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Note

A re-examination of the gas chromatographic determination of α-*d*-propoxyphene

A number of reports have been published on the gas chromatographic determination of α-*d*-propoxyphene (Darvon) in blood[1] and plasma[2–4] samples. The need for a quantitation procedure in our laboratories led to an examination of these methods, which were found to be generally inadequate. Significant amounts of decomposition at low masses of α-*d*-propoxyphene were reported on different column packings[2,3]. However, WOLEN AND GRUBER[4] described the successful determination of plasma levels of α-*d*-propoxyphene using a rather short (30 cm) column of UC-W98 on Diatoport S. Decomposition was not observed[4], but low column efficiencies were obtained (*ca.* 375 theoretical plates), which limits separation of complex mixtures such as might be encountered with blood or plasma extracts. More importantly the column packing used in this procedure (Diatoport S, Hewlett-Packard Co., Avondale, Pa.) is no longer commercially available.

When we attempted to repeat the work of WOLEN AND GRUBER using UC-W98 on Chromosorb W, significant amounts of decomposition were observed. A study was thus undertaken to ascertain the cause of decomposition, and to devise another feasible gas chromatographic procedure for the quantitative determination of α-*d*-propoxyphene. The only difference between the conditions of WOLEN AND GRUBER and those in our work was the type of column support employed. Since Diatoport S is no longer available* we examined several existing, similar supports. These supports, like Diatoport S, and "W" type supports, are flux-calcined diatomaceous earths, which have been acid washed and silanized.

Materials and methods

α-*d*-Propoxyphene was extracted from Darvon capsules. The product was homogeneous on TLC (two systems) and the R_F value was identical to authentic *d*-propoxyphene (a gift from Eli Lilly Co.). The UV spectrum was likewise identical to published data[5].

Analyses were conducted using a Fisher Victoreen 4400 series gas chromatograph equipped with a flame ionization detector. Glass columns (30 cm × 4 mm I.D. of 1.8 m × 2.5 mm I.D.) were silanized with Silyl 8 (Pierce Chem. Co., Rockville, Ill.) or dimethylchlorosilane (DMCS) and then rinsed with methanol, acetone, and dried. The short columns contained: 3.8% UC-W98 on Chromosorb W (AW/DMCS) 80–100 mesh, lot 241 (column I); 3.8% UC-W98 on Chromosorb W (HP) 80–100 mesh,

* Upon completion of this work, we were able to obtain some Diatoport S (a gift from Hewlett-Packard) from which we constructed a column identical to that reported by WOLEN AND GRUBER[4]. Repeated injections of low masses (*ca.* 40 ng) of α-*d*-propoxyphene led to decomposition patterns similar to those shown in Fig. 1A.

lot 261 (column II); 3.8% UC-W98 on Supelcoport 80–100 mesh, lot 070 (column III).

The longer columns were packed with: 3.0% OV-17 on Chromosorb W (HP) 80–100 mesh, lot 261 (column IV); 3.0% OV-17 on Chromosorb W (AW/DMCS) 80–100 mesh, lot 241 (column V); 3.0% OV-17 on Supelcoport 80–100 mesh, lot 070 (column VI); 3.0% SE-30 on Chromosorb W (HP) 80–100 mesh, lot 261 (column VII); 3.0% SE-30 on Chromosorb W(AW/DMCS) 80–100 mesh, lot 241 (column VIII).

Instrumental parameters for short columns (I–III) were: oven temperature, 172°; injection port, 205°; detector bath, 195°; and a carrier flow (nitrogen) of 60 ml/min. For the longer columns (IV–VIII) the parameters were: oven temperature, 180° or 200°; injection port, 240°; detector, 220°; and carrier flow 55 ml/min. Carbon disulfide was used as the solvent.

