Derivatization of Ethylene Dibromide with Silica-Supported Silver Picrate for Improved High-Performance Liquid Chromatographic Detection

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Silica-supported silver picrate was used as an off-line, precolumn derivatization reagent for ethylene dibromide (EDB). Two products were obtained, the ratio of which, as a function of reaction conditions, is characteristic of EDB. The derivatives were monitored with UV, reductive electrochemical, and photolysis/oxidative electrochemical detection. Sub-parts-per-billion detection limits were obtained. The method was used to quantitate EDB in leaded gasoline, and the results were confirmed with gas chromatography with electron capture detection (GC/ECD). The method was further validated with a single blind analysis of spiked EDB in gasoline. This is the first report of an HPLC method for EDB.

Large-scale production and use of ethylene dibromide (EDB) began in the 1920s for its use in leaded gasoline as a scavenger for lead oxide released in the combustion process, and millions of pounds are used annually in the United States for this purpose (*1*, *2*). It also is currently used in the production of certain dyes, drugs, and vinyl bromide. EDB has been used as a general fumigant since 1948. In 1975, the National Cancer Institute found EDB to be toxic and carcinogenic. Additional studies have confirmed EDB's toxic effects (*3*, *4*), and permissible occupational exposure levels have been set (*5*). EDB has been detected in ambient air (*2*, *6–13*), water (*12*, *14*, *15*), foods (*7*, *13*, *16–21*), soil (*22*, *23*), and biological samples (*12*, *15*, *23*). The ability to constantly monitor EDB is very important, and several analytical techniques to detect and/or confirm the presence of EDB are needed.

EDB is usually determined by using gas chromatography (GC) with electron capture (ECD), mass spectrometric (MS), flame ionization (FID), and/or Hall electrolytic detection (*2*, *6–12*, *15–21*, *23*). Many of the analyses using gas chromatography were confirmed by using more than one GC column and/or more than one GC detector (*2*, *6*, *8*, *9*, *16–18*, *20*, *21*, *23*). The analyses not confirmed as above usually used a mass spectrometer as the detector and/or capillary columns (*10–12*, *15*, *19*). EDB can also be determined by colorimetric means (*13*, *24*) or by molecular emission cavity analysis (*22*, *25*), but these nonchromatographic methods cannot distinguish different brominated organic compounds. For those laboratories that lack a GC/MS or capillary GC system, or those that need a method other than GC to confirm the presence of EDB, a highly specific liquid chromatographic method is needed. Even if a GC is available, an LC approach may be more convenient because heavily contaminated samples are much less of a problem in LC.

There are few reports of liquid chromatographic methods for alkyl halides because of the lack of a good chromophore, fluorophore, or electrophore (*26–30*), and no reports exist of an LC approach specific for EDB. Alkyl halides have previously been determined by initial decomposition of the parent compound with ultraviolet (UV) radiation (*26*), a Pd catalyst (*27*), or sodium fumes (*28*), followed by ion chromatographic analysis of the halides formed. These methods, however, cannot distinguish different compounds containing the same halogen. Reports describing derivatization reactions of alkyl halides have been limited to two, (*29*, *30*) and neither of these were applied to the determination of EDB.

In this report, we have expanded our earlier work on the derivatization of various electrophiles (*31*) to include a very specific and sensitive LC derivatization approach for EDB and other bifunctional alkyl halides. Silver picrate adsorbed onto silica gel was reacted with EDB, and, depending upon the reaction conditions, 1-bromo-2-(picryloxy)ethane (BPE) and/or 1,2-bis(picryloxy)ethane [ethylene dipicrate (EDP)] can form. These derivatives can be monitored by UV detection, (HPLC-UV), reductive electrochemical detection (reductive LC-EC), or oxidative electrochemical detection after postcolumn photolysis (HPLC-*hν*-EC) (*32*). The reaction sequence is shown in Figure 1. The EDP/BPE ratio, as a function of reaction conditions, is characteristic of EDB. For the best improvement in detectability, the reaction conditions were engineered to convert EDB to EDP. Derivatization of an analyte to form two derivatives, the ratio of which being characteristic of that analyte, has been used in the analysis of the E and D prostaglandins, asymmetric carbonyls, steroids, and other compounds (*33*, *34*). All of the derivatizations in the current work were performed off-line, before the chromatographic separation.

Picrate was chosen as the labeling moiety because it allows the option of three different detection modes. This is advantageous, especially when the method is applied to real world samples often containing many interferents. Separating the interferents can often be accomplished through selective detection rather than through chromatography. The derivatization scheme introduced here is more selective than the existing GC methods. In GC, which is usually less selective than LC, the problem of possible coelution always exists, even with capillary and megabore columns. In this study, the specificity of the derivatization as a function of reaction conditions, combined with the selectivity of the chromatographic separation provides fingerprint identification of EDB. Identification is accomplished by using much more than retention time. This technique could be made even more selective by monitoring the peak height ratio of each of the derivatives as function of wavelength (using a dual channel UV detector) or potential (using a dual electrode EC detector). This ratio technique also is possible with GC/MS, but not with GC/ECD.

