

Separation of Explosives Using Capillary Electrochromatography

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The identification of explosives and their degradation products is important in forensic and environmental applications. Complete separation of these structurally similar compounds using reversed-phase liquid chromatography has proven to be a challenge. Here we present a demonstration of the use of capillary electrochromatography on the separation of a series of 14 nitroaromatic and nitramine explosive compounds. A separation with baseline resolution is achieved for all of the compounds in under 7 min, featuring efficiencies of over 500 000 theoretical plates/m. Using more aggressive running conditions, 13 of the 14 compounds are separated in under 2 min.

The closure and remediation of former ammunition plants and military facilities requires accurate characterization of the extent of soil and groundwater contamination. The most common pollutants found at these facilities are nitroaromatic and nitramine explosives and their biological and photolytic degradation products.¹ It has been found that the distribution of contamination is often highly heterogeneous, requiring numerous samples and analyses for these sites to be adequately characterized.¹ Moreover, the identification of constituents found in blast residues is critical in forensic investigations.² A number of different methods have been applied to the analysis of explosives including gas,³ ion,⁴ liquid,^{5,6} and thin-layer chromatographies,⁷ Raman spectroscopy,⁸ and immunoassay techniques.⁹

The United States Environmental Protection Agency (EPA) specifies method 8330 for the trace analysis of explosive residues in water, soil, or sediment matrixes.¹⁰ Following sonication, extraction with acetonitrile, and preconcentration, analysis for 14 species is performed using high-performance liquid chromatography (HPLC) and UV absorption. The 14 components of method 8330 are listed in Table 1. Isocratic HPLC separations using commercially available C18 columns typically take over 30 min and are unable to separate the two aminodinitrotoluene isomers and two of the three dinitrotoluene isomers.¹¹ To fully identify each of the 14 compounds, an additional HPLC run must be performed using a cyano column, leading to an increase in analysis time and sample handling complexity. These disadvantages have led to the search for alternative liquid chromatographic techniques to the traditional isocratic HPLC separation of explosives. Emmrich and co-workers¹² investigated the use of mobile-phase gradients using a single C18 column. Using a C8 stationary phase under isocratic conditions and photodiode array detection, Bouvier and Oehrle⁵ were able to identify all of the method 8330 components in under 25 min, but were unable to achieve baseline resolution. The use of micellar electrokinetic chromatography (MEKC) was demonstrated by Oehrle,⁶ who was able to fully resolve all 14 components of the 8330 mixture in less than 15 min.

Capillary electrochromatography (CEC) can be viewed as a hybrid technique between HPLC and capillary electrophoresis (CE) where a mobile phase is driven through a stationary phase using electroosmotic flow (EOF). In CEC, neutral species are separated on the basis of differential partitioning between the mobile and stationary phases, while charged species are additionally separated according to differences in electrophoretic mobility. The concept of CEC was first demonstrated by Pretorius et al.¹³ in 1974 using 1-mm-diameter columns. The technique was further pioneered by Jorgenson and Lukacs,¹⁴ and Knox and Grant.¹⁵

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Table 1. Names and Abbreviations for 14 Explosive Compounds

compound	abbr	compound	abbr
octahydro-1,3,5,7-tetranitro-1,3,5,7 tetrazocine	HMX	methyl-2,4,6-trinitrophenylnitramine	tetryl
hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	2,6-dinitrotoluene	2,6-DNT
1,3-dinitrobenzene	DNB	2-amino-4,6-dinitrotoluene	2-Am-DNT
1,3,5-trinitrobenzene	TNB	2-nitrotoluene	2-NT
nitrobenzene	NB	4-nitrotoluene	4-NT
2,4,6-trinitrotoluene	TNT	4-amino-2,6-dinitrotoluene	4-Am-DNT
2,4-dinitrotoluene	2,4-DNT	3-nitrotoluene	3-NT

Recently, CEC has been used exclusively in a microcapillary format, taking advantage of the benefits that are offered using small-diameter columns such as reduced Joule heating, smaller sample size, and reduced solvent consumption.^{16–20}

The advantages that the use of electrokinetically driven flow offers over pressure-driven flow have been well enumerated in recent review articles.^{21–24} The absence of a significant pressure drop across the column in CEC allows for the use of longer capillaries packed with smaller particles than is typically possible using pressure-driven flow. This can lead to higher resolution separations, higher column efficiency, and shorter chromatographic run times.