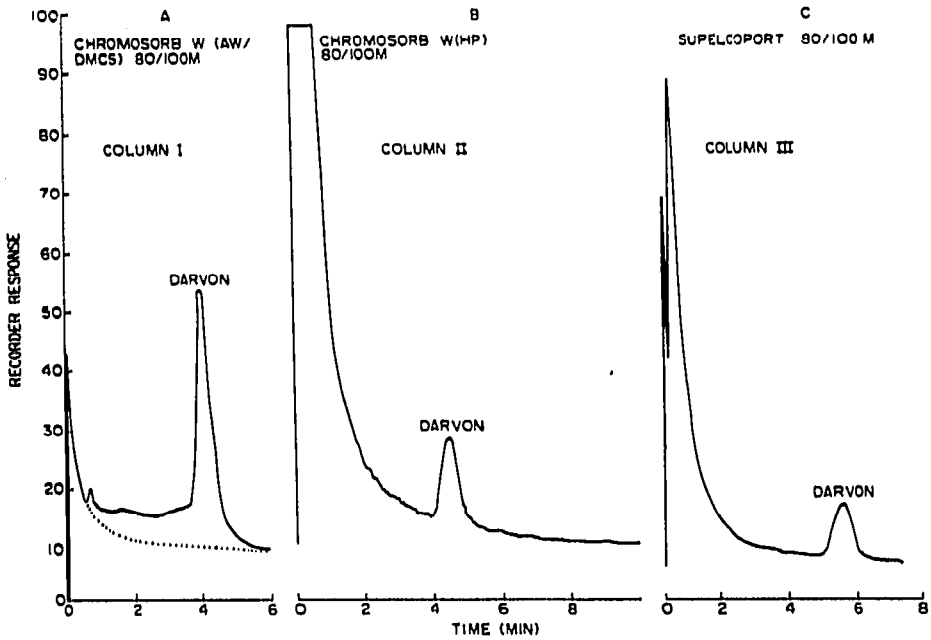

Fig. 1. Gas–liquid chromatograms of *α-d*-propoxyphene on UC-W98 liquid phase and various solid supports (0.3 M × 4 mm I.D. glass column). (A) 113 ng injected; $1.6 \cdot 10^{-10}$ a.f.s. (B) 28 ng injected; $4 \cdot 10^{-11}$ a.f.s. (C) 113 ng injected; $1.6 \cdot 10^{-10}$ a.f.s.

Results and discussion

The results shown in Fig. 1 indicate that the nature of the support affected the quality of chromatography for *α-d*-propoxyphene. Chromosorb W (AW/DMCS) caused breakdown of the substrate during its transit through column I, as is indicated by the drop in baseline just after the *α-d*-propoxyphene peak. Symmetrical peaks with no evidence of decomposition and good baseline characteristics were obtained on columns II [Chromosorb W (HP)] and III (Supelcoport). A linear dynamic study of mass *vs.* detector response was conducted using column II. A linear (least squares fit) response was observed over the entire range (17.8–357 ng) (Fig. 2). A near zero intercept for this curve indicated that essentially no decomposition of the substrate occurred. (Regression equation: $y = 0.6182 + 0.1678x$).