The advantages of off-line, precolumn solid phase derivatizations over solution (homogeneous) precolumn reactions have been summarized (*31*, *35*). Specifically for silica-support silver picrate (SSSP) reactions with EDB, the most obvious advantages of using a supported reagent include the following: (1) reagents can become more stable after immobilization on a support; (2) reagents that are dangerous (picrates are po-

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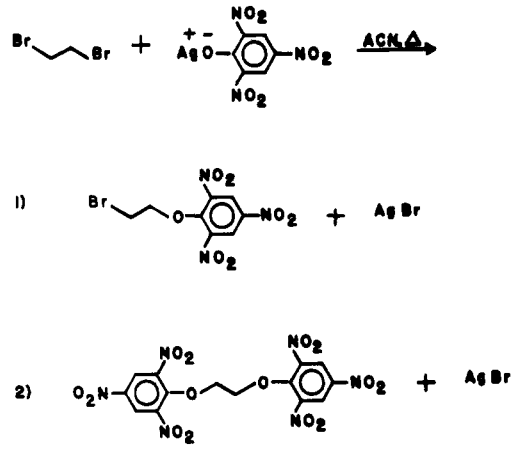

Figure 1. Reaction sequence forming the labeled picryl ethers from EDB. Ethylene dibromide (EDB) reacted with silver picrate in acetonitrile forms 1-bromo-2-(picryloxy)ethane (BPE) and ethylene dipicrate (EDP).

tentially explosive) can be diluted with the support making them much safer to work with; (3) derivatizations are much more convenient; since the reagent is present on the support, reagent solutions of known concentrations do not need to be prepared each time; and (4) reactions not possible in solution because of lack of solubility of the derivatization reagent can be carried out in high effective reagent concentration on a support.

EXPERIMENTAL SECTION

Chemicals. HPLC solvents were obtained from MCB Manufacturing Chemists, Inc. (Gibbstown, NJ), as their Omnisolv brand and were filtered through a 0.45-μm solvent filter and degassed with stirring under vacuum. The chemicals used were obtained from a variety of commercial suppliers, including: Aldrich Chemical Co., Milwaukee, WI; Chem. Service, West Chester, PA; and J. T. Baker Chemical Co., Phillipsburg, NJ.

Apparatus. The LC system consisted of a Waters Model 6000A solvent delivery system (Waters Chromatography Division, Millipore Corp., Milford, MA), a Waters Model U6K syringe loading injection valve, and a Linear dual pen recorder (Linear Instruments Corp., Reno, NV). The detectors used were a Waters Model 480 variable-wavelength UV detector and/or an amperometric detector which has been described (*31*). At times, the amperometric detector was used in conjuction with a postcolumn photolysis unit (*32*). For some applications, a Waters automated switching valve was used. Chromatographic columns consisted of a Waters μBondapak CN reversed-phase column, 0.39 cm × 30 cm, and a 0.78 cm × 30 cm (semipreparative), and a Radial-PAK RESOLVE CN column, 0.80 cm × 10 cm in an RCM-100 Radial Compression Module. The instrumentation used to characterize EDB and its derivatives consisted of a Varian T-60 NMR spectrometer (Varian Associates, Palo Alto, CA), a Perkin-Elmer 599B infrared spectrometer (Perkin-Elmer Corp., Norwalk, CT), a Perkin-Elmer Lambda 3B UV/VIS spectrophotometer equipped with the PE 3600 data station, a Nuclide magnetic sector mass spectrometer (Nuclide Corp., State College, PA), and a Thomas Hoover capillary melting point apparatus (Arthur H. Thomas Co., Philadelphia, PA).

Some studies used a Varian Model 3700 gas chromatograph equipped with a pulse linearized ^{63}Ni electron capture detector (ECD). The column was an OV 17, 10% loading on Chromosorb WHP, 80/100 mesh (HNU Systems, Newton, MA).

Preparation, Isolation, and Characterization of the Labeled Derivatives. 1-Bromo-2-(picryloxy)ethane was prepared by refluxing 1 g of silver picrate with a 10-fold molar excess of EDB in 15 mL of acetonitrile for 3 h. The preparation of silver picrate has been reported (*31*). The silver bromide precipitate was removed by filtration and the unreacted EDB was removed in a drying pistol over phosphorus pentoxide at 40 °C (reflux solvent, dichloromethane) for 18 h. The solid was dissolved in 10 mL of acetonitrile and subjected to semipreparative LC using a 7.8 mm i.d. μBondapak CN column, water:acetonitrile (65:35) at 8 mL/min. The peak of interest, eluting at a retention time

