

Comparative Study for the Analysis of Organic Gunshot Residues by Micellar Electrokinetic Chromatography

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ABSTRACT

This study was undertaken to separate three dinitrotoluene compounds using micellar electrokinetic chromatography (MEKC). The influences of a running buffer and sample matrices were investigated with respect to migration, efficiencies, peak shapes and resolution. In this work, three on-line sample preconcentration techniques, sweeping, high salt stacking, sweeping-high salt stacking were developed from MEKC using 25 mM phosphate at pH 7.5, 75 mM sodium dodecyl sulfate, 30 kV applied voltage, and on-column UV detection at 200 nm. High salt stacking had shown to be the most powerful tool for sample concentration and optimized concentration of NaCl was 150 mM which provided the best separation in terms of peak intensity and efficiency. The linear regression was greater than 0.995 (1.0 to 10.0 µg/g). For spiked the sample at 5 µg/g, the precision was ranging from 0.99 to 3.16 % relative standard deviation (RSD). Limit of detection was lower than 1.0 µg/g. The mean recoveries were between 68.03 and 90.78%. The optimized high salt stacking procedure has been shown to be reliable, accurate and precise for monitoring of DNT in unburned gun powder.

Keywords: micellar electrokinetic chromatography (MEKC), dinitrotoluene (DNT), high salt stacking, sweeping, sample matrix

INTRODUCTION

When a firearm is discharged, a residue is produced that can be deposited on the hands of the shooter. On targets and in weapons, the residue is made up of unburned powder, as well as metallic particles from the primer, the cartridge casing and the barrel of the gun (Romolo and Marget, 2001; Thorton, 1994 ; Meng and Caddy, 1997). There are three major reasons for analyzing explosive and gunshot residues: 1) to calculate firing distances; 2) to identify bullet holes; 3) to determine whether a person has fired a gun or participated in a terrorist blast. For these reasons, the analysis of explosives and gunshot residue has become one of the most crucial parts of forensic science. As referred to the work of Harrison and Gilroy, gunshot analysis was based on the evaluation of the metals lead, barium, and antimony, the primary metals found in bullets. These evaluations require various techniques of metallurgical analysis, including neutron activation analysis (NAA), atomic absorption spectroscopy (AAS), and inductively coupled plasma mass spectrometry (ICP-MS).

A review of all aspects of gunshot residue analysis included a summary of the specific nature of inorganic and organic residues, how they arise, and how they may be collected from a variety of matrices. Techniques discussed included scanning electron microscopy with energy dispersive X-ray analysis (SEM-EDX), flameless AAS, NAA, energy dispersive X-ray fluorescence (EDXRF) for inorganic residues and GC, HPLC and supercritical fluid chromatography (SFC) with special detectors, such as MS, for organic residues.

Up to date, the most accepted technique is SEM-EDX, which combines morphological (SEM) and elemental (EDX) identification of the metal residues. Although it is considered to be specific, heavy-metal analysis offers moderate diagnostic sensitivity, requires expensive instrumentation and is highly demanding in terms of professional skills, manpower, maintenance, and service. As a result, its application as a routine technique is difficult. Besides, it has been recently discovered that some particles found in gunshot residue can be misidentified, decreasing the specificity of SEM-EDX analysis (Garofano *et al.*, 1999). The modern trend to produce primers free of heavy metals is now causing a new problem to arise, possibly leading to an increase of false negative results. For these reasons, there is a need for a fast, cheap and specific technique that can be routinely used in laboratories with a high workload.

In recent years, capillary electrophoresis (CE) has been developed as a separate method suitable for routine applications in trace-analysis to determine either organic or inorganic elements in explosives and gunshot residues, according to the studies of Northrop and *et al* in 1991 and Hagarion and McCord in 1992. Its popularity may be attributed to its extremely high efficiency, short analysis time, and wide application range.

