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Rapid Screening of Selected Organic Explosives by High Performance Liquid Chromatography Using Reversed-Phase Monolithic Columns

ABSTRACT: This study presents the rapid screening of various high grade explosives by high performance liquid chromatography (HPLC) with monolithic stationary phases. Two gradient methods were developed, the first for quantitative analysis of eleven explosives: HMX; RDX; Tetryl; TNT; 2,3-DNT; 2,6-DNT; 3,4-DNT; 2-NT; 3-NT; 4-NT; and PETN in under 14 min. The second method separated seven explosives in under two min and is suitable for rapid screening to determine the presence of specific and/or class of explosive. The rapid screening methods were successfully applied to soils spiked with known amounts of target explosives. This technology showed excellent potential for forensic explosives detection and analysis.

KEYWORDS: forensic science, explosives, screening tests, high performance liquid chromatography, monolithic column

The need for forensic laboratories to detect and identify explosive materials is most often derived from incidents involving improvised explosive devices or the presence of residues of explosive compounds on a person or object. In the former case (post-blast situation) the zone of interest may be spread over a large area, so many samples may need to be collected and screened for unreacted residues. In the latter case (pre-blast situation) it may be desirable to have the ability to screen large numbers of people for explosive residues during vulnerable public events or places considered potential targets of terrorist attack. Therefore there is an immediate need for a technique to rapidly screen many samples for the presence of explosives residues. This need has been emphasized by recent worldwide events, including the bombings which occurred on the Indonesian island of Bali in 2002.

Some techniques used for the analysis of explosives include Thin Layer Chromatography (TLC), Gas Chromatography (GC), Infra-Red Spectroscopy (IR), Capillary Electrophoresis (CE), and High Performance Liquid Chromatography (HPLC). TLC has the advantages of low cost, low solvent consumption and moderate analysis time. However, the sensitivity of detection is often too low for the trace analysis of some explosives (1), and one cannot unambiguously detect explosives such as nitroglycerine and pentaethythritol tetranitrate (PETN) (2–4). TLC is generally used as a part of clean up procedures or as a screening test (5), but it is not considered a definitive test.

Gas Chromatography has high resolution and the ability to use a variety of detection methods, including chemiluminescence, mass spectrometry, electron capture, and flame ionization (1). This detection versatility is offset by the instability of explosives at high temperatures, leading to the requirement of precise operating conditions. Analysis times may be as long as 20–30 min duration, a significant disadvantage when rapid confirmation is required.

IR spectroscopy has been demonstrated as a suitable method for the identification of relatively pure explosives, but trace amounts of contaminants present in samples of forensic interest can obscure the spectra. In addition, mixtures of explosives may also cause problems (5).

CE in the micellar electrokinetic chromatographic (MEKC) mode can also be used for the analysis of explosives. Some advantages of MEKC are: high efficiency separations with moderate analysis times of approximately 10–15 min (6,7); less reagent use; and very small sample sizes, usually in the order of nanoliters. However, MEKC suffers from poor migration time reproducibility and questionable quantitation (8). MEKC has good mass sensitivity but lacks in concentration sensitivity in comparison to other methods (9).

HPLC is an excellent alternative method for the analysis of explosives. This is primarily because the analysis can be conducted at room temperature, resolving the problem of thermal instability encountered in GC. Detection systems are selective and of sufficient sensitivity to detect explosives in typical samples of forensic interest. Current separations are of similar analysis times as GC, hindering the use of HPLC for rapid screening.

In efforts to speed up liquid chromatographic separations, the usual approach is to use shorter columns containing increasingly smaller stationary phase particles. However, such traditional particle packed LC columns are limited to 1.5 μm particle size due to the high back-pressures generated even at low to moderate flow rates. This means shorter columns are necessary to counter this restriction, although this also means less theoretical plates (N). Silica based monolithic stationary phases have been developed recently to overcome this restriction. Such monolithic phases have been promoted as allowing increased mobile phase flow rates to be used without the usual build-up of restrictive column back-pressures associated with particle packed LC columns (10). Monolithic columns are formed from a single rod of porous silica and consist of a small sized

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silica skeleton (0.3–5 μm) and relatively large flow through pores (0.5–8 μm). The low pressure drop generated across the monolith means multiple columns can be used in-line to increase N, and moderate to high mobile phase flow rates also can be used to increase sample throughput.