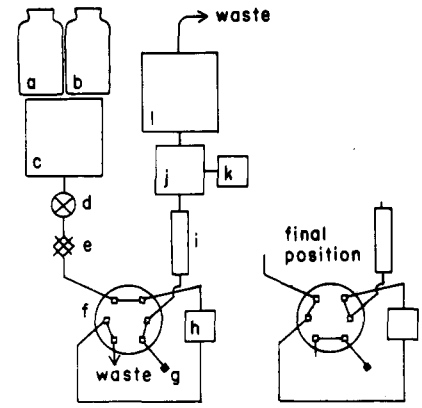

Figure 2. Instrumentation used to obtain the chromatogram in Figure 6: (a) solvent a (water); (b) solvent b (methanol:0.2 M NaCl, 65:35); (c) solvent delivery system; (d) injection valve (250-μL sample loop); (e) precolumn filter; (f) switching valve (shown in the initial and final position); (g) plug; (h) CN Guard-PAK precolumn insert; (i) CN Radial-PAK cartridge; (j) photolysis irradiation chamber; (k) photolysis power supply; (l) amperometric electrochemical detector monitoring 0.8–0.7 V. See text for details.

different from the starting compounds, was collected and rotoevaporated to dryness, leaving the pure product. The peak, having a non-Gaussian flat top, was collected from 5 to 8 min. Poor peak shape was a result of the UV detector monitoring the separation being overloaded, not a result of poor chromatography. The collected peak was later detemined to be a single component, and identified by several spectroscopic and physical methods (vide infra).

Ethylene dipicrate (EDP) was prepared by heating and stirring EDB in a capped reaction vial with a 2-fold molar excess of silver picrate in 4 mL of acetonitrile at 140 °C (oil bath). After 1 h, the precipitate was removed by filtration, and the resulting solution was subjected to semipreparative LC using the same column as above. The mobile phase consisted of water:acetonitrile (60:40) at 8 mL/min. EDP was collected from 8 to 13 min and rotoevaporated to dryness, as above. The physical and spectral characteristics of EDB and its two derivatives are summarized in Table I. All of these data are consistent with the structures reported in Figure 1.

Caution: Although we have not experienced any problem concerning the stability of these reagents, it is possible that an explosion may occur when using these reagents, and proper eye and body protection should be worn at all times.

Solid-Supported Reaction Conditions. Preparation of silica-supported silver picrate (SSSP) has been reported (*31*). The SSSP was loaded into a 0.3-mL reaction vial, 100 or 200 μL of EDB in acetonitrile was then injected onto the supported reagent, and the vial was capped, placed in an oil bath, and heated. After a predetermined specific period of time, the reaction mixture was cooled to room temperature by placing the vial in a beaker being flushed with cold water for 1 min. The vial contents were then washed through a 0.45-μm HPLC sample filter into a volumetric flask with the LC mobile phase. Percent reaction reported here is defined as the percentage of the original substrate converted to the labeled derivative, not the percentage of the original substrate gone at the completion of the reaction time, because this would also include the competing (and undesirable) elimination reactions. Percent reaction was calculated by measuring the peak height of the derivative formed and correlating this to a calibration curve generated by using the purified and characterized product of the reaction (the external standard method).

In the derivatization of 200 μL of a 40 ppb solution of EDB, the reaction mixture was washed with 2 mL of water:acetonitrile; (75:25) through a 0.45-μm filter into a 2-mL volumetric flask. Two hundred microliters of this was then injected into the HPLC. The instrumentation used to obtain this chromatogram is illustrated in Figure 2.

Switching Valve Operation. Operation of this system (Figure 2) was as follows: In the initial position, solvent a (water) flowed through the CN Guard-PAK precolumn insert cartridge to waste. After injection, the excess silver picrate of the reaction mixture

Table I. Physical and Spectral Characteristics of EDB and Its Labeled Derivatives

ethylene dibromide	1-bromo-2-(picryloxy)ethane	ethylene dipicrate
MW, 188	MW, 336	MW, 484
UV, 200 nm (ϵ = 885)	UV, 229 nm (ϵ = 17 800)	UV, 229 nm (ϵ = 38 300)
NMR: 4.2 ppm (s)	NMR: 4.1 ppm (2, t)	NMR: 4.86 ppm (2, s)
	5.0 ppm (2, t)	8.9 ppm (2, s)
	9.9 ppm (2, s)	
MP (liquid at 25 °C)	MP 77–79 °C	MP 196–197 °C
	elemental analysis[a]	elemental analysis[a]
	C: 28.80 (28.59)	C: 34.83 (34.72)
	H: 1.96 (1.78)	H: 1.74 (1.67)
	N: 12.20 (12.50)	N: 16.98 (17.35)
	O: 33.50 (33.33)	O: 46.45 (46.25)[b]
	Br: 23.80 (23.78)	
	mass spectrum[c]	mass spectrum[d]
	338 1%	484 5%
	337 5%	256 100%
	336 1%	212 100%
	335 5%	
	109 100%	
	107 100%	
	infrared spectrum[e]	infrared spectrum[e]
	3100 s; 1410 m; 920 m	3100 s; 1440 w; 1010 m
	2830 w; 1340 s; 900 w	2960 w; 1410 w; 940 w
	1600 s; 1250 m; 810 w	2860 w; 1340 s; 900 m
	1530 s; 1210 m; 750 w	1600 s; 1250 s; 810 m
	1460 w; 1050 m; 710 s	1530 s; 1160 w; 760 m
	1440 w; 970 w	1460 w; 1080 m; 710 s