CEC also offers advantages over other electrokinetically driven separations. Unlike open tube capillary electrophoresis, CEC separates neutral as well as charged species. The use of a true stationary phase in CEC rather than the pseudostationary phase used in MEKC allows for the use of a variety of specially functionalized packing materials and greater versatility in choice of mobile phases. Also, unlike MEKC, CEC can be easily coupled to a second dimension of analysis such as mass spectrometry or flame-based detection for component identification.

Here we present the results of the first application of capillary electrochromatography to the separation of the EPA 8330 mixture. Under isocratic conditions, using capillaries packed with ~20 cm of 1.5- μ m nonporous octyldecylsilica (ODS) particles and moderate electric fields (<1000 V/cm), baseline separations of all 14 of the components were achieved in under 7 min. Using shorter columns and stronger fields, 13 of the 14 components were separated in less than 2 min.

EXPERIMENTAL SECTION

Column Packing. CEC columns were produced in-house²⁵ or were obtained commercially from Unimicro Technologies, Inc. (Pleasanton, CA). Briefly, a temporary packing frit was made in one end of a 50-cm section of a fused-silica capillary (365- μ m o.d., 75- μ m i.d., purchased from Polymicro Technologies, Phoenix, AZ)

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by plugging it with a paste of 5- μ m silica particles. This was gently sintered in place using a butane microtorch. Next, a suspension of 1.5- μ m nonporous ODS II packing material (provided by Micra Scientific Inc., Northbrook, IL) in methanol was electrokinetically packed into the column. New inlet and outlet frits were made in the packed section under pressure using a resistively heated wire stripper (Teledyne Kinetics, Solana Beach, CA), and the temporary frit was removed. A 2-mm detection window was burned in the polyimide coating ~2 mm downstream of the outlet frit. Total column lengths used in this study were 25–34 cm, of which 12–21 cm was packed with the stationary phase.

Chemicals. Explosives samples (1000 mg/L in acetonitrile) were obtained from Ultra Scientific (Kingstown, RI). Methanol, 2-propanol (both HPLC grade), and 2-(*N*-mopholino)ethanesulfonic acid monohydrate (MES; >98%) were purchased from Sigma-Aldrich (Milwaukee, WI) and were used without additional purification. Electrophoresis grade (>99.5%) sodium dodecyl sulfate (SDS) was obtained from Life Technologies Inc., (Gaithersburg, MD). Water was purified using a Labconco Water Pro PS (Kansas City, MO) purification system.

CEC System. Packed capillaries were inserted into an on-column flow cell (model 9550-0155, Thermo Separation Products Inc., San Jose, CA) installed on a Linear Instruments (Fremont, CA) model 200 UV/vis detector. The flow cell contained a spherical lens which illuminated only a 100- μ m section of the capillary detection window. All measurements in this study were made using 254-nm absorption. Columns were conditioned with mobile phase using a manual syringe pump (Unimicro Technologies Inc., Pleasanton, CA). After conditioning, each end of the capillary was inserted into a vial containing mobile phase which had been degassed for at least 15 min using sonication under vacuum. Electroosmotic flow was established by applying a potential across the column using a 0–30-kV power supply (model 30R, Bertran, Hicksville, NY). The stability of the electroosmotic flow was indicated by measuring the current, which was determined by monitoring the voltage drop across a 10 000- Ω resistor in series with the capillary. Analog output from the detector was digitized and recorded with a PC-compatible computer using a program written in Labview (National Instruments, Austin, TX).