This study investigated the rapid separation of 11 commercial and military grade explosives by HPLC utilizing monolithic reversed phase columns of various lengths.

Materials and Methods

Instrumentation

Separations were carried out using a Waters 2690 equipped with photodiode array detector. The analytical separation columns were a 50 × 4.6 mm Chromolith SpeedROD RP-18e (Merck) and a 100 × 4.6 mm Chromolith Performance RP-18e (Merck). For the work requiring a 150 mm column, the above two monoliths were coupled together using a Chromolith Column Coupler (Merck). A 1.8 μm Zorbax Stable Bond Rapid Resolution HT column (Agilent Technologies) was also used for comparisons of plate height.

Reagents

Mobile phases were prepared using deionised water from a Milli-Q water purification system. HPLC grade MeCN and MeOH were obtained from Sigma. Prepared mobile phases were filtered using 0.45 μm nylon filters and degassed using sonication.

The explosives were purchased from either Radian International or Alltech as either a solid, liquid, or standard solution in acetonitrile. Eleven explosives were chosen for the analysis and are given in Table 1. These explosives were selected primarily due to their availability and provided a range of both high and low explosives.

Soil Extractions

Soil samples were collected and ground using mortar and pestle. One g of each soil sample was dried at ∼50°C for 1 h. Each soil was then spiked with 20 μg of each explosive. The spiked soils were then thoroughly mixed and re-dried at ∼50°C for another 1 h, followed by mixing with 2 mL of MeCN. The 2 mL volumes of MeCN containing the spiked soils were then sonicated for 30 min.

TABLE 1—*Abbreviation and classification of selected explosives.*

Explosive	Abbreviation	Classification and Use
2,3-Dinitrotoluene	2,3-DNT	Propellant, nitroaromatic,
2,6-Dinitrotoluene	2,6-DNT	constituent in smokeless
3,4-Dinitrotoluene	3,4-DNT	powder
2-Nitrotoluene	2-NT	Nitroaromatic
3-Nitrotoluene	3-NT	
4-Nitrotoluene	4-NT	
Octohydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX	Organic high explosive,
	RDX	nitramine, military
Hexahydro-1,3,5-trinitro-1,3,5-triazine		
2,4,6,N-Tetranitro-N-methylaniline	Tetryl	Organic high explosive, nitroaromatic, military
2,4,6-Trinitrotoluene	TNT	Organic high explosive, nitroaromatic, military
Pentaerythyritol Tetranitrate	PETN	Organic high explosive, nitrate ester, military

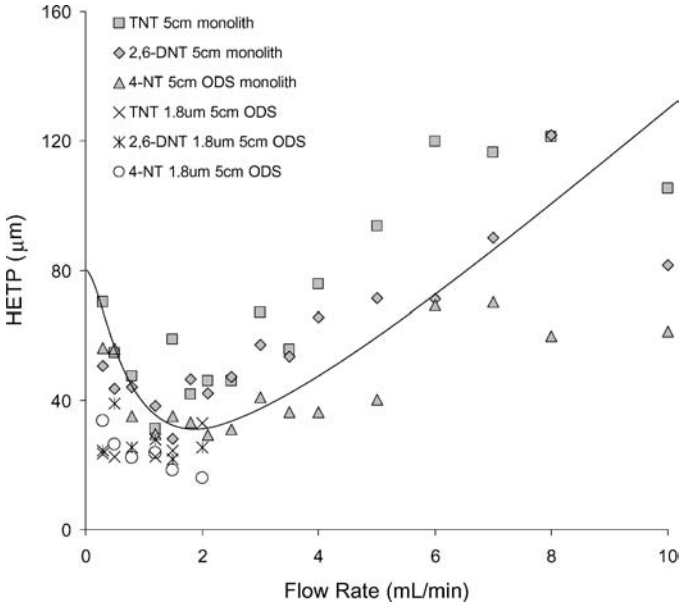

FIG. 1—*Van Deemter plot showing peak efficiency as a function of mobile phase flow rate for monolithic and particle type stationary phases. Conditions: Mobile phase = 35:75 MeOH: water. The trend line is a line of sight fit for monolithic data.*

Following sonication, the MeCN extracts were filtered with 0.45 μm filters, and each were injected subsequently.