[a] Elemental analyses were performed at Galbraith Laboratories, Inc., Knoxville TN. The numbers in parentheses represent the theoretical value. [b] Obtained by difference. [c] Electron impact (70 eV); source, 100 °C; probe, 140 °C; ion acc. pot., 4.5 kV. [d] Electron impact (20 eV); source = 200 °C; probe = 180 °C; ion acc. pot. = 4.5 kV. [e] Key: s, strong; m, moderate; w, weak.

was washed to waste, while the reaction product, ethylene dipicrate, was absorbed onto the head of the Guard-PAK cartridge. After a 3-min water wash, the switching valve was rotated to its final position, the solvent select valve on the pump was changed to solvent b (methanol:0.2 M NaCl) (65:35), and the power supply to the irradiation chamber was turned on. This back-flushed the Guard-PAK cartridge, and washed the labeled derivative onto the analytical column. A photolysis chamber for irradiation of the column effluent was located between the analytical column and the detector (*32*). In the initial position of the switching valve, there was no flow through the photolysis chamber. Since the Teflon tubing of the photolysis weave will not be cooled by the mobile phase, the light of the chamber was turned on only after the switching valve had been rotated to its final position, thereby avoiding heat damage to the tubing.

Applications. EDB was quantitated in gasoline by this derivatization approach, and the results were confirmed by GC/ECD. In the HPLC method, spiked and unspiked gasoline solutions were diluted 10-fold with acetonitrile, and 200-μL aliquots were reacted with the silica-supported silver picrate at 160 °C for 30 min. At the conclusion of the reaction, 0.5 mL of 0.1 M NaCl/methanol was added to precipitate the excess silver ion. It was necessary to remove the silver ion to prevent its precipitation with chloride present in the mobile phase. One half milliliter of a 0.1 M solution contained a slight excess of chloride necessary for the precipitation reaction. The reaction mixture (the support, the precipitated AgCl, and the solution containing the product) was then removed from the vial with a disposable pipet, and washed through a 0.45-μm filter into a 5-mL volumetric flask with water:acetonitrile (50:50). Three reactions were performed at three concentration levels, and each of the reaction solutions was injected into the HPLC three times (a total of 27 injections). The derivative was monitored with reductive LC/EC at an applied potential of −0.8 V vs. Ag/AgCl. The injector was modified for sample deoxygenation by using an arrangement published elsewhere (*36*) and by using a 250-μL sample loop. In the single blind analysis of EDB in gasoline, the reaction procedure was identical with the standard addition analysis with the exceptions that the reaction mixture was washed into the volumetric flask with acetonitrile, a 20-μL sample loop was used, and the applied potential was −0.5 V vs. Ag/AgCl. Operating at less negative potentials improves long term reproducibility in electrochemical detectors. A standard addition calibration plot was constructed to determine the concentration of the blind samples. The total amount of time required to analyze these three blind samples including construction of the calibration plot and data analysis was approximately 8 h.

The quantitation of EDB in gasoline was confirmed by GC using an OV 17, 10% loading on Chromosorb WHP column, nitrogen as the carrier gas at 85 mL/min, and the following temperatures: injector, 160 °C; oven 60 °C; detector, 260 °C. The spiked and unspiked gasoline samples were diluted 100-fold with acetonitrile and injected into the GC. Quantitation was performed, as above, using standard addition.

RESULTS AND DISCUSSION

Temperature and Time Optimization. In the earlier report on derivatizations of alkyl halides and epoxides, limits of 90 °C and 2 h were set. Under these conditions, EDB is only slightly reactive, and more strenuous conditions were employed. A plot of the relative peak heights of BPE and EDP as a function of temperature is shown in Figure 3. The ratio of the two peak heights, which is characteristic of EDB, is also shown. In the peak height ratio plot of Figure 3, the last point (at 175 °C) is not shown because this point represents a value obtained from a very large difference in peak heights and therfore is much more subject to error than at other points on the curve. Chromatograms showing different amounts of each product as a function of reaction temperature at constant time are illustrated in Figure 4. The optimum temperature of 160 °C was then held constant while time was varied, Figure 5. Again, the ratio of the peak heights of the products is characteristic of EDB. A compromise between percent derivatization to EDP and reaction time was made, and further derivatizations were carried out at 160 °C for 30 min.

Percent Derivatization as a Function of EDB Concentration. With the conditions of 160 °C and 30 min, the percent derivatization was monitored as a function of EDB