MEKC is a mode of CE that supports the separation of electrically neutral analytes. To operate MEKC, an ionic micelle is added to the running solution of CE without modifying the instrument. The principle of MEKC is to separate substances concentrated in the differential migration of the ionic micelles and the bulk running buffer under electrophoresis conditions and in the interaction between the analyte and the micelle. Accordingly, MEKC has the same separation principle as chromatography. MEKC is a beneficial technique for separating small molecules, both neutral and charged. The technique is also high efficiency for short time separations with minimum amounts of samples and reagents.

The purpose of this work is to develop a method to analyze the organic gunshot residues as a cheaper, faster, and more specific alternative than the conventional techniques used nowadays. In this study three on-line sample preconcentration techniques, sweeping, high salt stacking and sweeping -high salt stacking were developed using MEKC technique.

Sweeping, a new on-line sample concentration technique in MEKC, is defined as the sorption and accumulating of analyte molecules by the pseudo-stationary phase (PS) that enters and fills the sample zone upon application of voltage. High salt stacking, the effect of salt concentration used to dilute sample solution without injection surfactant. The sample stacking method efficiency is high as a result of the micelles being stacked before they enter the zone. While it is critical to maintain the highest possible surface tension in the sample matrix to

maximize hydrophobic interaction with analytes. The last method is sweeping-high salt stacking. This is the novel technique, invented by the researcher, through combining sweeping and high salt stacking. This technique shares the same principle as sweeping in terms of an equivalence of conductivity between sample matrix and running buffer. However, in this technique salt solution or co-ion was added in to sample matrix whereas sweeping contains no co-ion in the system.

Sweeping

Sweeping was referred to “the picking and accumulation of analytes by pseudo-stationary phasing that enter into the sample zone under an applied voltage.” The principle of the sweeping technique is that the conductivity of a sample matrix is equivalent to that of the running buffer, but an analyzed solution contains no surfactant sodium dodecyl sulfate (Quirini and Terebe, 1998, 1999). The definition of “sweeping” was later expanded by Terabe and coworkers (Quirini *et al.*, 2002), “sweeping” comprises of any condition in which a running buffer contains a separation vector and the sample matrix does not. Figure 1 shows the mechanism of stacking for an equal conductivity sample matrix and a system with negatively charged micelles and normal electroosmotic flow (EOF). Under a homogeneous electric field, neutral analytes are carried toward the negative electrode only to be swept up in the micelle at the boundary of the sample region. The efficiency of the stacking relies on an analytes’ affinity for a micelle, an increase in the affinity of micelles results in the higher extent of stacking. Terabe derived the following equation representing an extent of stacking as a function of an analytes affinity for micelle.

$$l_{\text{sweep}} = l_{\text{inj}}(1/1+k)$$

where l_{sweep} is the length of the swept analyte, l_{inj} is the length of the injected plug, and k is the retention factor for a given analyte.

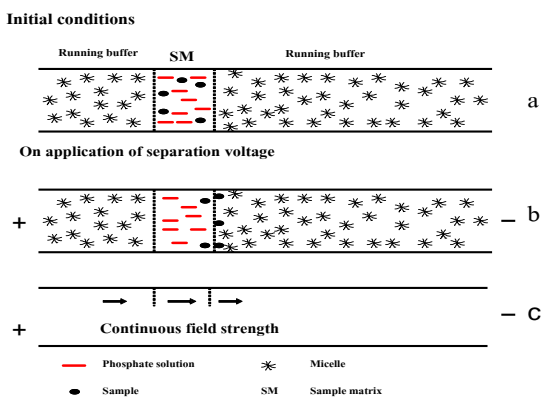


Figure 1 Schematic representations of sweeping (a) Starting situation: Sample solution prepared in sample matrix having conductivity similar to the running buffer. (b) Application of voltage at position polarity (c) Continuous buffer system (adapted from Landers, 2008)