Results and Discussion

Efficiency of Short Monolithic Silica Columns

According to LC plate theory, mobile phase flow rate has a significant effect upon plate height (HETP), and this is commonly illustrated graphically using a van Deemter plot. As monolithic columns have a much larger flow rate range than conventional particle packed columns, it is necessary to identify flow rates that could be used for rapid sample screening before unacceptable losses in efficiency. A van Deemter plot (see Fig. 1) was constructed for a short reversed-phase 5 cm Octadecyl Silica (ODS) monolithic column for three commercial explosives, TNT; 2,6-DNT; and 4-NT, under isocratic conditions using a 35:75 MeOH: Water mobile phase. Figure 1 also shows the plate height under identical conditions for a 5 cm 1.8 μm ODS particle packed reversed-phase column, to demonstrate the relative efficiencies of the two column types of similar length.

The mobile phase flow rate was increased from 0.2–10 mL/min ($n = 16$) using the monolithic column. At the maximum flow rate of 10 mL/min, the back-pressure was 2500 p.s.i., which is comparable to that found with a standard 25 cm 5 μm particle type reversed-phase column at 1–2 mL/min (as commonly used for explosive analysis). For the monolithic column, maximum efficiency (N) was found between the flow rates of 1.5 and 2.5 mL/min, illustrating how the column could be used at these elevated flow rates to increase sample throughput without loss in peak resolution.

At 2 mL/min the average number of theoretical plates for the monolithic phase was approximately 31 250 plates/m, decreasing to approximately 17 000 plates/m at a flow rate of 5 mL/min. This compares reasonably well to the highest average plate count seen with the 1.8 μm particle packed column, which was approximately 46 000 plates/m at an optimum flow rate of 1.5 mL/min. A number

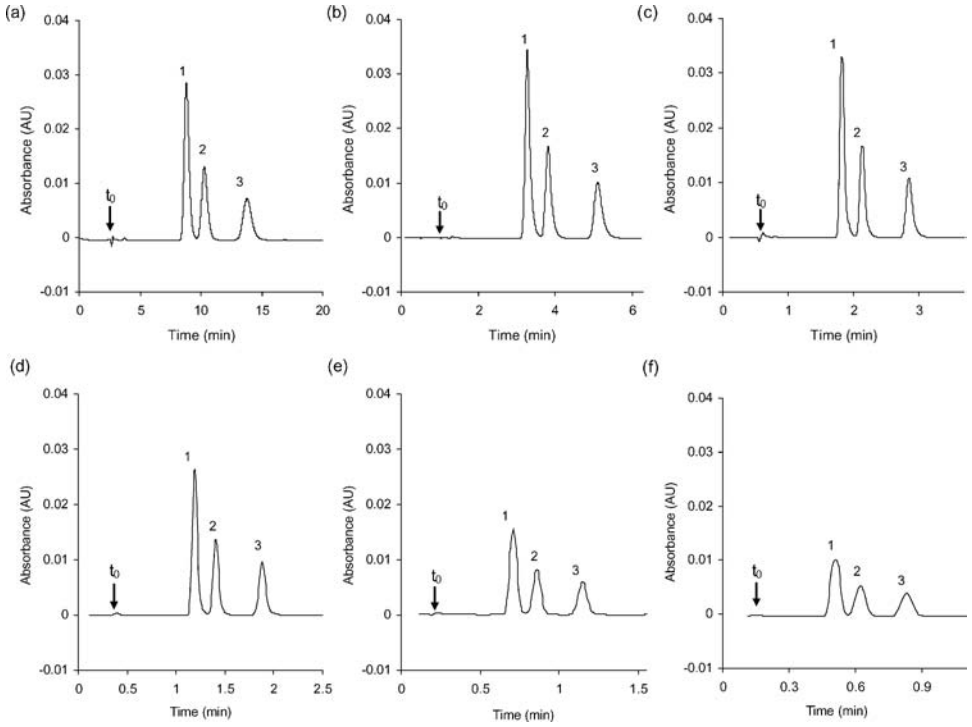

FIG. 2—*Chromatograms showing the separation of* (1) *TNT;* (2) *2,6-DNT; and* (3) *4-NT using a 5 cm ODS monolith at flow rates of* (a) *0.3,* (b) *0.8;* (c) *1.5,* (d) *2.5,* (e) *5.0, and* (f) *8.0 mL/min. Conditions: Mobile phase = 35:75 MeOH: water, Column = Chromolith Speed ROD RP-18e (5 cm ODS monolith).*

of chromatograms obtained using the monolithic column in the above study are shown in Fig. 2.

