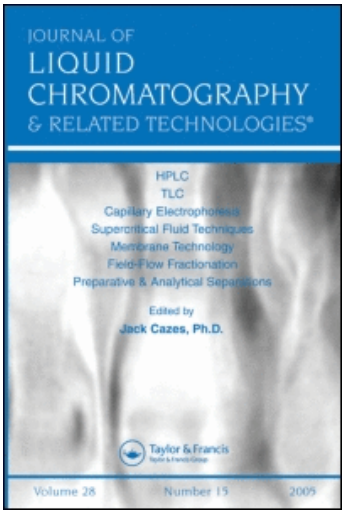

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Sample Collection, Preparation, and Quantitation in the Micellar Electrokinetic Capillary Electrophoresis of Gunshot Residues

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SAMPLE COLLECTION, PREPARATION, AND QUANTITATION IN THE MICELLAR ELECTROKINETIC CAPILLARY ELECTROPHORESIS OF GUNSHOT RESIDUES

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ABSTRACT

Application of micellar electrokinetic capillary electrophoresis (MECE) to gunshot residue (GSR) analysis was accomplished by developing appropriate sample collection and handling techniques. Masking adhesive-tape particle collection was used to minimize analyte losses and coextraction of sample matrix interferences associated with solvent swabbing collection methods. In addition, ethylene glycol (EG) was added to the extraction solvent to prevent analyte loss during evaporative preconcentration. β-naphthol, used as an internal standard, improved quantitative precision when EG was used in the MECE analysis of GSR's. Gunshot residues were collected from the hands of individuals who had discharged a firearm and analyzed by MECE using these sample collection and preparation techniques. Detection of gunpowder constituents, characteristic of the unfired gunpowder was obtained.

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INTRODUCTION

Recent work in this lab (1) and by others (2,3) has demonstrated the feasibility of using CE as an analytical tool for samples of forensic significance. Here we describe a specific protocol for gunshot residue sample collection and handling for analysis using the CE technique (1) tailored to the analysis of the organic constituents of gunpowder and explosives.

Methods used for the collection of gunshot residues (GSR's) depend on the final analytical measurement technique employed. The two most commonly used collection methods are solvent swabbing and adhesive-film lifts. Cotton swabs, moistened with dilute nitric acid, have been used to collect GSR's for analysis of the inorganic constituents by atomic absorption spectroscopy (AAS) (4), and adhesive-tape, attached to aluminum stubs, has been used to collect GSR's for the scanning electron microscopic (SEM) analysis of the inorganic constituents of the primer compounds (5). The analysis of the organic constituents of GSR's has been attempted using GC/MS (6), GC/TEA (7) and HPLC (8-9). In each of these procedures, sample collection was done using cotton swabs moistened with an organic solvent such as methanol or acetone.

In our previous study (1), micellar electrokinetic CE (MECE) was used to separate 26 organic compounds

which are constituents of gunpowder and explosives. In the present study, collection of GSR's using both swabs and adhesive-film lifts is examined for use in applying MECE to organic component GSR analysis. Important factors which were considered included: type of adhesive-film; choice of solvent for collection and extraction; the use of a non-volatile "keeper" to prevent sample loss during evaporative preconcentration/reconstitution; the effect of keeper concentration on peak quantitation; and the use of an internal standard to improve quantitative precision.

EXPERIMENTAL

Materials:

Sodium dodecylsulfate (SDS), boric acid, sodium tetraborate decahydrate, β-naphthol, ethylene glycol, and chromatographic-grade ethanol and acetone were obtained from commercial sources. Cotton and polytetrafluoroethylene (PTFE) wool were used for swabbing, and Scotch brand cellophane tape, Post-it brand adhesive tape (both from 3M Co., St. Paul, MN), and Tuck brand masking tape (Tesa Tuck Inc., New Rochelle, NY) were evaluated for use as adhesive-film lifts. Standards of possible gunpowder constituents, listed in **Table I**, were obtained as a gift from either the Bureau of Alcohol, Tobacco, and Firearms (Rockville, MD) or the U.S. Army