High salt stacking

High salt stacking is a mechanism of analyte preconcentration provided by the stacking of micelles at the negative electrode side of the sample plug (Palmer *et al.*, 1999). Analyte enters into the stacked micelle region experiencing a local high retention factor caused by the high micelle concentration. High salt stacking is achieved under the following conditions:

$$\begin{aligned} \mu_{\text{sample}} E_{\text{sample}} &< \mu_{\text{ev}} E_{\text{ev}} \\ \mu_{\text{sample}} &> \mu_{\text{ev}} \end{aligned}$$

Where μ_{sample} is the mobility of the sample stacking co-ion, E_{sample} is the electric field in the sample zone, μ_{ev} is the mobility of electrokinetic vector (micelle), and E_{ev} is the field in a running buffer. To achieve the condition that the mobility of the sample stacking co-ion is higher than the mobility of the micelles, the field in the running buffer must be higher than the field in the sample zone; this is imperative to significantly increase conductivity of the sample zone associated with the running buffer. The stacking by high salt mechanism is presented in Figure 3.

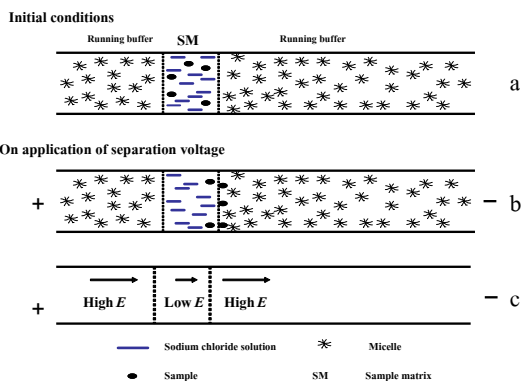


Figure 2 Schematic representations of high salt stacking (a) Starting situation: The conductivity of the sample zone was greater than that of the running buffer. (b) Application of voltage at position polarity (c) Discontinuous buffer system (adapted from Landers, 2008)

Sweeping –high salt stacking

A combination of the different on-line sample preconcentration techniques increases concentration efficiency or simultaneous concentration of different types of analysis. The aim of this study is an attempt to develop two analyzing techniques, sweeping and high salt stacking by controlling conductivity of sample matrix and running buffer as can be seen in Figure 3.

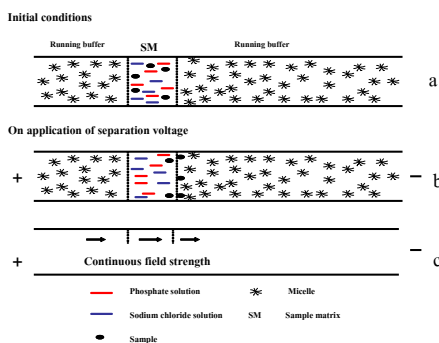


Figure 3 Schematic representations of sweeping-high salt stacking (a) Starting situation: The mixture was adjusted for the same conductivity as a running buffer. (b) Application of voltage at position polarity (c) Continuous buffer system (adapted from Landers, 2008)

EXPERIMENT

Chemicals

Monosodium, disodium phosphate and sodium dodecyl sulfate (SDS) from Carlo Erba were used in the running buffer (25 mM phosphate buffer/75 mM SDS/pH 7.5). 2,4- dinitrotoluene (2,4- DNT) was obtained from Acros Organics. 2,6- dinitrotoluene (2,6- DNT), 3,4- dinitrotoluene (3,4- DNT), sodium fluoride, chloride, bromide, iodide and chlorides of lithium, sodium and potassium, sodium sulfate and magnesium sulfate were obtained from Sigma-Aldrich. All of the substances were analytical grade.

Instruments

All experiments were carried out using a Hewlett-Packard^{3D} capillary electrophoresis system (Agilent Technologies, Germany) equipped with diode-array detection (DAD). pH of solutions were measured by a Sartorius pH meter (Germany). Conductivities were measured using a Eutech Instruments conductivity meter (Netherlands). All the separations were performed on a fused-silica capillary of 64.5 cm (56 cm effective length) x 75 μ m I.D. (Agilent, USA).

