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Note

Packed-column supercritical fluid chromatographic separation of highly explosive compounds

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Supercritical fluid chromatography (SFC) is complementary to gas chromatography (GC) and high-performance liquid chromatography (HPLC) because of its ability to mobilize compounds not readily chromatographed by GC and the greater ease by which it can be interfaced with GC-like ionization detectors and mass spectroscopy[1,2] than can HPLC. A case in point is highly explosive compounds, including nitrosubstituted aromatic hydrocarbons, nitramines, and nitroesters. The thermal lability of these compounds limits the applicability of GC and they are traditionally analyzed using reversed-phase HPLC[3]. The feasibility of applying SFC to high explosives is suggested by the work of West and Lee[4] in the separation of nitro-substituted polycyclic aromatic hydrocarbons and the separation of high molecular weight, nitro-substituted dyes by Jackson and Later[5] using capillary column SFC with carbon dioxide and nitrogen-selective thermionic detectors or pentane and UV absorbance detection, respectively. Chromatograms showing the SFC of several high explosives have been published in a book of chromatograms[6] from the 1988 Workshop on Supercritical Fluid Chromatography. As far as we can determine, this paper is the first report of a packed-column SFC separation of highly explosive compounds and their manufacturing byproducts, and the first report of the SFC of N-methyl-N,2,4,6-tetranitroaniline and pentaerytheritoltetranitrate. The results indicate that SFC can supplant HPLC as an analytical tool for high explosives.

EXPERIMENTAL

Equipment

The SFC system was a Suprex (Pittsburgh, PA, U.S.A.) Model SFC 200A equipped with an electrically actuated 1-μl Valco injection valve, a 250 mm × 1 mm I.D. Deltabond Cyano column with 5-μm particles (Keystone Scientific, State College, PA, U.S.A.), a Kratos Model 757 variable-wavelength UV absorbance detector with a 12-μl flowcell, and a flame ionization detector arranged in series. A 150-mm column of Deltabond Cyano phase also was used in some experiments. The injection valve was

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maintained at room temperature by pumping water through the cooling jacket with a laboratory pump. A tapered restrictor was drawn at the tip of the 25 μm I.D. fused-silica flame ionization detector interface tubing to achieve a gaseous flow-rate of *ca.* 30 ml/min, and a breakthrough time of 1.12 min with carbon dioxide at 162 atm and 100°C. The flame ionization detector was held at 350°C. SFC was performed using carbon dioxide at a column oven temperature of 100°C and a pressure program of 162 atm (0.607 g/cm^3) held for 1 min, programmed to 250 atm (0.822 g/cm^3) over 13 min (6.77 atm/min), held at 250 atm for 1 min, and programmed to 350 atm (0.906 g/cm^3) over 15 min (6.67 atm/min). Only the UV absorbance detector was used in this work. The 1-V signal of the UV detector output was collected with an IBM XT personal computer equipped with a Cyborg (Newton, MA, U.S.A.) 41I interface and an I150 16-bit board, and was processed using Maxima 2.0 software (Dynamic Solutions, Ventura, CA, U.S.A.). The data collection rate was 3 points/s.

Materials

The SFC-grade carbon dioxide was purchased from Scott Specialty Gases (Plumstead, PA, U.S.A.) in an aluminum cylinder with a helium-pressurized headspace, and was used as received. All highly explosive compounds and byproducts were obtained from the Picatinny Arsenal (Dover, NJ, U.S.A.) in their Standard Analytical Reference Material grade. The compounds and their abbreviations are 2,6-dinitrotoluene (2,6-DNT), 2,4-dinitrotoluene (2,4-DNT), 2,4,6-trinitrotoluene (TNT), trinitroglycerin (NG), pentaerytheritoltetranitrate (PETN), N-methyl-N-2,4,6-tetranitroaniline (TETYRYL), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). TNT, 2,6-DNT, 2,4-DNT, TETRYL, and RDX were used without further purification to prepare stock solutions of *ca.* 1 mg/ml in acetonitrile, and dilutions in the range 0.5–20 μg/ml in methylene chloride. The NG and PETN were prepared from older stock solutions in ethanol, and their concentrations were approximately 4 and 8 times (respectively) those of the other explosives. The standards were stored in the dark at 1°C. All solvents were distilled in glass grade from Burdick and Jackson (Muskegon, MI, U.S.A.).