TABLE I: Standards for MECE Analysis

Gunpowder Constituents	
Dibutylphthalate	DBP
N,N'-diethyl-N,N'-diphenylurea (ethyl centralite)	EC
2,3-dinitrotoluene	2,3-DNT
2,4-dinitrotoluene	2,4-DNT
2,6-dinitrotoluene	2,6-DNT
3,4-dinitrotoluene	3,4-DNT
Diphenylamine	DPA
Glycerol trinitrate (nitroglycerin)	NG
Nitroguanidine	NGU
2-nitrodiphenylamine	2-nDPA
N-nitrosodiphenylamine	N-nDPA
Internal Standard	
β-naphthol	Naph

Toxic and Hazardous Materials Agency (Aberdeen Proving Grounds, MD).

The buffer for the electrophoretic separation was 25 mmol/L SDS, 2.5 mmol/L sodium tetraborate adjusted to pH 8.5 with boric acid, and was degassed daily by ultrasonic agitation under vacuum. Solutions of GSR standards were prepared by dissolution in ethanol and dilution with the MECE buffer as described previously (1).

Test solutions for the quantitative studies were prepared by combining 2.5 μL of 10^{-3} mol/L ethyl centralite (EC - analyte), and 5 μL of 10^{-3} mol/L β-naphthol (an internal standard) with either 0, 5, 10 or 15 μL of ethylene glycol (EG - to prevent evaporation), and diluting to a final volume of 60 μL with buffer.

GSR's were collected from: a Beretta Model 92F, 9mm semiautomatic pistol, firing Federal American Eagle 125 grain ammunition; a Smith and Wesson, Bulldog 44 special revolver, firing Remington 44 Smith and Wesson special 245 grain ammunition; a Colt Series 80, 45 caliber semiautomatic pistol, firing Federal 45 auto, American Eagle 230 grain ammunition; and a Ruger "Security Six" .357 revolver firing Winchester Western 145 grain magnum ammunition.

Apparatus and Methods:

Experiments were conducted by using a commercially available CE instrument that consisted of a 0-30 kV power supply, an autosampler, and absorbance and fluorescence detectors. This instrument provided three automatic injection modes (electrokinetic, pressure, and gravity). Use of a deuterium light source as well as microprocessor control of the variable-wavelength grating monochromator allowed for wavelength optimization during runs. The wavelength used was 200 nm.

Polyimide-coated, fused silica capillary tubing, 350 μm O.D. and 100 μm I.D. was used for all experiments. The column length was 820 mm with on-column detection 50 mm from the ground end of the capillary, making the effective separation length 770 mm. The running voltage was constant at 25 kV. Gravity injection at 50 mm for either 5 or 10 seconds was used to avoid the small bias that we observed when using electrokinetic injection.

Firing Range. Sample blanks were collected from the cleaned hands of each volunteer prior to weapon firing. Each individual fired only one weapon so as to prevent cross-contamination. Each weapon was fired 3 times after which samples were collected from the firing hand, as well as from the hand that did not hold the weapon. The sample area of interest was the back of the hands along the thumb and forefinger and the webbing between those two digits, which has been identified as the area where GSR's are most likely to be found (10). Unfired ammunition from each weapon was collected, as well as spent shell casings fired from each weapon. Adhesive film lifts were also collected from the trigger area of the 44 caliber revolver and the 45 caliber semiautomatic pistol. Finally, post-firing blanks were also collected from the hands of each volunteer after washing thoroughly following the experiment.

Swabbing. Alcohol-cleaned cotton, polyester, or PTFE wool, moistened with ethanol or acetone and held with stainless steel tweezers, was used to swab the hands of the individual that fired each weapon. Each swab was placed in a separate, labeled, sealed glass vial and stored under refrigeration until analysis. Samples for MECE analysis were obtained by ultrasonic agitation of the swab in 500 μL of ethanol containing 1% ethylene glycol for 15 minutes. Collection of the GSR's was done by centrifugal filtration through a 1 μm PTFE filter. The extract was concentrated to about 2 μL under a stream of nitrogen and then diluted to 25 μL with buffer.