Preparation of MEKC buffer and standard solutions

The running buffer system was composed of a combination of monosodium and disodium phosphate, adjusted with sodium hydroxide to maintain a pH of 7.5 at 25 mM phosphate concentration. SDS, as a micellar system, was added to the buffer at a concentration of 75 mM, and then the final volume was adjusted with deionized water. After a thorough mixing in a sonicator for 10 minutes, the final running buffers were filtered through a 0.45 μ m membrane filter.

Organic standards were dissolved in methanol to provide 1000 ppm stock solutions.

Preparation of sample solutions

Normal-MEKC: standard solutions were prepared using running buffer (25 mM phosphate buffer/75 mM SDS/ pH 7.5).

Sweeping: standard solutions were prepared in diluted phosphate solution (no SDS micelles). The mixture was adjusted to the same conductivity as a running buffer.

High salt stacking: standard solutions were prepared with sodium chloride or other salt as indicated by using deionized water. The conductivity of the sample zone was greater than that of the running buffer.

Sweeping-high salt stacking: standard solutions were prepared in diluted phosphate solution (no SDS micelles) and sodium chloride solution. The mixture was adjusted for the same conductivity as a running buffer.

Analytical performance of ultrasonic extraction (USE) and the high salt stacking analysis

The unburned gun power samples were prepared by spiking appropriate amount of the DNT standards solution to the gun powder to get the final concentration of 5 $\mu\text{g/g}$ -dry weight. Extractions were performed in triplicates ($n=3$) and included a method blank, whereas USE efficiencies of the experiments were checked by calculating of recoveries. The spiked unburned gun powder (0.05 g) was accurately weighed into a centrifuge tubes and 5 mL of methanol was added. The sample was extracted a time by ultrasonication for 15 min in an ultrasonic bath. The extracts were evaporated by a gentle stream of nitrogen gas at room temperature to near dryness and the residues were re-dissolved in 1 mL of 150 mM NaCl solution. After that, the solution was filtered by a 0.45 μm nylon membrane filter. The amount of DNT was determined by capillary electrophoresis system.

The linearity of the method was tested by extracting five spiked unburned gun powder samples with increasing concentrations over a range between 1 and 10 $\mu\text{g/g}$ -dw. The accuracy was estimated by means of recovery experiments, analyzing unburned gun power samples ($n=5$) spiked at 5 $\mu\text{g/g}$ -dw. The limit of detection (LOD) was estimated as the analyte concentration that produced a peak signal of three times the background noise from the electropherogram at lowest fortification level tested.

RESULTS AND DISCUSSION

Optimization of normal-MEKC

Method development by capillary electrophoresis involved the optimization of many experimental parameters, any of which can be critical to the ultimate performance of the method. The general strategy and factor considered in the optimization of the important parameters involved in the optimized method are described as followed.

Effect of anionic surfactant

CE of nonionic analytes could not be performed in a free solution due to the lack of electric charges on the analytes. This problem could be overcome by employing a charged additive that forms micelles which could be used as a

psuedostationary phase such as SDS. The concentration of SDS in the running buffer was varied from 25 mM to 100 mM for the separation of the three dinitrotoluene compounds of interest in Figure 4. The SDS concentration of 75 mM was chosen for all further MEKC work.

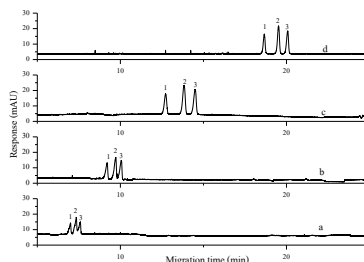


Figure 4 Electrophoregrams of the effect of SDS concentration on separation of three dinitrotoluene compounds in MEKC. Conditions used: buffer compositions; 50 mM phosphate buffer, pH 7.0 with; a) 25 mM, b) 50 mM, c) 75 mM and d) 100 mM SDS, separation voltage +30 kV at 200 nm, $L=64.5/56$ cm effective length, Peaks identification 1: 2,4 DNT, 2: 2,6 DNT, 3: 3,4 DNT

Effect of pH

EOF can be controlled, or suppressed to effect a variety of different separations. pH is a very important factor involved in the separation, because it can control the charge state of solutes as well as the level of EOF. The overall migration time of a solute is related to both the mobility of the solute, and the EOF. The effect of pH in the running buffer was examined from 6.0 to 8.0, see Figure 5, it was found that pH 7.5 gave the best result in terms of peak shapes and migration time.