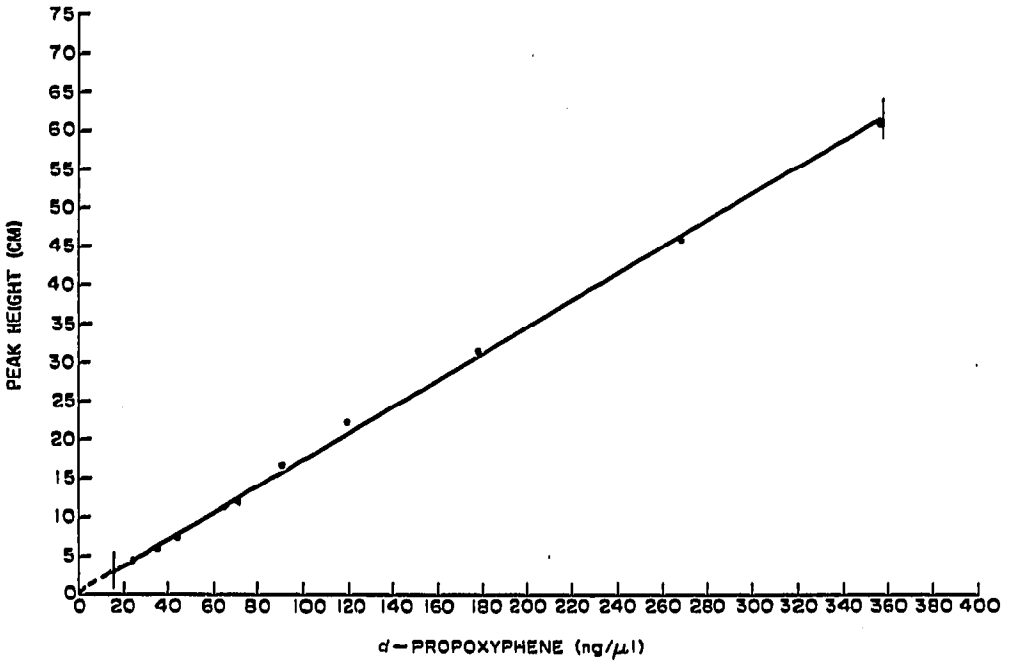

Fig. 2. Standard curve for *α-d*-propoxyphene using column II. Average of two injections with each concentration.

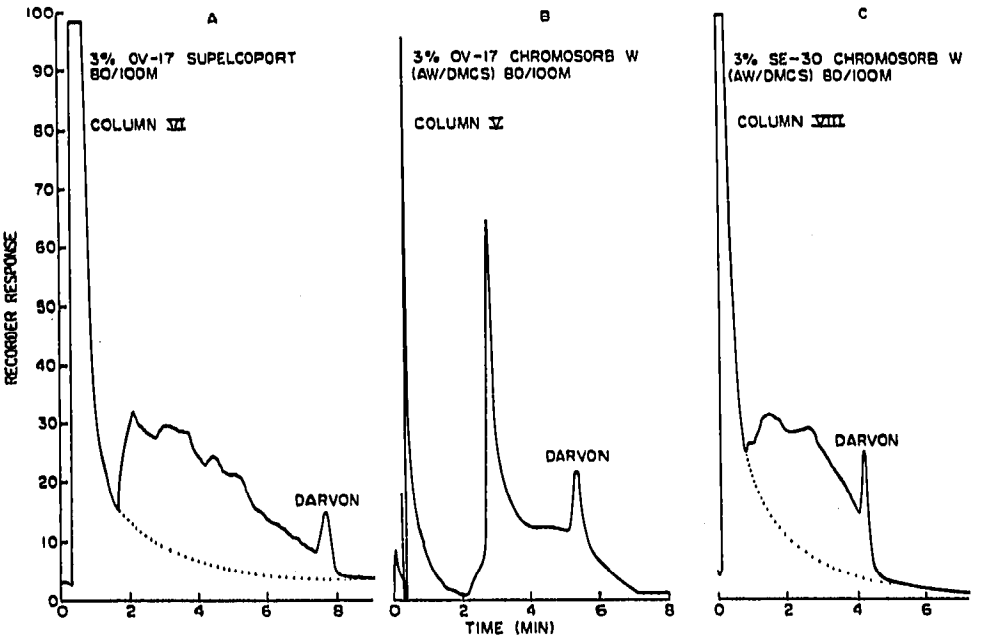

Fig. 3. Effect of solid supports on gas–liquid chromatography of *α-d*-propoxyphene. (A) 140 ng injected; $1.6 \cdot 10^{-10}$ a.f.s. (B) 140 ng injected; $3.2 \cdot 10^{-10}$ a.f.s. (C) 140 ng injected; $1.6 \cdot 10^{-10}$ a.f.s.

Examination of *α-d*-propoxyphene on 1.8-m columns containing Chromosorb W (AW/DMCS) at temperatures up to 200° revealed more striking amounts of decomposition, and now Supelcoport also appeared to be inferior (Fig. 3). In contrast, the breakdown pattern was not observed on Chromosorb (HP) (Fig. 4—small peaks due to solvent impurities).