Adhesive Film Lift. 1 inch square sections of masking tape were used to collect the GSR's. Each film lift was placed in a separate, labeled, sealed glass vial, and stored under refrigeration until analysis. A binocular stereoscope was used to examine each lift for GSR particles. Suspect particles were removed from the adhesive tape with tweezers and placed in a glass micro-vial. 50 μL of ethanol was added to the vial and extraction of the particle was accomplished by ultrasonic agitation for 30 minutes. Approximately 1 μL of ethylene glycol was added to the vial and the ethanol was evaporated under a stream of nitrogen. 25 μL of buffer was then added to the sample in preparation for analysis by the MECE method.

A second approach was also used to collect GSR's from the tape lifts by cutting a 2 cm^2 section from the tape, placing it in a microvial and extracting as above. The extracted tape was removed from the ethanol before the ethylene glycol was added. Solvent and adhesive tape blanks were prepared in the same manner. Also individual grains from unburned gunpowder samples were extracted in this manner by placing a single gunpowder grain in a microvial with ethanol.

RESULTS AND DISCUSSION

Sample Collection and Handling

Several requisites must be met when tailoring sample preparation to the requirements of MECE analysis. The samples must have: (1) an analyte concentration range of 1-100 μmol/L (owing to poor detection limits when using absorbance detection); (2) an ionic strength similar to the CE electrolytic buffer (to avoid poor peak shape); and (3) been dissolved in a solvent similar in both polarity and viscosity to the MECE buffer (to avoid peak distortion, migration time shift, and injection volume variability) (1,11). In addition, we found several unique difficulties in applying MECE to the determination of the GSR components from human skin. Two major problems were identified: (1) analyte losses by adsorption during collection and handling, and (2)

effects of the dissolution solvent and matrix on the sample viscosity. Careful choice of the sample collection and extraction protocol circumvented these difficulties.

Sample collection by solvent swabbing proved to be a poor approach to collecting the organic GSR. Although cotton, polyester and PTFE swabbing materials were tested using ethanol for the recovery of standards from skin, all demonstrated large analyte losses. The losses are primarily adsorptive since analyte losses from volatilization or decomposition were found to be minimal under the experimental conditions. In addition, swabbing recovered unwanted quantities of skin fats and oils causing a matrix interference when evaporative concentration was used (as required for MECE analysis). The remaining gelatinous concentrate prevented redissolution of the hydrophobic analytes in the MECE buffer. In addition, the significant concentration of gelatinous material created a high sample viscosity resulting in analyte peak shape distortion for any redissolved residue, thus interfering with MECE quantitation.

Collection of the GSR particulate material using adhesive-tape lifts provided a much more appropriate sampling approach for organic residue analysis, and took advantage of the very small sample size requirements of CE. We found that it was not necessary to collect all

of the GSR from a hand, since a single particle provides sufficient material for MECE analysis. In addition, only minute quantities of the interfering fats and oils were found using tape lifts.

We found that the sample collection film needed to meet several criteria: (1) possess adequate adhesive character for collection of GSR from sweaty hands, (2) provide resistance to extraction solvents, and (3) exhibit a MECE blank that was free from coeluting interferences. A test of several adhesive tape formulations showed that masking tape met all of these criteria. The adhesive used on the cellophane and Post-it brand tapes was readily extracted with ethanol. Although a masking-tape extraction blank showed the presence of plasticizers such as dibutylphthalate (DBP) with $k'=\infty$, no interference was observed in the GSR components of interest. Given the negligible interferences from the tape blanks, it is possible to extract residues from sections of the tape that may not contain discernible residue particles.

In the course of evaluating analyte recovery, we discovered losses during the evaporative concentration and reconstitution of ethanolic standard solutions. Two changes alleviated these losses from the concentration step: (1) replacement of the adsorptive polyethylene sample collection and analysis vials with glass vials,

and (2) addition of a small percentage of a nonvolatile "keeper" to prevent the sample from going to dryness. Ethylene glycol (EG) was tested as a keeper by virtue of its high boiling point (B.P.=198°C) and high polarity. The use of EG as a keeper has the added advantage that in the MECE procedure it is not retained in the micelles, thus it migrates with the electroösmotic flow providing a convenient marker for determining k'.