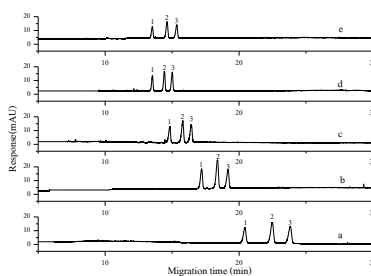


Figure 5 The effect of pH buffer on separation of three dinitrotoluene compounds in MEKC. Conditions used: 25 mM phosphate buffer containing 75 mM SDS with various pH values, separation voltage +30 kV at 200 nm, $L=64.5/56$ cm effective length, Peaks identification 1: 2,4 DNT, 2: 2,6 DNT, 3: 3,4 DNT \

Effect of buffer concentration

The phosphate buffer composed of a combination of monosodium and disodium phosphate. Figure 6 shows the separation of three dinitrotoluene

compounds obtained at different phosphate concentrations. The separation was achieved at all concentrations of phosphate buffer; increasing the concentration of phosphate resulted in longer migration times whilst the separation could be obtained at the phosphate concentration of 25 mM.

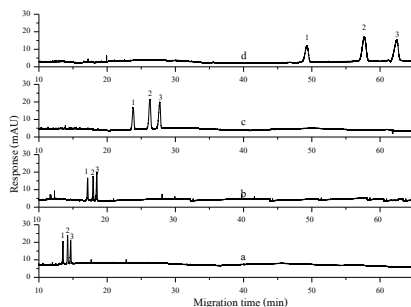


Figure 6 Electrophoregrams of the effect of phosphate buffer on separation of three dinitrotoluene compounds in MEKC. Conditions used: 75 mM SDS, pH 7.0 with; a) 25 mM, b) 50 mM, c) 75 mM and d) 100 mM phosphate buffer, separation voltage +30 kV at 200 nm, L=64.5/56 cm effective length, Peaks identification 1: 2,4 DNT, 2: 2,6 DNT, 3: 3,4 DNT

Effect of applied voltage

The voltage applied during the run time of separation was arrived at by applying a series of voltages to the running buffer and monitoring the resultant current. The effect of varying the applied voltage (10 kV to 30 kV) on the separation of three dinitrotoluene compounds was studied, as can be seen in Figure 7. An operating potential of 30 kV gave the minimal analysis time. Therefore, the potential of 30 kV was applied for further optimization step.

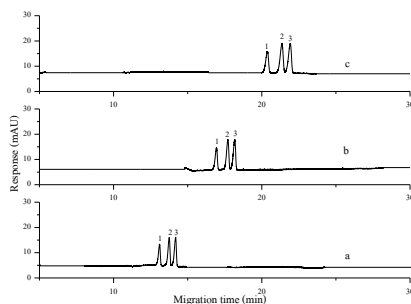


Figure 7 The effect of applied voltage on separation of three dinitrotoluene compounds in MEKC. Conditions used: buffer compositions; 25 mM phosphate buffer pH 7.5 containing 75 mM SDS, L=64.5/56 cm effective length, 200 nm. Separation voltage; a) 30 kV, b) 20 kV and c) 10 kV, Peaks identification 1: 2,4 DNT, 2: 2,6 DNT, 3: 3,4 DNT

Sweeping

Sweeping is an on-line sample preconcentration technique for neutral analytes, charged analytes, or ions. It is initially implemented by preparing analytes in a sample matrix of equal conductivity to the running buffer but devoid of surfactant. In sweeping, there is no micellar stacking observed, and the velocity of analytes in the sample zone in the presence of micelles is the same as the velocity of the analytes in the running buffer. Figure 8 shows the effectiveness of sweeping in the sample matrix. Samples were prepared in various concentrations of phosphate solutions (25 mM phosphate, 25-time-diluted 25 mM phosphate and 50-time-diluted 25 mM phosphate). Electropherograms show the maximum sensitivity with no peak broadening; therefore 25-time-diluted 25 mM phosphate was selected to use as a sample matrix.