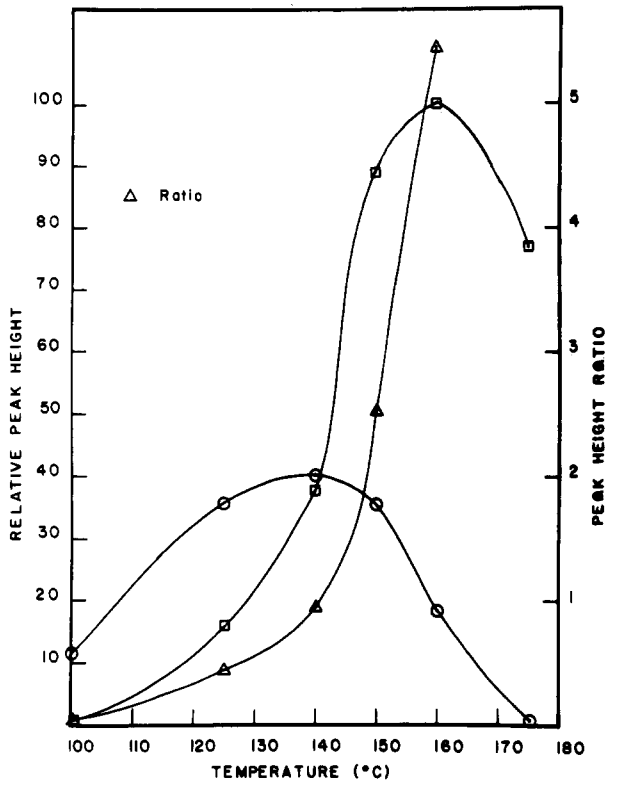

Figure 3. Relative peak height, and ratio of BPE (O) and EDP (□) as a function of temperature. Reaction conditions: volume 100 μL, time 60 min. HPLC conditions: water:acetonitrile, 65:35, 2 mL/min, μBondapak CN column, UV detection (229 nm).

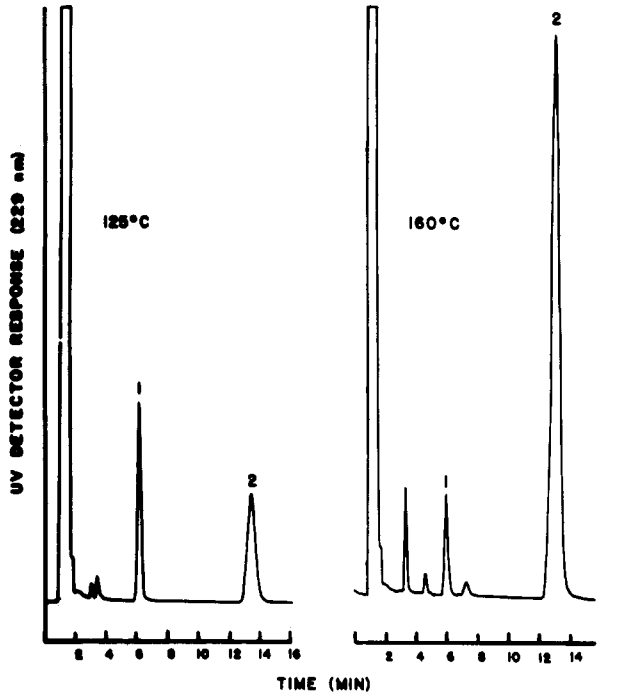

Figure 4. Chromatograms illustrating the formation of BPE and EDP as a function of temperature. Reaction and HPLC conditions are listed in Figure 3. (1) BPE (1-bromo-2-(picryloxy)ethane), (2) EDP (ethylene dipicrate).

concentration. At high concentration, the percent derivatization was fairly constant, but increased as concentration decreased (Table II). It is desirable if the percent derivatization is constant over a wide concentration range, but in the case of derivatization of EDB to ethylene dipicrate, this was not the case. In and of itself, this is not a problem if standard addition is employed, and the percent derivatization

Table II. Percent Derivatization of EDB to Ethylene Dipicrate as a Function of EDB Concentration

EDB, ppm	% derivatization[a]
1118	34.0 (1.2)
112	33.5 (3.1)
11.2	41.8 (5.0)
1.12	65.4 (1.8)
0.112	82.0 (3.2)

[a] Reaction conditions: 100 μL of EDB in acetonitrile, 10% w/w loading of silver picrate on silica gel, 160 °C (oil bath), 30 min.

Table III. Minimum Detection Limits of Ethylene Dibromide and Its Labeled Derivatives[a]

	detection limits, ppb		
	HPLC-UV	HPLC-$h\nu$-EC	reductive LCEC
ethylene dibromide	574[b]	ND[c]	ND[c]
1-bromo-2-(picryloxy)ethane	19[d]	16[e]	10[f]
ethylene dipicrate	13[g]	0.65[h]	1.6[i]

[a] Signal/noise = 2.5/1; 200-μL injections. [b] Calculated at 200 nm using ε ratios at 200 and 229 nm. [c] ND, not detectable. [d] 229 nm, μBondapak CN, 55/45 water/acetonitrile, 2 mL/min. [e] +1.0 V vs. Ag/AgCl, μBondapak CN, 65/35 0.2 M NaCl/MeOH, 2 mL/min. [f] −0.9 V vs. Ag/AgCl, μBondapak CN, 80/20 0.2 M NaCl/MeOH, 2 mL/min. [g] 229 nm calculated from ε values. [h] +1.0 V vs. Ag/AgCl, μBondapak CN, 65/35 0.2 M NaCl/MeOH, 2 mL/min. [i] −0.8 V vs. Ag/AgCl, μBondapak CN, 65/35 0.2 M NaCl/MeOH, 2 mL/min.