Since the sample viscosity will be modified by the addition of EG, the effect of EG concentration on the sample injection volume and peak shape was studied. We also studied the improvement in quantitation RSD's by using β-naphthol as an internal standard. Test solutions containing a fixed quantity of β-naphthol and ethyl centralite (EC - a GSR constituent), and varying quantities of EG were analyzed using the MECE method. **Figure 1** shows the effect of EG concentration on the peak areas, heights, area/height ratios, and efficiencies for both β-naphthol and EC. Changes in peak shape are revealed by the area/height ratio and efficiency, thus demonstrating an effect on the electrophoretic behavior of the sample. We found that the changes in analyte (EC) peak area, resulting from changes in EG concentration, could be minimized by using an internal standard (β-naphthol) as can be seen in **Figure 2.**

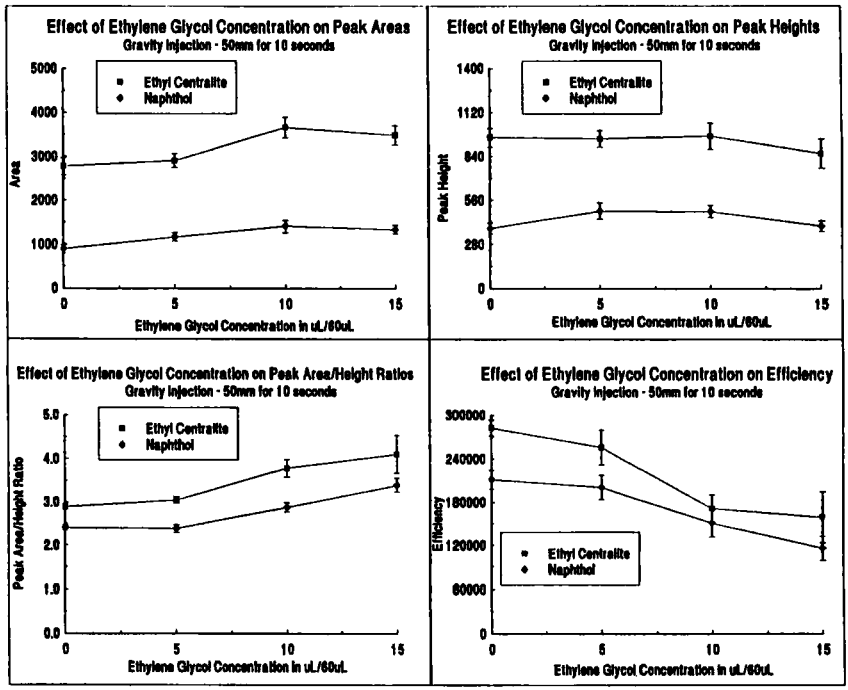

FIGURE 1: Effect of ethylene glycol (EG) concentration on peak areas, peak heights, area/height ratios, and peak efficiencies. 5 μL β-Naphthol + 2.5 μL ethyl centralite + EG in buffer. Buffer - 25 mmol/L SDS, 2.5 mmol/L borate, pH 8.5. Gravity injection at 50 mm for 10 seconds. Voltage - 25 kV. Detection - 200 nm.

The issue of migration time and quantitative reproducibility in MECE is important. It has been previously shown (1) that the use of k' to index migration provides more precise qualitative information than the use of migration times. Quantitative precision in MECE is also an issue as can be seen from the current data. Variance

