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## Note

### Gas-liquid chromatographic analysis and purification of chloroform

M. MALAIYANDI, K. L. TANG and J. P. BARRETTE

Plant Products Division, Agriculture Canada, C. E. F. Ottawa, Ontario K1A 0C5 (Canada)

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During the development of a highly sensitive analytical gas-liquid chromatographic (GLC) electron-capture procedure for the estimation of diethylstilbestrol (DES) (as its trifluoroacetate) in animal tissues and plant materials, chloroform-benzene mixtures were used at the extraction step. Extractions of a sample containing known amounts of the oestrogenic material with varying amounts of chloroform-benzene mixtures were processed, derivatized and analysed for DES. From the chromatograms it was observed that the ratio of the peak heights of both *cis*- and *trans*-DES (34.6:65.4) was varying. Not only was the ratio of the geometric isomers unpredictable, but also at times the values were reversed. After considerable investigation concerning this anomaly it became apparent that the solvent mixture benzene-chloroform (93:7) mixture was responsible for these spurious results.

Attempts to purify chloroform by conventional methods<sup>1</sup> did not eliminate the above anomaly. In this communication we wish to report a simple distillation method for purifying chloroform so that minimal residues are carried along with the distillate and chromatograms are provided in support of this technique.

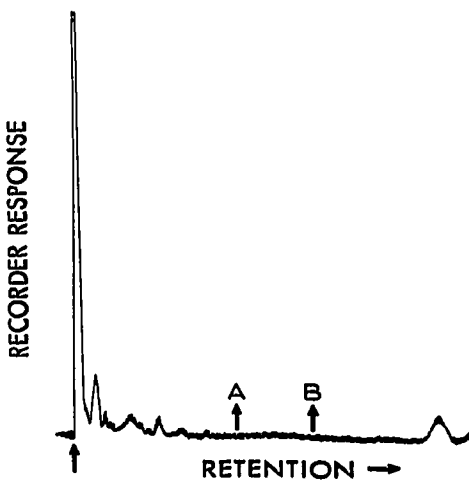
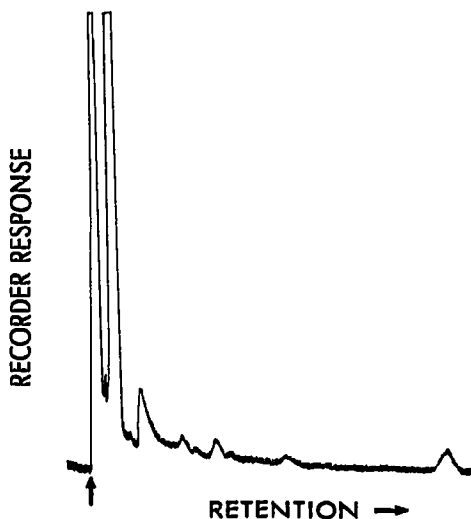


Fig. 1. Electron-capturing residues of re-distilled trifluoroacetic anhydride.

Fig. 2. Electron-capturing residues of re-distilled pesticide-grade benzene. A and B indicate the trifluoroacetates of *cis*- and *trans*-diethylstilbestrol, respectively.