Table IV. Minimum Detection Limit of EDB following Derivatization[a]

method of detection	detection limit, ppb	detection limit without derivatization, ppb
HPLC–UV (229 nm)	5.7	574 (200 nm)
HPLC–$h\nu$–EC	0.28	ND[b]
reductive LCEC	0.95	ND
GC/ECD		1.4[c]
GC/MS		1[d]

[a] Assumes 82% derivatization of EDB to ethylene dipicrate. [b] ND, not detectable. [c] 5-μL injection, see text for details. [d] Reference 11.

is constant over the range of concentrations derivatized. The correlation coefficient of 0.994 from the standard addition plot of spiked and derivatized EDB in gasoline (vide infra) is, in fact, consistent with the percent derivatization being constant over the concentration range employed for the study. It also is important to note that it does not matter if the amount or concentration is determinant of the yield as long as the volume of the solution is kept constant throughout a standard addition study.

Minimum Detection Limits (MDLs). Minimum detection limits are here defined as the concentration that yields a signal to noise ratio of 2.5/1 for a 200-μL injection into the HPLC. Table III lists the MDLs of EDB and its two derivatives. Underivatized EDB can only be seen with UV detection, and its wavelength of maximum absorbance (200 nm) is less than ideal, because many compounds are detectable. Its MDL at this wavelength is too high for most applications. EDB is undetectable with the reductive electrochemical (EC) or photolytic-HPLC approaches. Assuming an 82% derivatization of EDB to EDP, the MDL of EDB after derivatization is summarized in Table IV. All detection modes provide 2 to 3 orders of magnitude improvement in LC detectability using this derivatization approach. For comparison purposes,

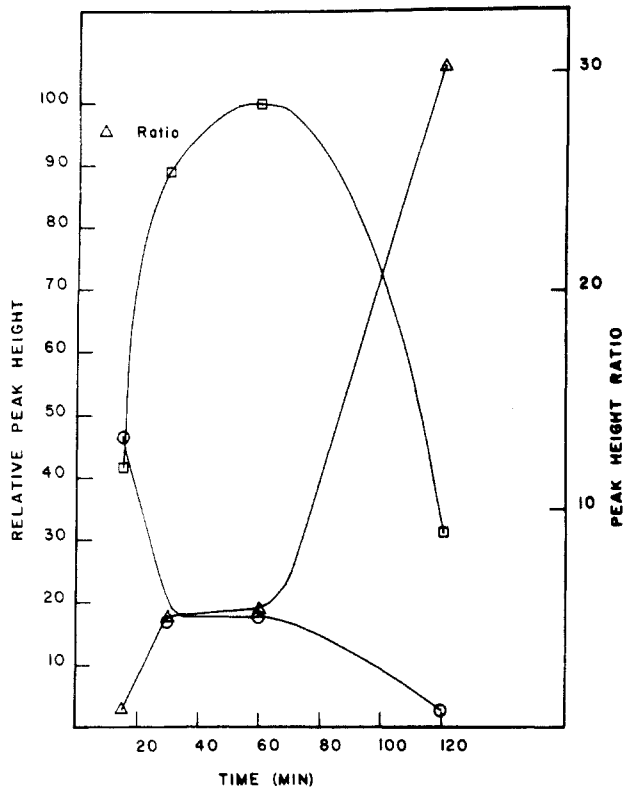

Figure 5. Relative peak height and ratio of BPE (O) and EDP (□) as a function of reaction time. Reaction temperature was 160 °C, all other reaction and HPLC conditions are identical with those listed in Figure 3.

the MDL of EDB using GC/ECD and GC/MS are also included in Table IV. The GC/ECD MDL was determined in our laboratory by using a 5-μL injection, S/N = 2.5, and the packed column chromatographic conditions listed in the Experimental Section. The MDL probably would be lower if a capillary column had been employed. The GC/MS MDL was obtained from ref 11 and used a 2 mm i.d. column and a Finnigan 3200 GC/MS system. MDL was defined as the amount of material required to obtain an area of 1000 counts under the selected ion chromatogram. The derivatization technique introduced here has similar MDLs as the GC techniques, concomitant with higher specificity. After derivatization, the products can be monitored with three separate and distinct LC detectors.

A chromatogram showing the product derived from the derivatization of 200 μL of a 40 ppb solution of EDB in acetonitirle at 160 °C for 30 min is illustrated in Figure 6. The arrow indicates ethylene dipicrate, the product of the reaction. The other peaks present in the reagent blank are system peaks from the column switching apparatus used to obtain this chromatogram (Figure 2). When working at low levels of EDB, high detector sensitivity settings were employed, and the large excess of reagent showed tailing in the area where the derivative eluted. This was undesirable, and therefore this column switching apparatus was employed (Experimental Section).