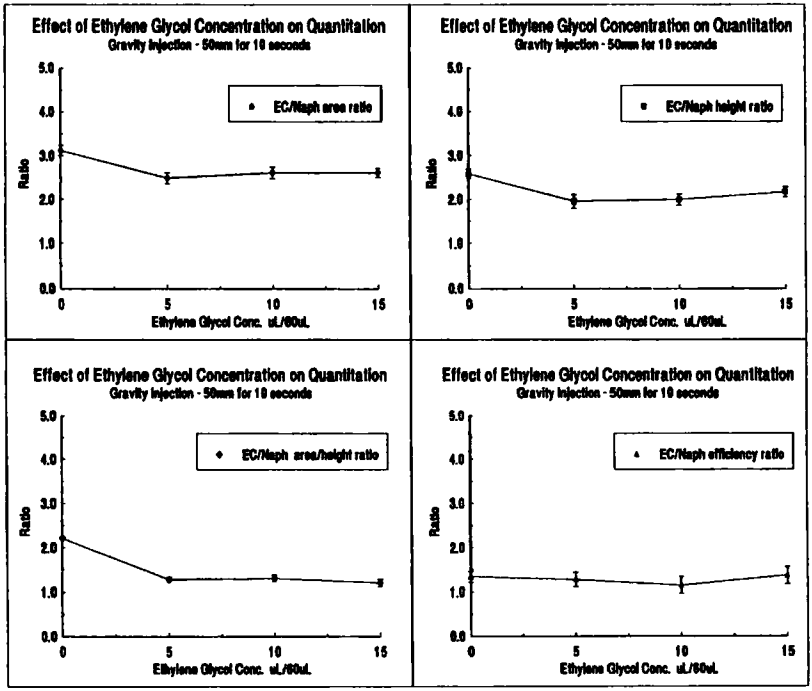

FIGURE 2: Effect of ethylene glycol (EG) concentration on the ratio of analyte peak areas, heights, area/height ratios, and efficiencies to internal standard peak areas, heights, area/height ratios, and efficiencies. Conditions - same as in FIGURE 1.

in the quantitative measurement of peak areas and heights (without using an internal standard) was found to be in the range of 5-25% as compared to better than 1% for normal free-zone capillary electrophoresis on the same instrument. The source of the quantitative variance is largely indeterminate. The MECE precision was the same on other commercial CE systems and appear to be

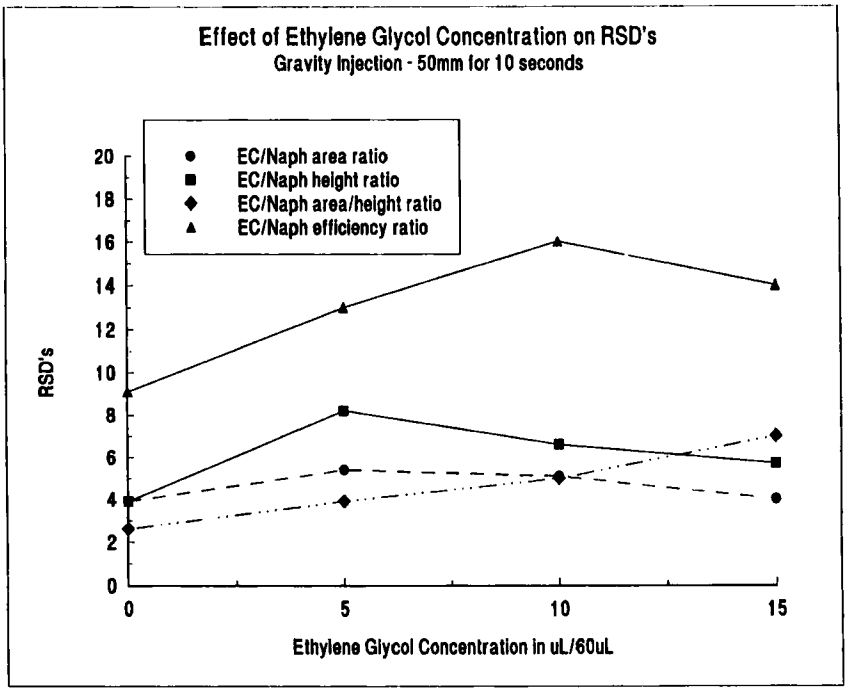

FIGURE 3: Effect of ethylene glycol (EG) concentration on the RSD's for analyte/internal standard peak area, height, area/height, and efficiency ratios. Measurements made on 8 replicates. Conditions - same as in FIGURE 1.

independent of the cooling method used. The addition of an internal standard improves the quantitative precision. **Figure 3** shows a plot of the RSD's for the normalized peak characteristics. It should also be noted that we found peak area ratios to be more precise than peak height ratios, thus peak areas ratios are recommended for quantitation.