RESULTS AND DISCUSSION

Figure 1 shows a CEC separation of 14 nitroaromatics and nitramines using a 30 cm (17 cm packed) \times 75 μ m i.d. column at a running voltage of 12 kV. In capillary electrochromatography, the field strength in the open portion of the capillary is different from that in the packed portion. The latter value is generally of greater experimental interest and can be calculated according to the following method. The total column resistance (R_T) can be

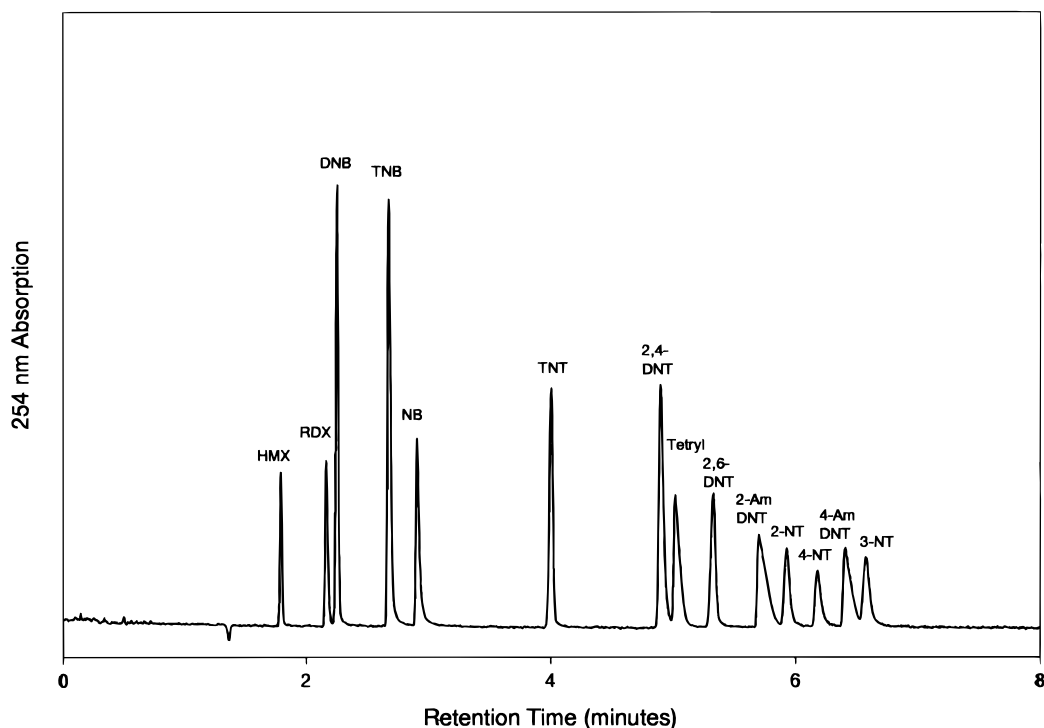


Figure 1. CEC separation of 14 explosive compounds. Column: 30 cm \times 75 μ m i.d., 17 cm packed with 1.5- μ m nonporous ODS II particles. Mobile phase: 15% methanol, 85% 10 mM MES. Running potential: 12 kV (550 V/cm in packed portion). Injection: 1 s at 2 kV of 50 mg/L (each component) sample.