The detector used for this application was a parallel, dual electrode amperometric detector operated in the difference mode. Electrochemical detectors are more sensitive than UV detectors to changes in mobile phase composition, flow rate, and temperature. If the electrochemical detector was operated in the usual manner with direct (not difference) response at the electrode, the base line perturbations were too severe at high sensitivity settings when the switching valve was rotated to its final position. If, however, the difference between two parallel electrodes was monitored, the base line perturbations

Table V. Dipicryl Substituted/Monosubstituted Ratio as a Function of Substrate[a]

substrate	$Pi(CH_2)_nPi/Pi(CH_2)_nX$ ratio[b] (std dev)
$I(CH_2)_2I$	24 (2.8)
$Br(CH_2)_2Br$	0.94 (0.02)
$Cl(CH_2)_2Cl$	0 (0)
$Br(CH_2)_3Br$	0.60 (0.03)
CH_2Br_2	0.18 (0.01)

[a] Reaction conditions: 140 °C; 60 min; 100 μL substrate in acetonitrile; solution concentrations equal molar to 1000 ppm allyl iodide. HPLC conditions: μBondapak CN column, 65:35 water: acetonitrile; 2 mL/min, peak height ratio. Each data point represents an average of three reactions, each reaction mixture was injected in triplicate. [b] Pi = picrate; n = 1, 2, or 3; X = I, Br, or Cl.

Table VI. Retention Time of Picryl Derivatives as a Function of Substrate[a]

substrate	retention time of derivative,[b] min	
	$Br(CH_2)_nPi$	$Pi(CH_2)_nPi$
CH_2Br_2	2.55	10.40
$Br(CH_2)_2Br$	5.56	11.84
$Br(CH_2)_3Br$	6.83	14.98

[a] Reaction and HPLC conditions are identical with those listed in Table V. [b] Pi = picrate; n = 1, 2, or 3.

Table VII. Quantitation of EDB in Leaded Gasoline

method	EDB concn, ppm (std dev)
GC/ECD	81 (1.8); n = 9
HPLC	77 (5.2); n = 27
actual value	24–124

canceled, and a smooth base line was obtained (*37*). For this application, the difference in response between 0.8 V and 0.7 V was monitored. At these potentials, the background interferent signals from the column switching apparatus were largely canceled, but the derivatives's signal was seen, because its response was different at these potentials.

Reactive Specificity. The specificity of this derivatization approach is illustrated in Table V. Under identical reaction conditions, five different disubstituted halides yield characteristic ratios of the disubstituted and monosubstituted products. Ethylene diiodide, which has the best leaving group, was almost fully converted to EDP. Ethylene dichloride formed only the monosubstituted derivative, and ethylene dibromide, as expected, had intermediate reactivity under these conditions. 1,3-Dibromopropane and dibromomethane had reactivity similar to EDB but still could be distinguished on the basis of their product ratios. In addition, the derivatives formed from these electrophiles can be separated chromatographically from EDB's derivatives (Table VI). One specific set of conditions was chosen to distinguish these compounds, but we expect that, under a variety of other conditions, these and other dihalogenated compounds will yield unique disubstituted/monosubstituted ratios. This approach identified compounds not only by their retention time but also by their reactivity as a function of reaction conditions. In effect, fingerprint identification is possible with a general detector (i.e., UV–vis), concomitant with significant improvements in detectability. There are other potential interferents, but the combined specificity of the derivatization and chromatographic separation should be able to differentiate EDB from these compounds.

Application. As an application of this technique, EDB was quantitated in leaded gasoline. The results were con-

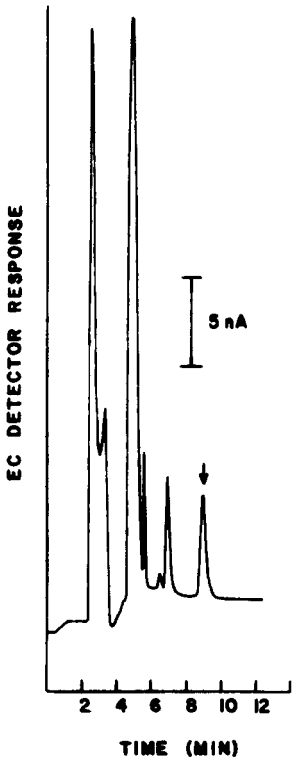

Figure 6. Chromatogram showing the reaction mixture from derivatization of 200 μL of a 40 ppb solution of EDB. The arrow indicates ethylene dipicrate. See text for reaction conditions and other details; see Figure 2 for HPLC conditions.