RESULTS AND DISCUSSION

Fig. 1 demonstrates that different chemical classes of highly explosive compounds and their manufacturing byproducts (*i.e.*, nitrosubstituted aromatics, nitroesters, and nitramines) can be successfully chromatographed and separated using packed-column SFC with a carbon dioxide mobile phase. With the exception of the latest-eluting compound, RDX, the peak shapes are reasonably symmetrical and the resolution is good. Retention times, capacity factors, selectivity, and peak resolution are listed in Table I. To our knowledge, this is the first report on the SFC of TETRYL and PETN, and the first report of packed-column SFC for any high explosives.

The elution order 2,6-DNT < 2,4-DNT < NG < TNT < PETN < TETRYL < RDX < HMX is generally in increasing polarity and molecular weight, as might be expected for a mobile phase with no dipole moment[2]. The 2,4-DNT is retained more than the 2,6-isomer, probably reflecting the steric hindrance of the methyl group upon the interaction of the two neighboring nitro groups in the latter with the stationary phase. NG and TNT, with three nitro-groups, are retained further. PETN and

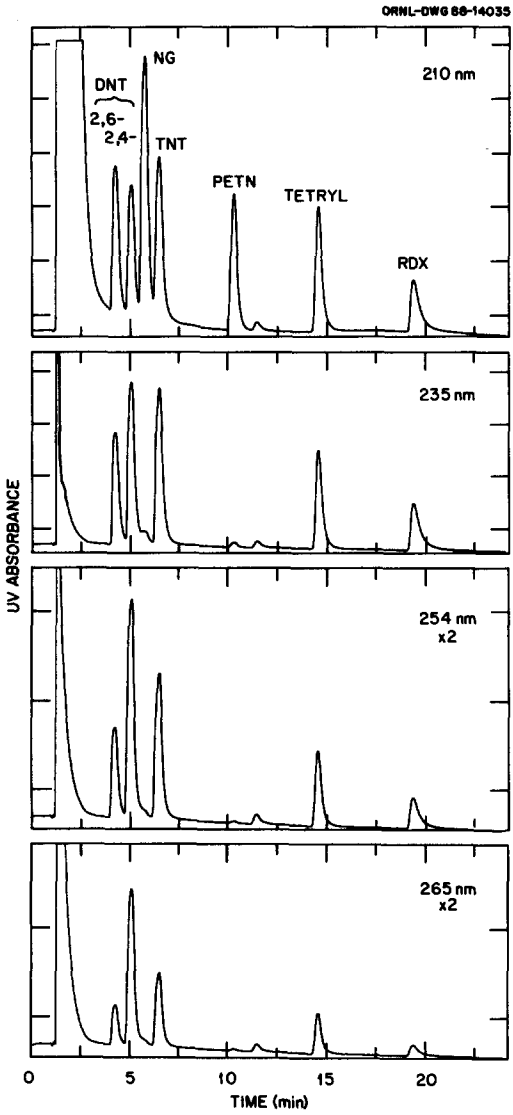

Fig. 1. Packed-column SFC of highly explosive compounds with UV absorbance detection at 210, 235, 254 and 265 nm. Vertical scale expanded two-fold at 254 and 265 nm. Abbreviations listed in Experimental.

TETRYL each have four nitro-groups and elute even later. RDX elutes after TETRYL, yet it contains only three nitro-groups. Its strong retention perhaps is conferred by the polarity from the heterocyclic nitrogen. Under the conditions used for these experiments, HMX, which contains an additional nitro-group and in-ring nitrogen and methylene, would not elute from the 250-mm column. In earlier work, a non-reproducible, tailing chromatographic peak for HMX eluted after RDX on the 150-mm column. These observations suggest that carbon dioxide is not sufficiently

TABLE I
CHROMATOGRAPHIC DATA FOR HIGH EXPLOSIVES AND BYPRODUCTS

Compound	*Retention time (min)*	*Capacity factor*[a], *k'*	*Selectivity*[b], *α*	*Resolution*[c], *R*
2,6-DNT	4.32	2.86		
			1.28	1.33
2,4-DNT	5.12	3.57		
			1.17	1.06
NG	5.81	4.19		
			1.16	1.12
TNT	6.54	4.84		
			1.70	6.32
PETN	10.33	8.22		
			1.46	6.58
TETRYL	14.61	12.04		
			1.36	5.67
RDX	19.43	16.35		

[a] $k' = (t_R - t_0)/t_0$, where t_R = retention time of the compound and t_0 = retention time of the leading edge of the solvent peak.

