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Note

Separation and use of the perdeuterated analogue as an internal standard for the analysis of ethylene dibromide

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The use of stable isotopes as internal standards for the accurate analysis of organic molecules is a well-established procedure[1] in gas chromatography–mass spectrometry (GC–MS). Isotopically labeled materials are ideal internal standards for trace analysis because they are nearly chemically identical to the analyte. Thus, systematic errors introduced in the assay during sampling, extraction, concentration, derivatization and detection are greatly minimized by using these materials[1]. Stable isotope dilution is particularly advantageous for biochemical assays because it eliminates any radiation problems associated with the use of radioactive internal standards[2].

It is generally assumed that special detectors such as radioactivity detectors or mass spectrometers are required if isotopically labeled materials are used as internal stadards for GC analyses; however, this is not the case if the isotopically labeled material can be separated from its unlabeled congener. Under these circumstances, simple, less expensive devices such as flame ionization or electron-capture detectors could be used. The advantages of precision and accuracy associated with stable isotope dilution GC–MS would still be realized with these GC detectors.

GC separations of isotopes of low-molecular-weight compounds have been previously reported[3–13]. However, these separations involved the use of special instrumentation or specially prepared columns, and the experimental conditions used produced retention times that were too long for routine analyses requiring high sample throughput.

In this communication, we report the facile separation of ethylene dibromide (1,2-dibromoethane, denoted EDB-H_4) from its deuterated analogue (1,1,2,2-[^{2}H]$_4$-1,2-dibromoethane, denoted EDB-D_4), using commercially available fused-silica capillary columns. Furthermore, we demonstrate that stable isotope dilution GC using a conventional electron-capture detector, can be conveniently utilized for the accurate ultra-trace (ppb*) measurement of this pesticide[14].

* Throughout this article, the American billion (10^9) is meant.

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EXPERIMENTAL

Materials

The EDB-H_4 (stated purity 98%) and EDB-D_4 were obtained from MCB and Cambridge Isotope Labs., respectively. The electron ionization mass and the ^{13}C NMR spectra confirmed that both compounds were the 1,2- and not the 1,1-dibromo isomer.

Stock solutions of EDB-H_4 and EDB-D_4 were prepared by accurate weighing and serial dilution in hexane or pentane (HPLC grade, Fisher Scientific, Cincinnati, OH, U.S.A.) to give final calculated concentration errors of +0.1%.

The isotopic purity of both materials was determined by GC–MS with selected ion monitoring. The ions at nominal mass 107 and 109 ($C_2H_4Br^+$) were monitored for EDB-H_4, while the ions at nominal mass 111 and 113 ($C_2{}^2H_4Br^+$) were monitored for EDB-D_4. Approximately 200 pg of each component was separately injected onto the capillary column, and the selected ion currents were integrated as a function of time. The integrated peak areas at the appropriate GC retention times confirmed that the unlabeled material was not contaminated with EDB-D_4, and had an undetectable contribution at *m/z* 111 from the naturally occurring isotopes. EDB-D_4 had an isotopic purity of *ca.* 97%.

Gas chromatography–mass spectrometry conditions

All GC–MS results were obtained on a Hewlett-Packard 5985B GC–MS system equipped with an HP-5840 gas chromatograph. The gas chromatograph was interfaced directly to the mass spectrometer through a heated (275°C) fused-silica line (*ca.* 1 m × 0.25 mm I.D.) threated directly to the ionization source. Electron ionization mass spectra were obtained at 70 eV with an ion source temperature of 200°C. For the quantitative studies, 10 μl of sample (split 10:1) was injected (250°C) onto a J&W 30 m × 0.326 mm I.D. DB-5 fused-silica column (1 μm film thickness). The column was maintained at 70°C. Helium was used as the carrier gas with a flow-rate of 1.5 ml/min to the mass spectrometer. Ion dwell times of 100 msec were employed for the selected ion monitoring experiments, and the electron multiplier was operated at 2800 V. For maximum sensitivity applications, only a single ion was monitored for each component. Under these conditions, it was possible to detect 1.8 pg EDB on column with a signal-to-noise ratio $\geqslant$ *ca.* 2.

Gas chromatography conditions

A Perkin-Elmer gas chromatograph equipped with a quartz injector trap[15] was used for the analysis of large volumes of gaseous or liquid samples. The analysis was performed with a 50 m × 0.32 mm I.D. Hewlett-Packard OV-101 fused-silica capillary column (0.25 μm film thickness). The effluent was split 1:1 between flame ionization detector and electron-capture detector. The flow-rate to each detector was set to 10 ml/min with helium, and an additional 100 ml/min of nitrogen was added to the stream going to the electron-capture detector. The electron-capture detector used is a constant frequency, ^{63}Ni detector (Antek Instruments, Model 245, Houston, TX, U.S.A.). This unit was operated at 300°C, with a period of 500 μsec and a pulse-width of 4 μsec. The resulting peaks were displayed on a Hewlett-Packard recorder (Model 7132A) and were integrated via a Nelson Analytical 4400 data system (Nelson Analytical, Cupertino, CA, U.S.A.).