The addition of an internal standard can minimize the changes in the qualitative and quantitative results in MECE analysis, resulting from the addition of up to 25% EG (v/v), however, the most precise results were obtained when the concentration was around 8% (v/v) or less. EG concentrations of about 5% (v/v) were chosen for the adhesive film lift studies. An added benefit of using the EG as a keeper is that it acts as a viscosity buffer. Small amounts of extracted fats and oils do not have a pronounced effect on the viscosity of the sample with EG as without.

Application to Gunshot Residue Analysis

Having developed appropriate particle sampling techniques, we then applied the MECE method to the analysis of field sample GSR's. There are several goals of GSR analysis. The first is to determine whether or not an individual has fired a weapon. This can be done by establishing the presence or absence of characteristic GSR constituents on specific areas of the hands, clothing, etc. of the suspect. We have already demonstrated that MECE is capable of identifying these components based on migration times and spectral characteristics of the analytes (1). A second objective of GSR analysis is to provide information concerning the commercial source of the gunpowder and perhaps specific

TABLE II: Samples Analyzed

Sample	Weapon	Hand	Firing Mode	Shots	Sample Type*
#1	None	L	Before firing		T blank
#2	None	R	Before firing		T blank
#3	None	L	Before firing		T blank
#4	None	R	Before firing		T blank
#5	None	L	Before firing		T blank
#6	None	R	Before firing		T blank
#7	45	L	Right Hand	3	T
#8	45	R	Right Hand	3	P
#9	45	R	Right Hand	3	T
#10	44	L	Two Handed	3	T
#11	44	R	Two Handed	3	P
#12	44	R	Two Handed	3	T
#13	9	L	Two Handed	3	T
#14	9	R	Two Handed	3	T
#15	9	R	Two Handed	3	P
#16	45		Unfired powder		P
#17	44		Unfired powder		P
#18	9		Unfired powder		P
#19	None	L	After washing		T blank
#20	None	R	After washing		T blank
#21	None	L	After washing		T blank
#22	None	R	After washing		T blank
#23	None	L	After washing		T blank
#24	None	R	After washing		T blank

* T = 2 cm^2 section of tape.
P = 1 particle from taken from tape.

batch or lot information. Again, our previous work (1) has shown that there are compositional differences between gunpowders from different manufacturers.

Firing range experiments were conducted as described in the Methods section. Adhesive film lifts, using masking tape, were collected from the hands of 3 individuals, each of whom fired a different weapon. Each lift was placed in a clean, labeled, sealed glass vial and placed in the dark under refrigeration until