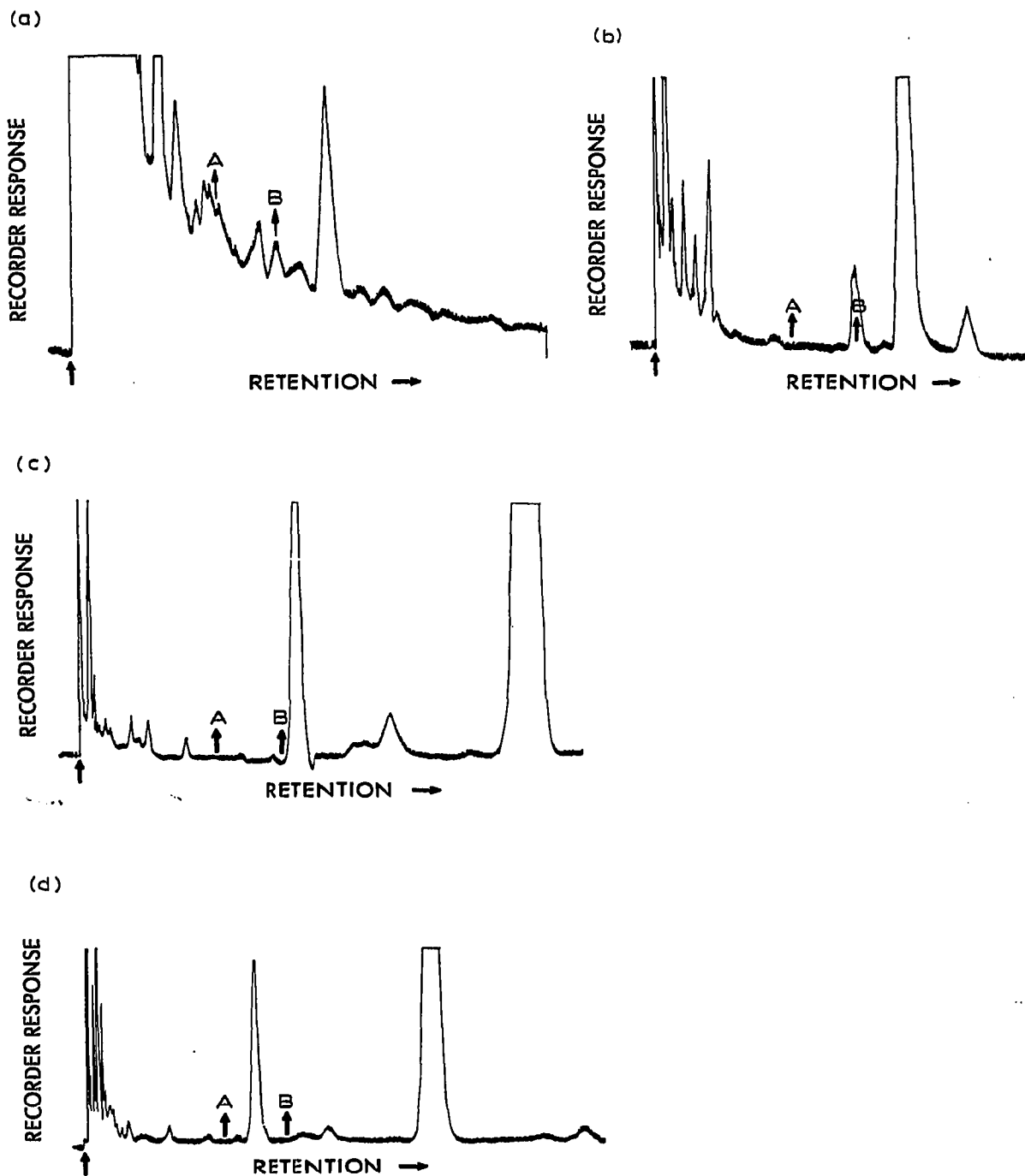


Fig. 3a-d. Electron-capturing residues of four types of commercially available pesticide-grade chloroform prior to purification. A and B, as in Fig. 2.

## MATERIALS AND METHODS

The benzene used in this experiment was of pesticide-grade quality; it was further distilled over anhydrous  $\text{Na}_2\text{CO}_3$  and dried over anhydrous alumina (Fisher Scientific, Pittsburgh, Pa., U.S.A.) grade 1 (previously heated to  $540^\circ$  for 14–16 h and cooled). Four brands of commercially available pesticide-grade chloroform were used in this experiment, as obtained.

About 200 ml of chloroform from each source was separately evaporated using a Büchi Rotavapor (W. Büchi, Flawil, Switzerland) to about 3 ml and after quantitative transference to a 5-ml centrifuge tube the residual solvent was evaporated almost to dryness at  $30^\circ$  with a gentle stream of ultra-pure dry nitrogen. About 1 ml of re-distilled trifluoroacetic anhydride (b.p.  $39^\circ$ ) (for purity, see Fig. 1) was added to the residue in the centrifuge tube at  $30^\circ$ . After 1 h, excess reagent was evaporated off using a nitrogen stream to leave residue (free of trifluoroacetic acid, if any, and its anhydride), which was then dissolved in 1 ml of purified, anhydrous toluene (distilled over  $\text{LiAlH}_4$ ) and analysed using a Hewlett-Packard Model 5700A gas chromatograph (Hewlett-Packard, Avondale, Pa., U.S.A.) with a  $^{63}\text{Ni}$  electron-capture detector under the following conditions: column packing, 11% OV-17+QF-1 on Gas-Chrom Q, 80–100 mesh (supplied by Applied Science Labs., State College, Pa., U.S.A.) in 6 ft.  $\times$  1/4 in. borosilicate glass column; column temperature,  $180^\circ$ ; injection port temperature,  $200^\circ$  (on-column injection); detector temperature,  $200^\circ$ ; carrier gas, argon–methane (90:10) at 60 ml/min; chart speed, 0.25 in./min; attenuation,  $\times 8.0$ .

Fig. 2 shows the impurities, as electron-capturing materials, present in the re-distilled pesticide-grade benzene, whereas Figs. 3a–d depict the impurities present in four samples of commercially available pesticide-grade chloroform. One of the samples of chloroform showing a large number of impurities (see Fig. 3a) was distilled under dry nitrogen atmosphere and low actinic light using a 12 in.  $\times$  1 in. glass column packed with borosilicate helices and the rate of distillation being maintained at 5–7 ml/min. The distillate was stored in amber-coloured glass containers under nitrogen. (*Note.* Irradiated chloroform with normal daylight lamps seems to contain the same impurities as found in the commercial solvents.)

About 200 ml of the distillate was processed in the manner previously

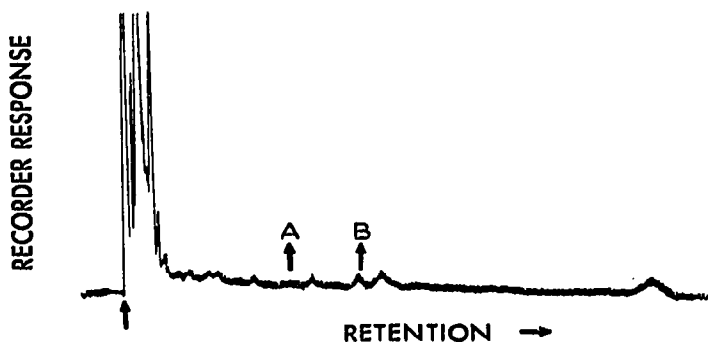


Fig. 4. Electron-capturing residues of one of the chloroform samples after purification. A and B, as in Fig. 2.

described to determine the electron-capturing residues. Fig. 4 shows the impurities present in chloroform purified by the proposed procedure.

It is apparent that, by this proposed technique, it is possible to obtain high-purity chloroform for extraction purposes involving GLC-electron-capture analysis of organic compounds. No attempts have been made to identify and characterize the impurities found in pesticide-grade chloroform.

#### REFERENCE

- 1 J. A. Riddick and W. B. Bunger, *Techniques of Chemistry, Vol. II, Organic Solvents*, Wiley-Interscience, New York, 3rd ed., 1970, pp. 771-773.