Experiments designed to measure the temperature dependence of the relative retention, α, were performed on a SiChromat 2 GC (Siemens, ES Industries, Marlton, NJ, U.S.A.). Analyses were performed with two Quadrex columns (Quadrex, New haven, CT, U.S.A.): a 25 m × 0.32 mm I.D. cross-linked 007 methylsilicone column (5 μm film thickness) and a 25 m × 0.32 mm I.D. SLP Carbowax 20 M (0.5 μm film thickness).

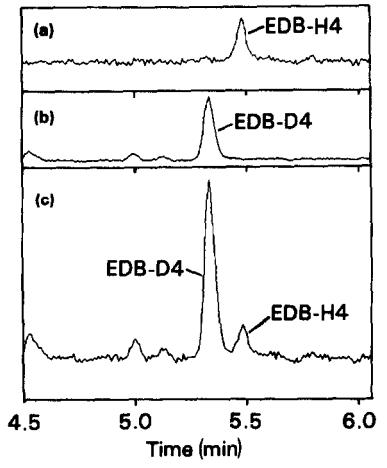
RESULTS AND DISCUSSION

Separations

The separation of EDB-D_4 from EDB-H_4, obtained in the GC–MS system, is illustrated in Fig. 1. These data were obtained by selected ion monitoring of a standard containing 11.4 pg/μl of EDB-H_4 and 68.1 pg/μl of EDB-D_4 in hexane. The upper trace (*m/z* 107) is specific for the unlabeled material, the middle trace (*m/z* 113) is specific for the fully deuterated (D_4) material, and the lower trace is the reconstructed total ion chromatogram. Baseline resolution is evident in the total ion chromatogram. Comparable resolution is maintained in analyses where full mass spectral scans are collected (200 ng on column). It is noteworthy that the separation is achieved isothermally, in less than 6 min, allowing high sample throughput.

The separation factor, α, in the perkin-Elmer GC system is 1.03 at 60°C, as calculated from Fig. 2. The temperature dependence of α in the cross-linked methyl silicone phase is given by the expression:

$$\alpha = -1.219 \cdot 10^{-4} T + 1.038$$



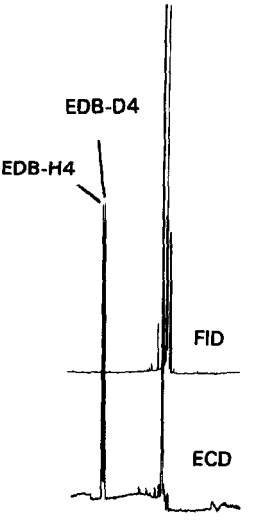


Fig. 1. Reconstructed total ion chromatogram (c) and selected ion chromatograms (a, *m/z* 107; b, *m/z* 113) obtained from the GC–MS analysis of an EDB-H_4 and EDB-D_4 mixture.

Fig. 2. Chromatogram of a pentane solution containing 180 pg/μl of EDB-H_4 (retention time: 7.50 min) and EDB-D_4 (retention time: 7.36 min). Injection volume, 0.9 μl; mass to detectors, 80 pg of each compound. OV-101 column, 70°C. FID = Flame-ionization detection; ECD = electron-capture detection.

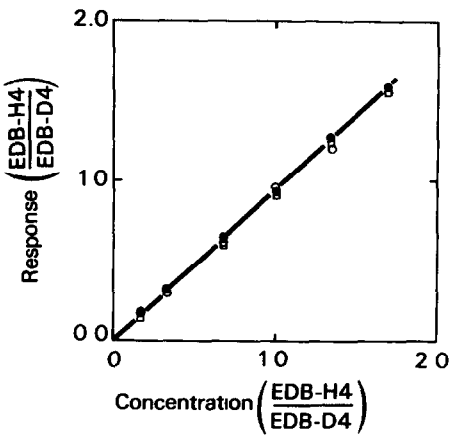
where T is the column temperature in °C. The correlation coefficient, r^2, was 0.99. This expression is valid over the 70–120°C range. The calculated α is in good agreement with the value measured in the OV-101 column. The separation factor remained reasonably constant (1.033 + 0.001) in the Carbowax 20M column over the temperature range 60–90°C.

The large separation factors indicate that the separation of EDB-H_4 from EDB-D_4 is easily achieved using commercially available capillary columns. Furthermore, these factors are larger than expected on the basis of simple mass-difference considerations[13], and we are currently exploring the reason(s) for this large effect. Notice also that the fully deuterated compound elutes earlier than the protiated species. This is an example of a reverse isotope effect[12].