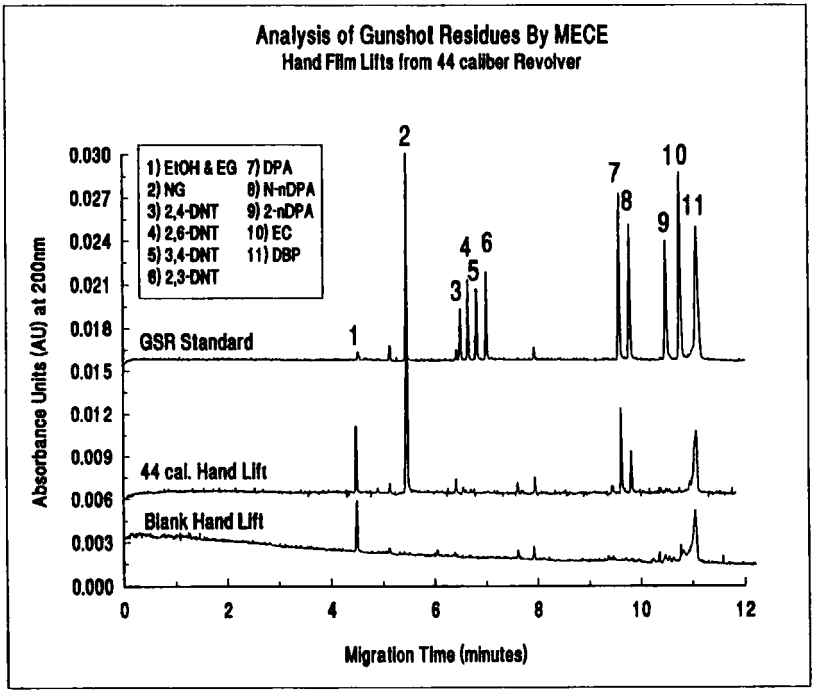

FIGURE 4: MECE analysis of a solution of GSR standards, an extract of a single GSR particle on a film lift from a hand which fired a 44 caliber revolver, and an extract from a film lift blank. Buffer - 25 mmol/L SDS, 2.5 mmol/L borate, pH 8.5. Gravity injection at 50 mm for 5 seconds. Voltage - 25 kV.

each sample could be analyzed. **Table II** provides a list of the samples collected. Each tape lift was examined for the presence of suspected GSR particles. Qualitative MECE analysis was conducted on both extracts from collected particles and extracts from small sections of the tape itself. **Figure 4** shows a comparison of elec-

TABLE III: GSR Constituents in Analyzed Samples

	GSR Constituents					
Sample #	NG	DPA	N-nDPA	2-nDPA	EC	DBP*
#1						x
#2						x
#3						x
#4						x
#5						x
#6						x
#7						x
#8	xxx				x	x
#9	xxx				x	x
#10	x					x
#11	xxx	x	x			x
#12	xxx	x	x			x
#13	x					x
#14	x					x
#15	xxx	x	x	x	x	x
#16	xxx				x	x
#17	xxx	x	x	x	x	x
#18	xxx	x	x	x	x	x
#19						x
#20						x
#21						x
#22						x
#23						x
#24						x

* DBP or any phthalate plasticizer
xxx = excess quantity

tropherograms from MECE runs of GSR standards, an extract of a particle from **sample #11**, and an extract of a section of the tape from **sample #12**. The results seen in **Table III** show that characteristic GSR constituents were found on each adhesive film lift on which GSR's would have been expected, and no evidence of GSR's was found on blanks and post-firing blanks. Identification of GSR constituents was made by comparison to the migration of standards, using EG and DBP as markers for the

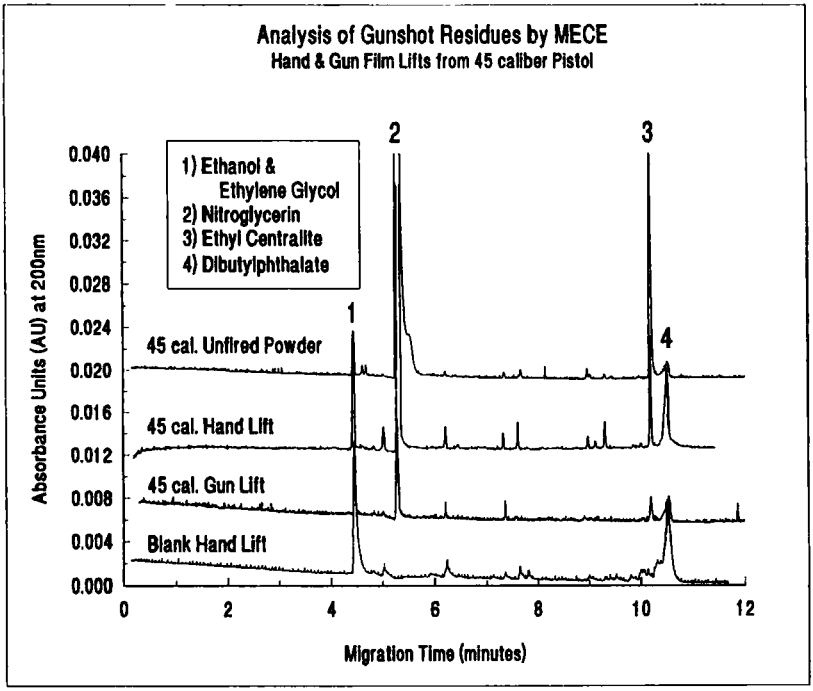

FIGURE 5: MECE analysis of an extract of a single, unburned flake from gunpowder for a 45 caliber semiautomatic pistol, an extract from single GSR particle on a film lift from the hand that fired the 45 caliber pistol, and extract of a section of the film lift from the trigger of the 45 caliber pistol, and an extract from a film lift blank. Conditions - same as in FIGURE 4.