[b] $\alpha = (t_{R2} - t_0)/(t_{R1} - t_0)$, where t_{R2} = retention time of the later-eluting compound.

[c] $R = (t_{R2} - t_{R1})/(w_2 + w_1)$ (0.5), where w = peak width at the baseline.

polar to efficiently mobilize HMX in packed columns, which are recognized[7] to be more active than capillary columns. Experiments are under way with polar modifiers such as hexanol which have been found[8,9] to mask the activity of the silica surface and improve the solubilizing characteristics of carbon dioxide. The success of capillary columns in eluting HMX with carbon dioxide at lower densities[6] indicates that the former is probably the predominant factor limiting the packed column. Although the Deltabond cyano phase has been shown[10] to be far superior to conventional, non-crosslinked cyano phases for the SFC of basic nitrogen-containing compounds, there appears to be a residual silanol activity for nitramines.

It is significant that the elution order of the explosives by SFC on the Deltabond cyano phase is similar to that reported[3] for HPLC on a cyanopropyl stationary phase using a reversed-phase eluent of water–methanol (50:50, v/v), which is considerably more polar than carbon dioxide. The elution order in HPLC is almost reversed when an octadecylsilane phase is used[3]. This suggests that interactions with the cyano groups in the stationary phase are quite selective among the chemical types represented in these classes of explosive compounds, regardless of the physical state of the mobile phase. The strong retention of TETRYL, RDX, and HMX both in SFC and HPLC on the cyano phase is consistent with unpublished data from D. C. Leggett (noted by Jenkins and Walsh[3]) showing that the solubilities of nitramines are increased 20- to 30-fold in acetonitrile *versus* methanol.

It is evident that the SFC separation is less rapid but more selective than the HPLC separation. The capacity factors of the explosives range from 2.86 to 16.4 for SFC *versus* from 1.37 to 2.28 for HPLC[3]. The SFC separation is *ca.* two-fold longer than the HPLC separation under these conditions. However, SFC is more selective. For example, the selectivity for the pair 2,6-DNT/2,4-DNT is 1.28 for SFC *versus* 1.08 for HPLC[3] and for 2,4-DNT/TNT the corresponding selectivities are 1.51 *versus* 1.05 for SFC and HPLC, respectively. The greater retention and selectivity of the SFC probably reflects the lesser polarity of carbon dioxide *versus* methanol–water. Use of a binary mobile phase in SFC would be expected to decrease the capacity factor and the selectivity, and facilitate the efficient elution of the HMX.

The reproducibility of the packed-column SFC separation is quite good considering the critical requirement for precise pressure/density control over hundreds of atm of programmed mobile phase density. The retention time reproducibility was determined for ten analyses employing six concentration levels of the seven high explosives at 210 nm. The reproducibility, as expressed by the relative standard deviation of the retention times for each compound, was only 0.2–0.3%. This level of precision was readily repeatable on a daily basis. This is equivalent to that reported by other workers[10] using packed-column SFC. Over longer periods of time, however, there is a gradual drift of retention times. The retention time of TNT increased 1.2% over a period of five days of heavy instrument use, and over nineteen days, the retention time increased by 3.3%. This increase in retention is attributed to a slow accumulation of material in the tapered restrictor opening. The net effect would be to decrease the mobile phase flow-rate under pressure-controlled conditions. Increasing the temperature of the restrictor might reduce this effect. The peak area reproducibility was investigated only briefly. The relative standard deviations of the peak areas for the compounds in the 20 μg/ml standard run three times at 210 nm ranged from 0.18% for 2,6-DNT to 2.8% for TETRYL. Inclusion of a greater number of runs probably would decrease the relative standard deviations, but this degree of precision appears to be typical[10].

The UV absorbance detector is well suited for determination of the highly explosive compounds. Fig. 1 shows the separations recorded at 210, 235, 254, 265, 280, and 290 nm. At 210 nm, all seven compounds are detected, but the tail of the solvent peak intrudes into the chromatogram to a much greater extent than at the other wavelengths. This causes a serious downward baseline drift at high sensitivites. At 235 nm, the solvent peak tail is greatly diminished and the nitroesters are barely detectable because they lack the strong UV chromophore of the aromatic group in the nitrotoluenes. However, the nitrotoluenes, nitroaniline, and nitramines are detected with nearly the same sensitivity as at 210 nm, as demonstrated by the peak area ratios plotted in Fig. 2. In fact, for 2,4-DNT, the sensitivity at 235 nm is greater than that at 210 nm. At 254 nm, the NG and PETN are relatively undetectable. The optimum