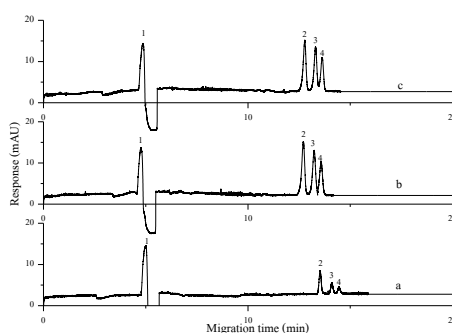


Figure 8 Electropherograms obtained from sweeping a) 25 mM phosphate b) 25-time- diluted 25 mM phosphate and c) 50-time- diluted 25 mM phosphate. Conditions used: buffer compositions; 25 mM phosphate buffer pH 7.5 containing 75 mM SDS, separation voltage +30 kV at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: EOF, 2: 2,4 DNT, 3: 2,6 DNT, 4:3,4 DNT.

High salt stacking

High salt stacking by focalizing the micelles in the sample-running buffer interface. Consequently, these analytes are swept by the grouped micelles at the interface thus the hydrophobic analyte is concentrated at this region. Stacking in high salt matrix will be apparent when the conductivity of a sample matrix is higher than that of a running buffer. To achieve stacking, ionic strength of sample matrix is higher than that of a running buffer. The concentration of NaCl in the sample matrix was varied from 50 mM to 200 mM. It was found that 150 mM NaCl in the sample matrices gave the best result with baseline separation in Figure 9.

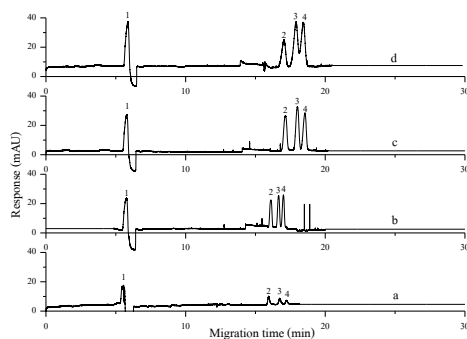


Figure 9 Electrophoregrams obtained from high salt stacking a) 50 mM b) 100 mM c) 150 mM and d) 200 mM NaCl. Conditions used: buffer compositions; 25 mM phosphate buffer pH 7.5 containing 75 mM SDS, separation voltage +30 kV at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: EOF, 2: 2,4 DNT, 3: 2,6 DNT, 4:3,4 DNT.

Sweeping –high salt stacking

This technique is a combination of sweeping and high salt stacking by controlling equivalent conductivity of the sample matrix and the running buffer. Three concentrations of NaCl solution (25, 50 and 75 mM) were added into 25-time-diluted 25 mM phosphate solutions (from sweeping). As can be seen in Figure 10, the mixture of 25-time-diluted 25 mM phosphate solution with 50 mM NaCl solution provided the best separation in terms of peak intensity and efficiency.

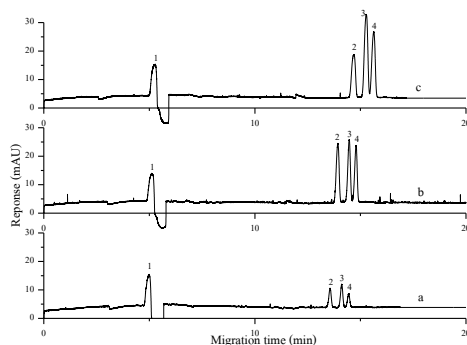


Figure 10 Electrophoregrams obtained from sweeping-high salt stacking a) 25-time- diluted 25 mM phosphate+ 25 mM NaCl. b) 25-time- diluted 25 mM phosphate +50 mM NaCl and c) 25-time- diluted 25 mM phosphate + 75 mM NaCl. Conditions used: buffer compositions; 25 mM phosphate buffer pH 7.5 containing 75 mM SDS, separation voltage +30 kV at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: EOF, 2: 2,4 DNT, 3: 2,6 DNT, 4:3,4 DNT.