Table 2. Analysis of Figure 1 Chromatogram

peak	K	no. of theor plates/m	peak	K	no. of theor plates/m
HMX	0.31	312 000	tetryl	2.67	289 000
RDX	0.58	244 000	2,6-DNT	2.90	436 000
DNB	0.65	372 000	2-Am DNT	3.18	135 000
TNB	0.96	343 000	2-NT	3.34	491 000
NB	1.13	358 000	4-NT	3.52	533 000
TNT	1.93	562 000	4-Am DNT	3.69	225 000
2,4-DNT	2.59	489 000	3-NT	3.81	457 000

determined from the current and voltage drop across the entire column (V_T). Neglecting the relatively small 10 000- Ω resistor placed in series with the column, R_T is the sum of the resistance from the packed portion (R_p) and the open portion (R_o) which are associated with potential drops V_p and V_o , respectively. The resistance per unit length of open column and, therefore, R_o can be determined by measuring the current and voltage drop across a 75- μ m-i.d. length of open capillary filled with mobile phase. R_p is the difference of R_T and R_o and can be used to calculate V_p according to

$$V_p = (R_p/R_T) V_T \quad (1)$$

V_p is divided by the length of the packed portion to give the field strength. For the separation in Figure 1, the field strength over the packed part of the column was 550 V/cm. The mobile phase was 15% methanol and 85% 10 mM MES. Table 2 shows the capacity factors for each compound and the number of theoretical plates per meter. (Theoretical plate numbers were calculated according to $N = 5.54(t_R/w_{1/2})^2$, where N is the number of

theoretical plates per column, t_R is the retention time of the analyte, and $w_{1/2}$ is the width of the peak at the half-height.) The sample was made by diluting a 1000 mg/L solution of each component in acetonitrile to 50 mg/L in the mobile phase. As had been noted earlier,^{26,27} due to the low capacities of the nonporous media, the column could be easily overloaded with analyte, adversely affecting resolution. Injections were made electrokinetically for 1 s at 2 kV, which corresponded to a total injected volume of \sim 1.5 nL.

Since each of the analytes is neutral over the pH range examined (6–8.5), none of the separation was due to differences in electrophoretic mobility. Although the rate of EOF is known to be dependent on the pH of the mobile phase due to its effect on the extent of dissociation of surface silanol groups,²⁸ no significant effects on the separation were observed on changing the pH from 6 to 8.5. EOF theory predicts that the thickness of the electrical double layer should increase with decreasing buffer ion concentration, leading to a greater EOF velocity. A small increase in speed was indeed observed with a lower ion concentration in the mobile phase. This, however, came at the expense of a loss of resolution. The factor that had the biggest effect on the separation was the type and concentration of organic additive in the mobile phase. As observed in previous studies of HPLC separations with nitroaromatic compounds using C18 stationary phases, methanol was the mobile-phase modifier that gave the

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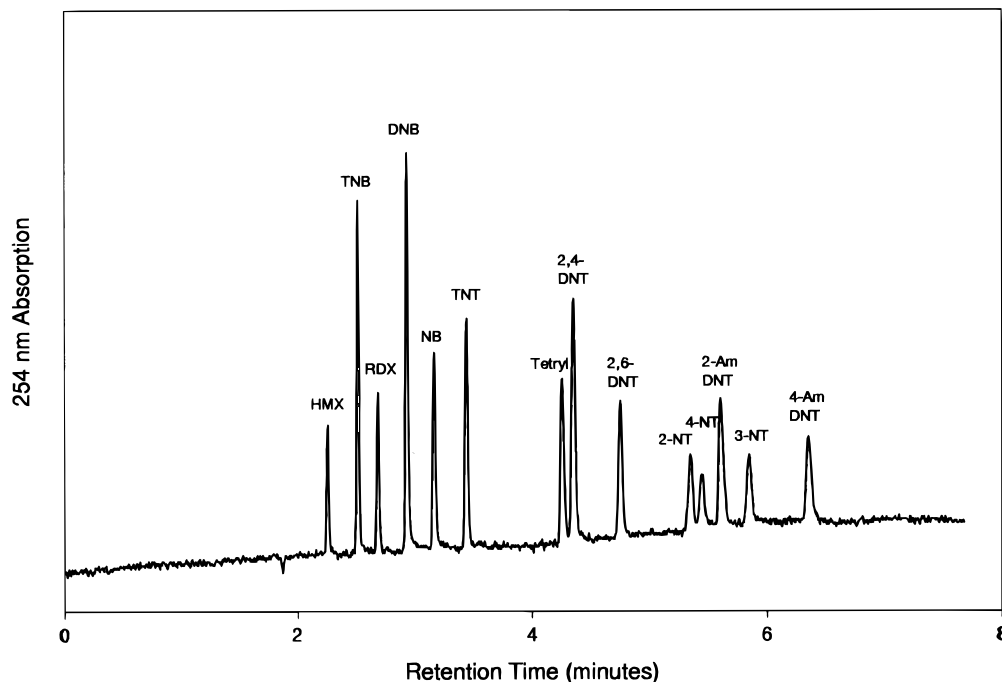