As can be seen from Fig. 2, the resolution of the three explosives is maintained even at flow rates of >8 mL/min, although detector response clearly decreases dramatically at the higher flow rates. However, the decrease in detector response with increasing flow rate is partially offset by the improvement in efficiency seen when working at the optimal flow rate. Hence at a flow rate of 1.5 mL/min, peak heights are equal to those seen at 0.8 mL/min and greater than those observed at 0.3 mL/min.

Rapid Gradient Separation of Commercial Explosives

Due to the large variation in structures between different classes of the target explosives and close similarities in structures within each class (including structural isomers), gradient elution was required to achieve separations of more than 3–4 commercial explosives in a single analysis and to maintain reasonable runtimes.

Initial work investigated simple MeCN: water gradients using the 5 cm ODS monolithic column. However, under identical conditions, resolution of later eluting peaks was much improved when using MeOH in place of MeCN. Therefore MeOH was used for subsequent separations; despite the higher background absorbance at 210 nm for MeOH. Two mobile phase solutions were prepared, solvent A = 10% MeOH and 90% water, and solvent B = 70% MeOH and 30% water. Using a linear gradient of 90(A):10(B) to 75(B): 25(A) over 10 min at room temperature at a moderate flow rate of 3 mL/min, the separation of HMX (0.72 min); RDX (1.27 min); TNT (3.62 min); Tetryl (3.86 min); 2,3-DNT (4.35 min); 3,4-DNT (4.86 min); 2-NT (5.17 min); 4-NT (5.45 min); 3-NT (5.59 min); and PETN (5.81 min), was possible in under 6 min. However, under these conditions all four isomers of DNT could not be separated, with 2,3-DNT; 2,4-DNT; and 2,6-DNT co-eluting.

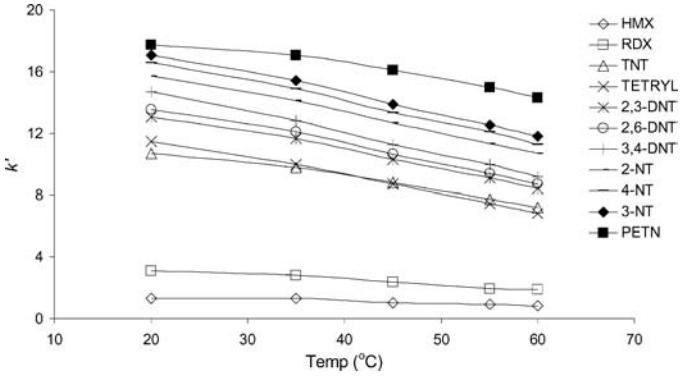

FIG. 3—*Effect of column temperature on retention (capacity factor, k′) of commercial explosives on 5 cm ODS monolithic column. Conditions: Mobile phase gradient = 90(A):10(B) to 75(B):25(A) over 10 min, flow rate of 3 mL/min (solvent A = 10% MeOH, 90% water and solvent B = 70% MeOH, 30% water).*

Effect of Temperature

In an attempt to improve resolution of co-eluting isomers, column temperature was investigated over the range 20–60°C. Increasing temperature caused a decrease in retention for all explosives of between 15–30%. However, there was no improvement in the resolution of the above three isomers, although removal of 2,4-DNT from the mixture did allow the baseline separation of the remaining three DNT isomers. The effect of temperature upon the separation is shown as Fig. 3.