determination of k', and by spectral characterization using sequential analysis at several wavelengths. Similarities between unfired gunpowder, particles from an adhesive lift off of a hand, and off of a weapon can be seen in **Figure 5.** In general, our results show that

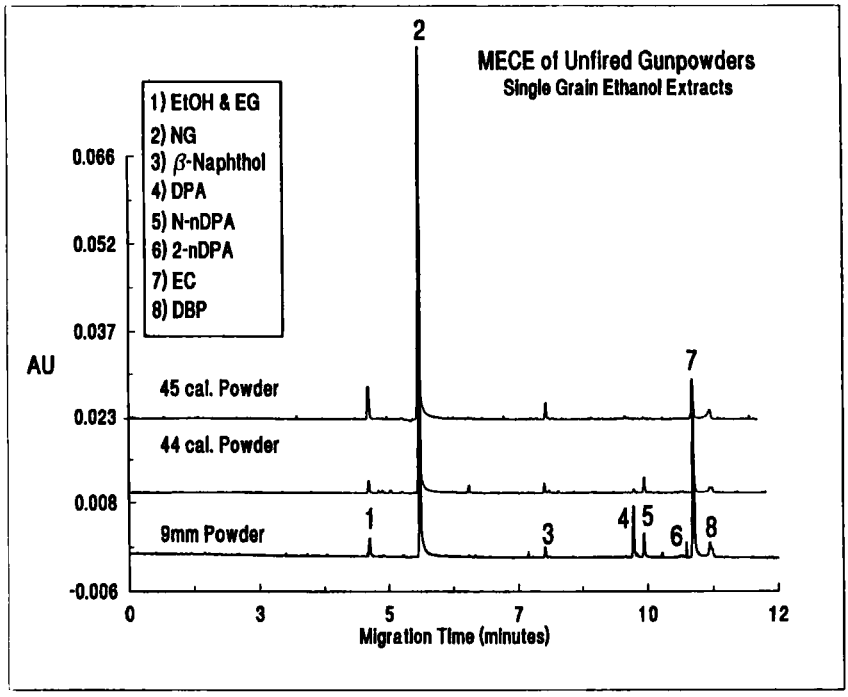

FIGURE 6: MECE analysis of extracts from single flakes of 3 gunpowders: 9mm powder, 44 caliber powder, and 45 caliber powder. Conditions - same as in FIGURE 4.

both the unfired gunpowder and the post-firing GSR's from the same gunpowder were similar in composition. This is consistent with previous findings (7). The differences which were observed with some of the samples may be due to particles coming from different lots of the same gunpowder. **Figure 6** shows the differences between the 3 different gunpowders used in this study.

It should also be noted that the addition of β-naphthol as an internal standard, for use in quantitation, does not interfere with any of the potential GSR constituents, and its migration time is approximately midway between the two k' markers.

CONCLUSION

The results presented in this study demonstrate the feasibility of using MECE as an analytical technique for the analysis of GSR's. Proper sample collection and handling techniques can eliminate sample matrix interferences which arise from changes in viscosity, ionic strength, and analyte composition. Adhesive film lifts were found to be an effective sample collection method, and the use of EG as a nonvolatile keeper and viscosity buffer eliminated many of the sample matrix interferences. Quantitative results were improved by using an internal standard.

This study also demonstrated that there are differences between gunpowders from different manufacturers and possibly lot-to-lot variations. Further experiments with a wide range of gunpowders are being conducted to determine the quantitative characteristics of each constituent. However, because gunpowder and GSR constituents can decompose over time, quantitative evidence must be interpreted with great care.

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