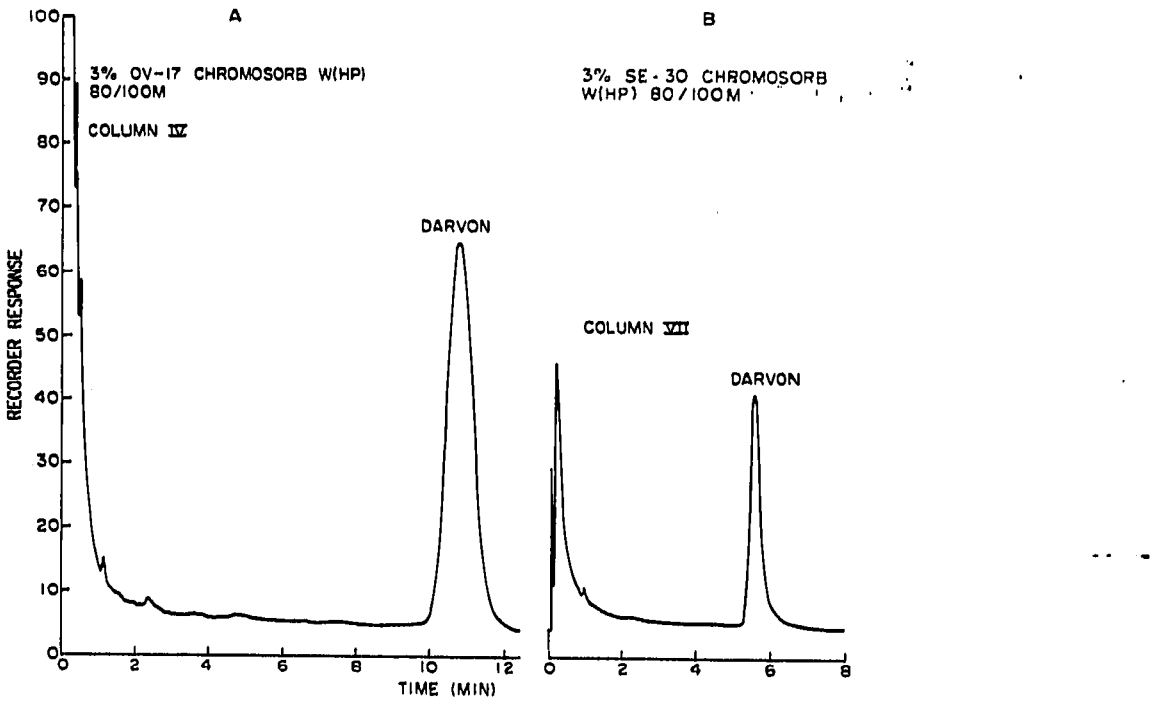

Fig. 4. Gas–liquid chromatography of *α-d*-propoxyphene on Chromosorb W (HP). (A) 140 ng injected; $1.6 \cdot 10^{-10}$ a.f.s. (B) 80 ng injected; $1.6 \cdot 10^{-10}$ a.f.s.

The above results indicate that the breakdown of *α-d*-propoxyphene was attributable to the acidic surface activity of the solid support, and was not associated with the use of higher temperatures. Further evidence for this explanation was provided by *in situ* silanization experiments. Silanization with reagents such as DMCS, which yields an acidic by-product (HCl), produced decomposition of *α-d*-propoxyphene, while this breakdown did not occur before silanization. Thus columns IV and VII (Fig. 4) produced the chromatograms shown in Fig. 5 after injection of 40 μl of DMCS followed by an equivalent of methanol. In contrast, when silanization was conducted with a reagent such as Silyl-8 (Pierce Chem. Co.) which yields ammonium chloride as one of the products, no change in the chromatographic pattern of *α-d*-propoxyphene was noted. The same results were obtained with hexamethyldisilazane (HMDS) (which yields ammonia as the by-product). Deactivation of surface sites for *α-d*-propoxyphene analysis was thus best accomplished with reagents such as HMDS or Silyl-8.