As can be seen from Fig. 3, there is an improvement in the resolution of 3-NT and PETN at higher column temperatures and a reversal of retention order seen for TNT and Tetryl. The use of elevated column temperature also led to a slight increase in peak

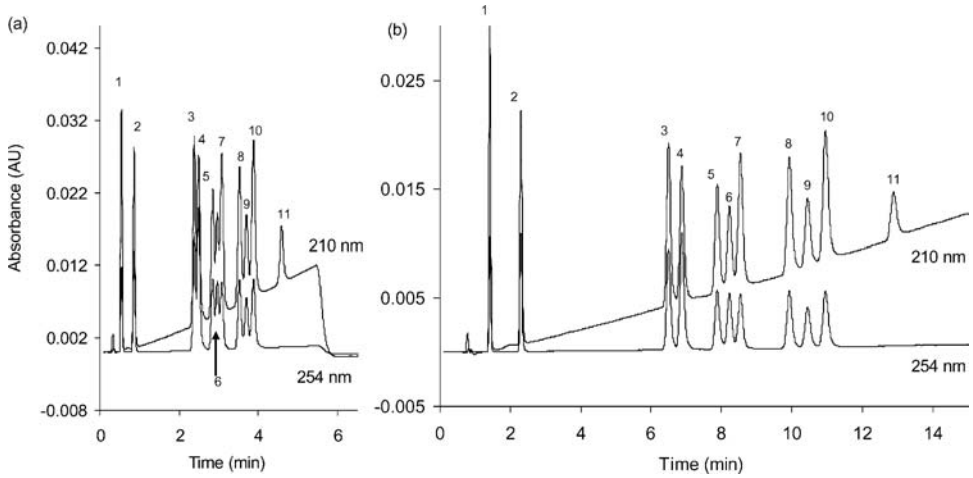

FIG. 4—*Chromatograms showing the separation of 11 commercial explosives on a:* (a) *5 cm ODS monolith and* (b) *15 cm ODS monolith. Peaks = 1 = HMX; 2 = RDX; 3 = Tetryl; 4 = TNT; 5 = 2,3-DNT; 6 = 2,6-DNT; 7 = 3,4-DNT; 8 = 2-NT; 9 = 4-NT; 10 = 3-NT; 11 = PETN. Conditions:* (a) *As Fig. 3, except column temperature = 60°C;* (b) *Gradient from 90*(A)*:10(B) to 75(B):25(A) over 30 min, flow rate = 3 mL/min and column temperature = 60°C.*

efficiency for several explosives, and so a temperature of 60°C was used for the remainder of this study.

Length of Monolithic Column

Three column lengths were investigated at the moderate flow rate and elevated column temperature using the above gradient. Monoliths of 5 cm, 10 cm, and 15 cm length were used, which at a flow rate of 3 mL/min produced back-pressures of only 555, 658, and 863 psi respectively. Separation of 11 explosives in under 4.5 min was possible on the short 5 cm monolith, although baseline resolution was not complete for several peaks. Total run times of 7 min (10 cm monolith) and 9 min (15 cm monolith) resulted from the use of the longer columns, although using the same rapid gradient, resolution of peaks was only marginally improved. However, for rapid screening purposes, resolution of the 11 explosives was acceptable, and Fig. 4*a* shows the separation obtained and monitored at both 254 nm and 210 nm (required to see PETN).

In order to achieve baseline resolution for quantitative analysis, a slower gradient program was used with the 15 cm monolithic column. This involved a gradient from 90(A):10(B) to 75(B):25(A) over 30 min. Flow rate was maintained at 3 mL/min, and column temperature kept at 60°C. The resultant chromatogram is shown as Fig. 4*b*, again monitored at both 254 and 210 nm. As can be seen from the chromatograms shown, the slower gradient produced a baseline separation of all 11 explosives in only 13 min. This method could be therefore used to quantitate peaks identified using the previous rapid screening method.

Separation of Seven Explosives in Under Two Minutes

To illustrate the potential for ultra-rapid multi-analyte sample screening, a short monolith (5 cm) was used with an elevated flow rate and rapid gradient program. This involved a gradient from 90(A):10(B) to 75(B):25(A) over just 5 min combined with a flow rate of 8 mL/min and a column temperature of 60°C. Under these conditions it was possible to obtain the baseline separation of HMX (18 s), RDX (24 s), TNT (69 s), 2,4-DNT (81 s), 2-NT (96 s), 3-NT (105 s), PETN (117 s) in under 2 min, including resolution from the solvent dip (9 sec). Under these conditions, peak baseline widths ranged between 7 and 10 s, and so a high detector sampling frequency (>10 Hz) was required. The resultant separation is shown as Fig. 5. To the authors' knowledge this separation represents by far the fastest practical separation of this many explosives. An added advantage of the high flow rate possible with the monolithic phases