Quantitation

Calibration curves were prepared using the EDB-D_4 as the internal standard. In the GC–MS experiments, the concentration of this material was held constant at 68 pg/μl and the concentration of EDB-H_4 was varied from 9 to 114 pg/μl. Calibration curves were obtained by plotting the calculated ratio of EDB-H_4/EDB-D_4 *versus* the experimentally measured ratio. The open data points in Fig. 3 represent duplicate analyses performed on a given day, while the closed points are averaged results of three determinations performed on the following day. Linear regression analysis by least squares gave a typical calibration line $y = 0.93x - 0.004$ with a linear correlation coefficient of 0.999. It is evident from Fig. 3 that the day-to-day reproducibility of this method is very good, and within any given day, the relative standard deviation at the low end of the calibration line is on the order of 10%.

Data were also acquired by GC–electron-capture detection (ECD), Fig. 4. A similar set of standards was used and a typical regression analysis gave a least squares fit of $y = 1.02x + 0.009$, with a linear correlation coefficient of 0.992. The higher



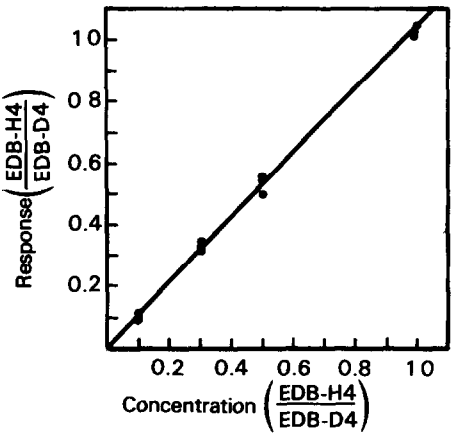
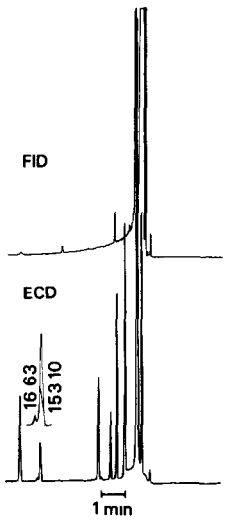


Fig. 3. GC–MS calibration curve obtained from the analysis of standard mixtures of EDB-H_4 and EDB-D_4. The concentration of EDB-D_4 was held constant at 68 pg/μl.

Fig. 4. GC–ECD calibration curve obtained from the analysis of standard mixtures of EDB-H_4 and EDB-D_4. The data points represent individual injections with varying masses of EDB-H_4 and EDB-D_4. Mass range: EDB-H_4 7–265 pg, EDB-D_4 70–285 pg to ECD.



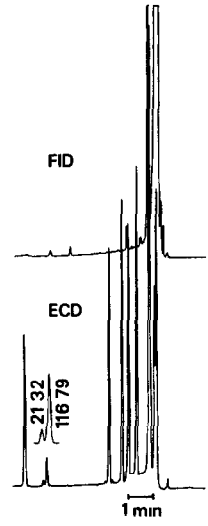


Fig. 5. Chromatogram of a 10-ml sample of headspace in equilibrium with water containing a 1:10 mixture of EDB-H_4/EDB-D_4. Mass of EDB-H_4 reaching ECD is *ca.* 8 pg. Experimental area ratio: 16.6/153.1 = 0.108.

Fig. 6. Chromatogram of a 10-ml sample of headspace in equilibrium with water containing a 1:10 mixture of EDB-H_4/EDB-D_4 and 1 g of flour. Experimental area ratio: 21.3/116.8 = 0.182.

slope and intercept reflect a slight non-linearity in ECD response at the high end of the calibration line. The ECD data are representative of the worst case analysis since the injection volumes and total sample mass were intentionally varied over a wide range (7.3–265 pg EDB-H_4, 70–285 pg EDB-D_4).

We have used this isotope dilution method, with either ECD or MS detection, to analyze a large number of samples prepared by a variety of sample preparation methods. We have utilized simple injections of hexane extracts or distillation extracts[16] as well as equilibrium headspace techniques. Fig. 5 shows a headspace analysis (10 ml of air) above a water standard containing a 10:1 ratio of EDB-D_4/EDB-H_4. The total mass of EDB-H_4 present in this system, 1 ng, corresponds to 1 ppb of EDB in flour when a 1-g sample is taken. The experimental area ratio is 0.108. Fig. 6 shows the increase in EDB-H_4 response caused by the presence of this material in a flour sample. The area ratio is 0.182 corresponding to a concentration of about 0.8 ppb in flour. This value was independently confirmed by a distillation–extraction procedure[16].

CONCLUSIONS

The large separation factors observed for EDB-H_4–EDB-D_4 mixtures indicate that these compounds can be separated over a wide range of temperatures, using commercially available capillary columns. Because of the similar behavior of these two compounds in bulk sample preparation procedures, EDB-D_4 is an ideal internal standard for the measurement of EDB-H_4 at trace and ultra-trace levels using inexpensive GC detectors. We have used this approach extensively in the analyses of

flour samples by a variety of sample preparation methods, including distillation–extraction, simple extractions with hexane, and headspace.

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