Figure 2. CEC separation of explosives with addition of SDS to mobile phase. Column: 34 cm \times 75 mm i.d., 21 cm packed with 1.5- μ m nonporous ODS II particles. Mobile phase: 20% methanol, 80% 10 mM MES, 5 mM SDS. Running potential: 12 kV (480 V/cm in packed portion). Injection: 2 s at 2 kV of 12.5 mg/L (each component) sample.

best overall separations.¹² Acetonitrile is known to have a higher elution strength than methanol. It was observed that addition of acetonitrile slightly increased the rate of EOF and significantly decreased the retention time, but had a generally deleterious effect on the resolution. Close examination of the peak shapes of Figure 1 reveals some tailing of the amino-substituted nitrotoluenes. The extent of the tailing was affected by the mobile phase, but its source, especially in light of the other rather symmetric peaks, is not entirely clear. It is possible that the polar amino groups interact more strongly with the non-end-capped silica surface of the stationary phase than the other compounds.

The number of theoretical plates shown in Table 2 is on the order of those obtained by Dadoo et al.²⁷ in their study of polycyclic aromatic hydrocarbons (PAHs) using 1.5- μ m nonporous packing and postfrit detection. Those authors noted that this number can be increased by a factor of ~ 2 by detecting before the frit in the packed part of the column, something that was not attempted in this study.

Due to the particularly nonwetting characteristics of the stationary phase and the highly aqueous mobile phase necessary for a baseline resolution, the CEC separations of explosives were occasionally hampered by the drying out of the capillary. This is a commonly encountered problem in CEC and is traced to bubble formation, which slows or stops the electroosmotic flow.²¹ Bubble formation most frequently occurs at irregularities in the stationary-phase packing which can lead to nonuniform flow and localized heating. This occurs particularly at frits where sintering decreases the flow channels between particles and alters the surface coating. The problem of bubble formation is exacerbated by a number of factors such as the use of a highly polar mobile phase, a particularly nonwetting stationary phase, and excessive

Joule heating due to the use of high running voltages or highly conductive buffers.²⁹

A number of techniques have been examined to address these difficulties. One is to use an increased organic content in the mobile phase. However, this typically decreases the retention time, leading to a faster separation and an unacceptable loss of resolution. Several groups have pressurized the buffer vials to suppress bubble formation.^{30–32} This, however, leads to increased operating complexity, particularly in performing injections. Another approach is to add a surfactant such as SDS to the mobile phase.³³ It is believed that the SDS molecules are adsorbed onto the surface of the reversed-phase packing, changing the osmotic flow characteristics. Further, the use of a surfactant decreases the surface tension at the solid–liquid interface, leading to increased wetting on the surface of the particles.