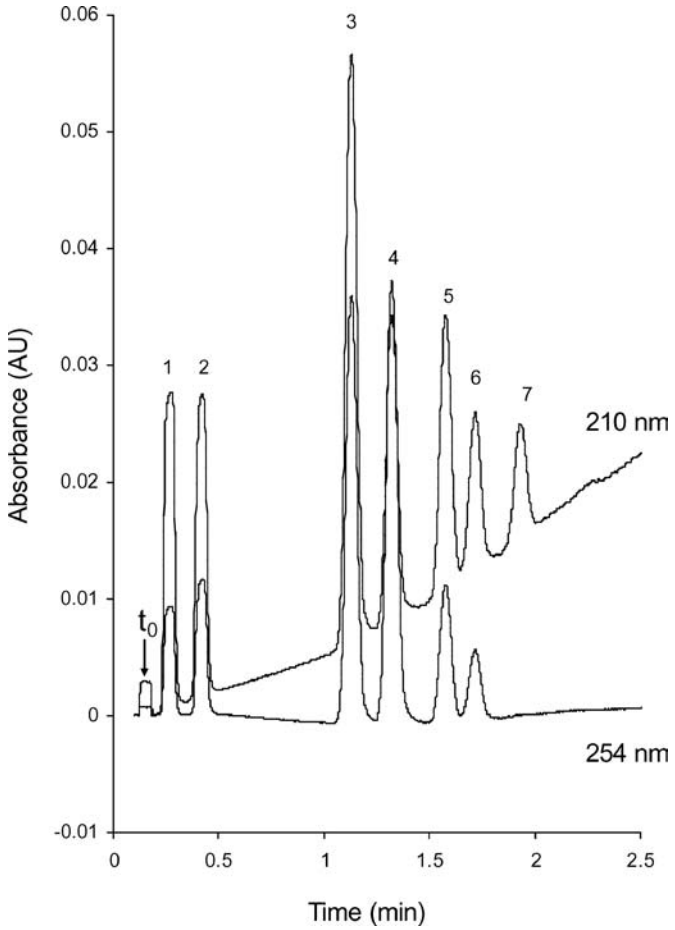

FIG. 5—*Chromatograms showing the rapid separation of 7 commercial explosives in under 2 min on a 5 cm ODS monolithic column. Peaks = 1 = HMX; 2 = RDX; 3 = TNT; 4 = 2,4-DNT; 5 = 2-NT; 6 = 3-NT; 7 = PETN. Conditions: Gradient from 90(A):10(B) to 75(B):25(A) over 5 min, flow rate = 8 mL/min and column temperature of 60°C.*

TABLE 2—*Method reproducibility (%RSD) based on 6 repeat injections.*

	Explosive										
	HMX	RDX	Tetryl	TNT	2,3-DNT	2,6-DNT	3,4-DNT	2-NT	4-NT	3-NT	PETN
Retention Time	0.23	0.23	0.39	0.34	0.36	0.33	0.36	0.33	0.35	0.34	0.30
Peak Area	2.18	2.17	2.23	1.10	1.50	2.37	2.35	1.28	1.43	1.48	2.56
Peak Height	2.08	2.25	1.97	1.81	1.48	1.19	1.63	1.25	1.31	1.15	1.66

Conditions: MeOH gradient over 5 min; using 5 cm ODS monolith.

is that re-equilibrating the column at the end of each run takes under 1 min. Therefore, under the above conditions the column can be re-equilibrated and ready for the next injection after only 180 s, meaning potentially 20 samples/h could be screened.

Analytical Performance Data

The analytical performance was assessed using a rapid gradient from 90(A):10(B) to 45(B):55(A) over 5 min and a flow rate of 3 mL/min with the 5 cm monolith. Table 2 shows the reproducibility data for 11 explosives determined from 6 repeat injections of a mixed 20 mg/L standard. As can be seen from Table 2, retention time precision was <0.4% for all 11 explosives. Peak area and height precision was <2.5%, again for all 11 peaks.

Method linearity was determined for a range of explosives over both high and low concentration ranges. Table 3 shows r^2 values obtained for calibrations over 2–20 mg/L ($n = 5$) and over the range 40–320 μg/L ($n = 5$). Over the lower concentration range, a 10 μL injection volume was used with no effect upon peak efficiency. Excellent linearity was seen for all explosives.