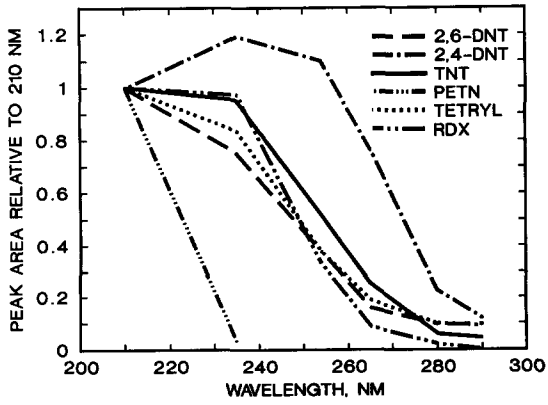

Fig. 2. Sensitivities of UV absorbance detection relative to 210 nm for highly explosive compounds at 210, 235, 254, 265, 280 and 290 nm.

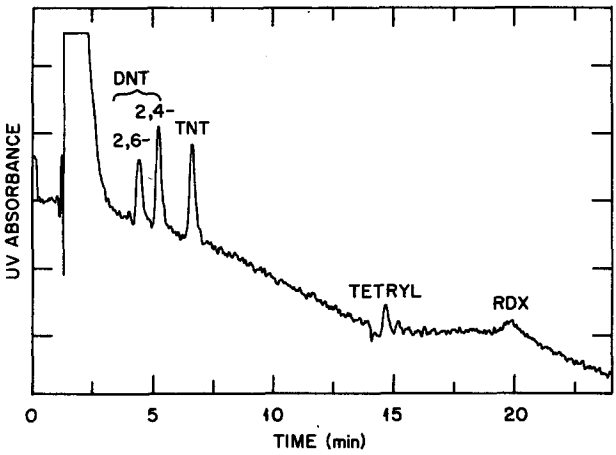

Fig. 3. SFC of lowest concentration standard (0.5 μg/ml, except for *ca.* 2 μg/ml of NG and *ca.* 4 μg/ml of PETN) with UV absorbance detection at 235 nm.

wavelength for detection would depend upon a number of sample- and analyte-specific factors, but 235 nm appears to offer good sensitivity and greater selectivity if NG and PETN are not sought. A chromatogram of the lowest concentration standard [0.5 μg/ml of all compounds except NG (*ca.* 2 μg/ml) and PETN (*ca.* 4 μg/ml)] recorded at 235 nm is shown in Fig. 3. The limits of detection estimated for a signal-to-noise ratio of 4 are 0.33 μg/ml for 2,6-DNT, 0.21 μg/ml for 2,4-DNT, 0.22 μg/ml for TNT, and 0.9 μg/ml for TETRYL. These are *ca.* 10-fold higher than the lowest reported[3] concentrations detected by HPLC with UV detection at 254 nm using a fixed-wavelength detector and a 100-μl injection volume. The much greater injection volume and probably greater source intensity of the fixed-wavelength detector may account for this difference in sensitivities.

Applications of the SFC technique to real-world samples have only been briefly tested. A 5-g soil sample known to be contaminated with explosives was extracted ultrasonically for 18 h in 40 ml of acetonitrile[3], and a portion of the extract, solvent-exchanged into methylene chloride, was injected into the SFC. No chromatographic peaks corresponding to explosive compounds were detected, and when an explosives standard was injected immediately afterward, the peak shapes and responses were badly degraded. Washing the column with carbon dioxide for several hours failed to regenerate the separation. This observation suggests that the soil sample extract contaminated the column and that carbon dioxide alone was insufficiently polar to clean out the contaminants. More polar mobile phases or sample pretreatments may be required to make packed column SFC more useful for the analysis of complex samples which contain polar, high molecular weight extraneous matter.

CONCLUSIONS

SFC can achieve good separations of explosive compounds and their by-products. Further work must focus on the selection of mobile phase modifiers to

extend the range of compounds (*e.g.*, HMX) which can be readily eluted from packed columns and to alleviate the effects of extraneous sample matrix components. More inert capillary columns may extend the elution range with carbon dioxide without modifiers. Sample pretreatments or chemical class isolations also may be necessary for analysis of complex environmental sample matrices.

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