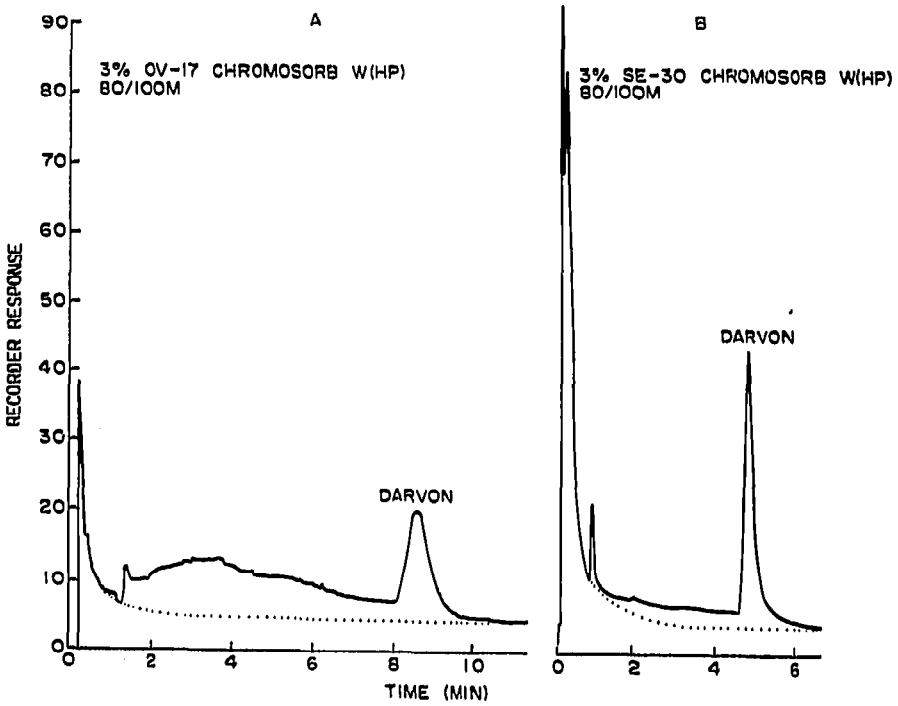

Fig. 5. Effect of *in situ* silanization on gas chromatographic column performance. (A) 140 ng injected; $1.6 \cdot 10^{-10}$ a.f.s. (B) 140 ng injected; $1.6 \cdot 10^{-10}$ a.f.s.

The authenticity of the gas–liquid chromatographic peak ascribed to *α-d*-propoxyphene was verified by combined gas–liquid chromatography–mass spectrometry, using an LKB 9000 gc mass spectrometer (LKB Produkter AB Stockholm-Brommal, Sweden). The mass spectrometric scan of the *α-d*-propoxyphene peak was identical, with respect to fragmentation, to the mass spectrum of authentic *α-d*-propoxyphene obtained via direct probe. A precise peak match of the [M−1]ion of the latter completed the verification. The parent ion was visible in this spectrum, but was much less intense than the [M−1).

Our results indicated that, of the solid supports examined, Chromosorb W (HP) was the best substitute for Diatoport S. The other supports, Chromosorb W (AW/DMCS) and Supelcoport, possess sufficient surface activity to render them undesirable for the analysis of small quantities of *α-d*-propoxyphene.

Another "W" type support, Gas Chrom Q (lot Q-66), which is acid and base washed, was briefly examined (3% SE-30) and found to give results similar to those obtained using the high performance (HP) columns.

The difference between the acid washed and acid washed high performance column packings, at least with regard to *α-d*-propoxyphene, lies in the degree of surface activity associated with each. Both supports were similarly prepared; the greater inertness of the HP grade packing may be a reflection of a higher degree of quality control in the preparation of this support.

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