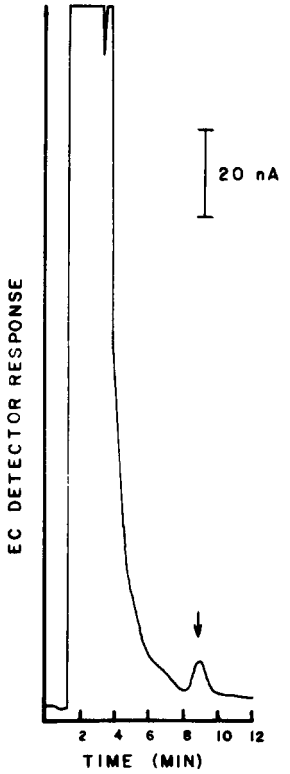

Figure 7. Chromatogram showing the reaction mixture of EDB derivatized in leaded gasoline. See text for derivatization conditions. HPLC conditions were as follows: methanol:0.2 M NaCl (45:55), 2 mL/min; CN Radial-PAK cartridge, 0.8 cm i.d., reductive electrochemical detection, applied potential = −0.8 V vs. Ag/AgCl, 250 μL injection. The arrow indicates ethylene dipicrate.

firmed by GC/ECD (Table VII). The standard deviation using the method introduced in this paper is larger than that in the GC method, but still is within acceptable limits. This was not unexpected because of the extra procedures inherent in a derivatization scheme. The benefits of increased specificity of the HPLC approach far outweigh the small decrease in standard deviation using the GC approach. Standard addition was utilized in both the GC and derivatization/HPLC analyses (Experimental Section). A reductive electrochemical LC detector was used because both UV and photolytic electrochemical detection detected interferents that eluted near ethylene dipicrate. Figture 7 shows the ethylene dipicrate product formed in the upspiked gasoline. The exact amount of EDB in gasoline is determined by the lead concentration, the upper limits of which are set by the Environmental Protection Agency (EPA) (*2*, *38*). At the time the gasoline was purchased, the Pb concentration limit was 0.5 g/gallon. This would correspond to an EDB concentration of 120 ppm. In January, 1986, the Pb limit was changed to 0.1 g Pb/gallon and the EDB level will be 24 ppm. The experimentally listed values in Table VII are consistent with these levels.

In addition to the above study, a single blind study was performed in which one person spiked EDB to another sample of the same brand of leaded gasoline (purchased on a different day) at three different levels, and a second person analyzed the samples not knowing the spiked concentrations. The quantitation ws done by using the derivatization technique. A standard addition calibration plot of peak height of EDP formed in the reaction vs. amount of spiked EDB was constructed and the concentrations of the unknowns were determined from their derivatives' peak heights. The standard addition plot was a straight line with an X intercept of −79.2, a Y intercept of 10.0, a slope of 0.127, and a correlation coefficient of 0.994. The experimentally determined EDB concentration found in this sample (79 ppm ± 6.2) is consistent with the previously purchased sample, and the values found in the blind samples agreed with the spiked levels within experimental error (Table VIII).

Table VIII. Single Blind Quantitation of EDB in Gasoline by Derivatization/HPLC

sample	spiked level, ppm	found level, ppm (std dev)	% difference
1	141.6	143.6 (12); n = 9	+1.4
2	98.1	92.4 (6); n = 9	−5.8
3	119.9	126.3 (11); n = 9	+5.3

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Registry No. BPE, 90111-00-5; EDP, 19404-12-7; EDB, 106-93-4; silver picrate, 146-84-9; silica, 7631-86-9.

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Activated Carbamate Reagent as Derivatizing Agent for Amino Compounds in High-Performance Liquid Chromatography

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Activated carbamate reagents were developed for the determination of amino compounds. The reagents, succinimido carbamates, were easily prepared by the reaction of disuccinimido carbonate (DSC) with the appropriate amine having a strong chromophore. Succinimido phenyl- and *p*-bromophenylcarbamates for spectrophotometric determination, and succinimido naphthylcarbamate for fluorometric determination, were synthesized as activated carbamate reagents. The carbamate reagents readily react with both primary and secondary amines such as alkyl amines or amino acids in mild conditions to give the corresponding urea derivatives. These derivatives are efficiently separated by reversed-phase liquid chromatography and were sensitively detected spectrometrically or fluorometrically.

Numerous derivatization reagents for amino compounds have been developed for high-performance liquid chromatographic (HPLC) study. Ninhydrin (*1*) was the most popular reagent for the colorimetric detection of amines only in postcolumn derivatization. Recently, fluorometric reagents such as *o*-phthalaldehyde (*2, 3*) or fluorescamine (*4, 5*), have applied to both precolumn and postcolumn derivatization procedures to replace ninhydrin. However, secondary amines do not fluoresce on reaction with these reagents. They should be converted into primary amines by an additional procedure such as oxidation prior to derivatization (*6, 7*). For the simultaneous determination of both primary and secondary amines, various types of reagents have been developed such as halogenosulfonate (*8–10*), formate (*11, 12*), nitroaryl halide (*13–15*), sulfonic acid (*16*), isocyanate (*17*), isothiocyanate (*18, 19*), etc.

Since isocyanate and isothiocyanate reagents readily react with amines under the mild conditions to give stable urea or thiourea derivatives without forming byproducts, various labeling reagents (*17–23*) of this type have been developed and applied to amino acid analyses, Edmann degradation (*18–21*), or chiral derivatization (*22–24*). However, these compounds were moisture-sensitive lachrymators, and their synthesis has required phosgen or thiophosgen.

On the other hand, a convenient synthetic method (*25*) for urea derivatives has recently been developed by using disuccinimide carbonate (DSC), which was essentially a synthetic reagent for the preparation of active carbonate in peptide chemistry (*26*). DSC reacts with amino compounds

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