Using the 10 μL injection, volume detection limits were calculated for all 11 explosives. These ranged from 21–94 μg/L at 210 nm and 20–55 μg/L at 254 nm (excluding PETN). These values are shown in Table 4 and were determined using peak height equivalent to 3× the measured baseline noise. Detection limits were also determined using the slower method developed with the 15 cm monolith, as described above. The longer column allowed increased sample volumes to be injected (25 μL). However, detection limits for most explosives were slightly higher due to broader peaks.

Extraction and Rapid Analysis of Spiked Soil Samples

To illustrate the potential of the rapid LC method for real sample screening, a number of soil samples were spiked with traces of various commercial explosives. These spiked soils were then ground, dried, and extracted with MeCN using sonication as described in Section 2.3. Four soil samples were spiked with 2–3 explosives each at concentrations of 20 μg/g. Recoveries of the extracted explosives were calculated against a 5 μg/mL standard. Each sample was initially screened on the 5 cm monolith using a rapid isocratic run with a 40% MeOH mobile phase and a flow rate of 4 mL/min. The screening of each soil extract in this way took approximately 90 s per sample. This method allowed the very rapid identification of the presence of members of each class of explosive, e.g., Tetryl, TNT, DNT, or NT. Following this a rapid gradient method was used with each extract that allowed the separation and identification of each explosive present. The gradient used was from 90(A):10(B) to 55(A):45(B) over 5 min, followed by equilibrating the column back to 90(A):10(B) over 5–6 min at a flow rate of 3 mL/min using the 5 cm monolith. The results of this study (in terms of recoveries for spiked explosives) are shown in Table 5.

TABLE 3—*Method linearity, determined using peak area for mixed standards (*n = *5) over both high (2–20 mg/L) and low (0.04–0.32 mg/L) concentration ranges.*

	Linearity (r^2)	
	2–20 mg/L 5 μL inj. vol.	0.04–0.32 mg/L 10 μL inj. vol.
HMX	0.9983	0.9985
RDX	0.9993	0.9578
Tetryl	0.9989	0.9909
TNT	0.9998	0.9981
2,3-DNT	0.9997	
2,6-DNT	0.9996	
3,4-DNT	0.9985	
2-NT	0.9996	
4-NT	0.9983	

TABLE 4—*Comparison of detection limits for 11 explosives using the rapid and standard gradient methods,* expressed as μg/L.*

	5 cm monolith–10 μL inj.		15 cm monolith–25 μL inj.	
	210 nm	254 nm	210 nm	254 nm
HMX	23	38	29	46
RDX	21	29	25	36
Tetryl	27	32	36	33
TNT	32	20	39	25
2,3-DNT	43	39	49	51
2,6-DNT	52	40	63	52
3,4-DNT	38	43	41	57
2-NT	41	42	39	45
4-NT	59	55	72	59
3-NT	29	42	35	45
PETN	94	ND	104	ND

* Detection limits calculated using peak height equivalent to 3× baseline noise.

TABLE 5—*% recoveries using solvent extraction under sonication for spiked* soil samples.*

	Sample Peak Area	Std. Peak Area (5 μg/mL std.)	% Recovery
Soil 1			
TNT	44930	24820	90.5
3-NT	28553	14694	97.2
Soil 2			
3,4-DNT	20485	12161	84.2
Tetryl	31409	16991	92.4
Soil 3			
2,4-DNT	49019	...	...
3-NT	23473	14694	79.9
Tetryl	34762	16991	102.3
Soil 4			
TNT	39530	24820	79.6
3,4-DNT	20681	14694	70.4

* Soils spiked at 20 μg/g.