Comparisons of sweeping, high salt stacking and sweeping-high salt stacking

Figure 11 shows the comparison of three dinitrotoluene compounds peak intensity obtained from MEKC conditions. As the figure 11, electropherogram b presented the best separation considering of peak intensity and efficiency. Therefore, high salt stacking would be the optimum condition for separation in the single run.

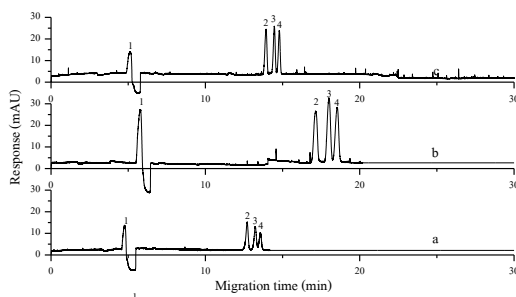


Figure 11 Comparison of concentration techniques in three dinitrotoluene compounds a) sweeping b) high salt stacking and c) sweeping-high salt stacking. Conditions used: buffer compositions; 25 mM phosphate buffer pH 7.5 containing 75 mM SDS, separation voltage +30 kV at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: EOF, 2: 2,4 DNT, 3: 2,6 DNT, 4:3,4 DNT.

Analytical performance

The repeatability of high salt stacking was obtained by analyzing five replicate spiked unburned gun powder samples consecutively at a concentration levels 5 $\mu\text{g/g-dw}$. The %RSD was between 0.99 and 3.16 %, and the precision of this method was good. The limit of detection (LOD) was evaluated by comparing the signal to noise ratio (S/N) of the lowest concentration to a S/N= 3. The results showed that the method allowed detection of DNT at concentration lower than 1.0 $\mu\text{g/g}$.

Series of five concentration levels were obtained by spiking unburned gun powder samples with all the DNT in a concentration ranging from 1.0 to 10 $\mu\text{g/g}$. Each solution was analyzed in three replicates. The linearity of the method has been investigated over the range of 1.0 to 10 $\mu\text{g/g}$ and the DNT had correlation coefficients of the calibration graphs greater than 0.995. The mean recoveries obtained for the DNT spiked in unburned gun powder samples. The recovery was determined as the peak area of sample spiked with analyte at a concentration levels (5 $\mu\text{g/g}$). The recoveries of all analyte ranged between 68.03 and 90.78%. This demonstrates that high salt stacking is simple, fast, precise and accurate technique for quantitative determination of the DNT in unburned gun powder samples when compared to the techniques in MEKC.

CONCLUSIONS

The objective of this study is to present fundamental concepts of on-line sample preconcentration techniques in capillary electrophoresis. Sample stacking in MEKC is a velocity change on partitioning into the micelles. In this case, preconcentration efficiency in MEKC is, at the least, dependent on the analyte affinity for the micelle and the mobility of the micelle. The separation of three dinitrotoluene compounds was achieved using 25 mM phosphate at pH 7.5, 75 mM SDS, 30 kV separation voltage, and on-column UV detection at 200 nm. High salt stacking gave successful separation of three dinitrotoluene compounds, with good peak shape. This developed method showed a simple, precise and accurate technique for quantitative determination of the DNT in sample when compared to the techniques in MEKC. The further work is to analyze organic components in fired gun powder by using high salt stacking.

ACKNOWLEDGEMENTS

This study was supported by the Forensic Science Police of Ubo Ratchathani Province, Royal Thai Police.

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