It was found that a 1–5 mM addition of SDS to the mobile phase aided in maintaining a consistent EOF. Figure 2 shows a separation of the 14 explosive compounds using a mobile phase in which 5 mM SDS had been added. The critical micelle concentration (cmc) of SDS in pure water at room temperature is 8 mM,³⁴ so it is unlikely that the separation in Figure 2 is due to micellar partitioning. The capacity factor and number of theoretical plates for each compound are listed in Table 3. Comparing Figures 1 and 2, it can be seen that there are shifts in the order of elution for some of the components. This is in

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Table 3. Analysis of Figure 2 Chromatogram

peak	<i>K'</i>	no. of theor plates/m	peak	<i>K'</i>	no. of theor plates/m
HMX	0.20	357 000	2,4-DNT	1.32	450 000
TNB	0.34	395 000	2,6-DNT	1.53	537 000
RDX	0.43	386 000	2-NT	1.85	500 000
DNB	0.56	363 000	4-NT	1.90	396 000
NB	0.69	464 000	4-Am DNT	1.91	420 000
TNT	0.83	501 000	3-NT	2.12	361 000
tetryl	1.27	431 000	4-Am DNT	2.38	426 000

contrast to the behavior observed by Seifar and co-workers,³³ who observed very little change in capacity factor upon addition of SDS. These authors, however, were using much more strongly eluting mobile phase containing 60% acetonitrile. Since it is known that the adsorption of SDS molecules to surfaces is highly dependent on the nature of the mobile phase, these differences are not entirely unexpected.³³

The use of nonporous stationary phases in CEC offers not only highly efficient separations but also very rapid analyses. In studying various mobile phases for the separation of explosive compounds it was noted that 2-propanol greatly reduced the retention time of the components with a partial loss of resolution. The use of a combination of 2-propanol and methanol in the mobile phase along with an increased field strength gave the chromatogram shown in Figure 3. Two of the isomers of nitrotoluene are no longer fully resolved, but what is noteworthy is that the entire separation took less than 2 min.

Dadoo et al.²⁷ have previously demonstrated remarkable CEC separations of five PAHs in under 5 s using short columns and high (2800 V/cm) electric fields. The separation shown in Figure 3 suggests that similar results should be attainable for the explosive compounds. In performing very rapid separations using short columns and high voltage injections, column manipulation and detection become awkward. Analyses of this type beg to be carried out on an integrated structure where fluids can be easily

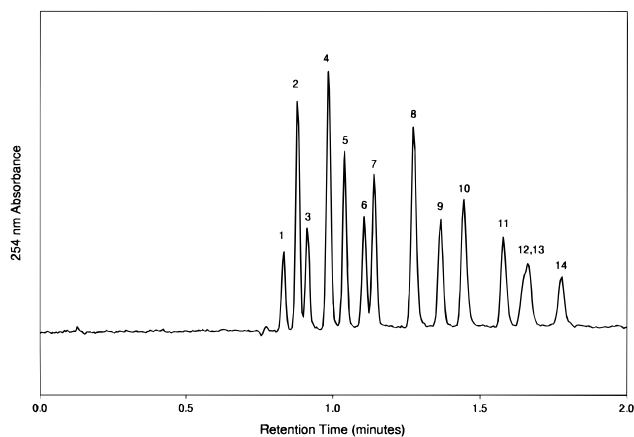


Figure 3. Rapid CEC separation of explosives. Column: 25 cm × 75 μm id, 12 cm packed with 1.5-μm nonporous ODS II particles. Mobile phase: 7.5% methanol, 7.5% 2-propanol, 85% 10 mM MES, 5 mM SDS. Running potential: 11 kV (690 V/cm in packed portion). Injection: 1 s at 2 kV of 12.5 mg/L (each component) sample. Peaks: (1) HMX, (2) TNB, (3) RDX, (4) DNB, (5) NB, (6) TNT, (7) Tetryl, (8) 2,4-DNT, (9) 2,6-DNT, (10) 2-NT, (11) 4-NT, (12) 2-Am-DNT, (13) 3-NT, and (14) 4-Am-DNT.

manipulated using controlled electric fields. Using precisely machined microchannels, further beneficial effects of microscale separations may be gleaned. Efforts toward this goal are currently under way in our laboratory.

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