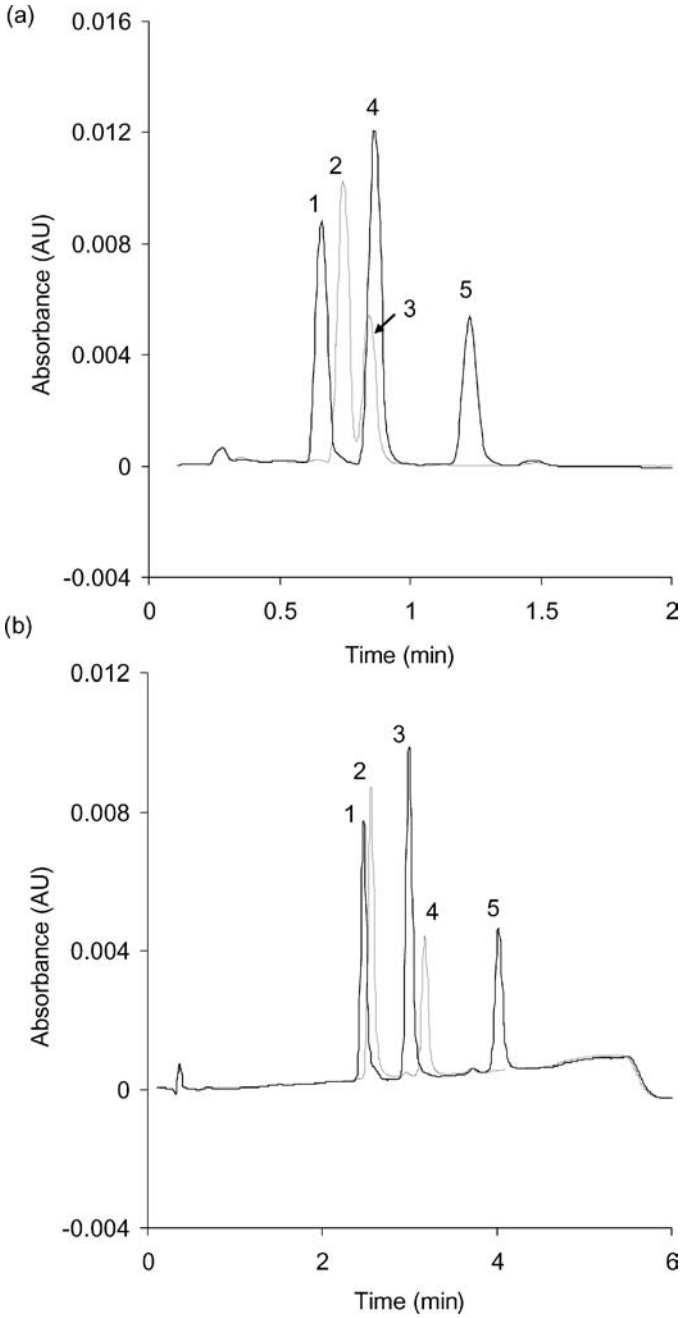

FIG. 6—*Overlaid chromatograms of soil extracts (soil no. 3 and no. 4). Conditions:* (a) *Column = 5 cm ODS monolith, mobile phase = 40% MeOH, flow rate = 4 mL/min, column temp = 60°C;* (b) *Column = 5 cm ODS monolith, mobile phase gradient from 90(A):10(B) to 55(A):45(B) over 5 min, 90(A):10(B) over 5–6 min, flow rate = 3 mL/min. Peaks = 1 = Tetryl; 2 = TNT; 3 = 2,4-DNT; 4 = 3,4-DNT; 5 = 3-NT.*

The recoveries for spiked explosives were all greater than 70% (TNT-79–91%, 3-NT-80–97%, 3,4-DNT-70–84%, Tetryl-92–102%). Typical chromatograms obtained for the soil extracts using both the rapid isocratic and gradient methods are shown in Fig. 6*a* and *b*. Two soil extracts have been overlaid in each figure. As can be seen from these chromatograms (which were monitored at 254 nm), the extracts were remarkably free of interfering species co-extracted from the soil samples, illustrating the clear potential of this rapid method for such an application.

Conclusions

Monolithic reversed phase columns were productively employed to provide a means for the rapid screening of commercial and military grade explosives. A fast gradient method was developed that separated seven explosives in under 2 min. This method could be used to identify the presence of classes of explosives and is capable of screening approximately 20 samples per h. Detection and quantitation of individual explosives was achieved by employing a slower gradient that allowed baseline resolution of all 11 target explosive compounds in less than 13 min, allowing unambiguous identification of the type of explosive.

The method was successfully employed to determine the presence of explosives in spiked soil samples with recoveries of approximately 70%, demonstrating the applicability of the method to real samples.

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