

## Gas Chromatographic Studies of Methylpentynol, Ethchlorvynol, Ethinamate, and Propinamate

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The estimation of volatile, acetylenic drugs by gas chromatography is studied. The compounds investigated are methylpentynol, ethchlorvynol, ethinamate, and propinamate. Three types of detectors and four different column materials are compared. Gas chromatograms obtained after the isolation of the drugs from biological material are presented.

The acetylenic compounds methylpentynol (3-methyl-1-pentyn-3-ol), ethchlorvynol (1-chloro-3-ethyl-1-penten-4-yn-3-ol), ethinamate (1-ethynyl cyclohexanol carbamate), and propinamate (1-propynyl cyclohexanol carbamate) have importance as central nervous depressants, hypnotics and sedatives. Chemical methods for the determination of these drugs in biological material are complicated. The ability of acetylenic compounds to form metal salts is the basis of some methods for the determination of methylpentynol (Perlman and Johnson,<sup>8,9</sup> Marley and Vane<sup>7</sup>), of ethinamate (*e.g.* Fischer and Specht,<sup>5</sup> Preuss and Mayer,<sup>10</sup> Kum-Tatt<sup>6</sup>), and of propinamate (Charlier *et al.*<sup>3</sup>). The reaction of an allyl group with phloroglucinol is described by Algeri, Katsas and Luongo<sup>1</sup> for the estimation of ethchlorvynol.

Cadman and Johns<sup>2</sup> briefly mention a gas chromatographic procedure for the determination of ethchlorvynol, using a UCON 50 HB 2000 column. In this paper, three types of detectors and a number of different column materials have been investigated for the analysis of each of the four sedatives concerned.

### EXPERIMENTAL

Methylpentynol from 0.5 g capsules of "Hexofen forte" (Hässle) was distilled at 20 mm Hg; the fraction distilling at 35°C was collected. Ethchlorvynol from 0.5 g capsules of "Placidyl" (Abbott) was distilled at 10 mm Hg; the fraction distilling at 73°C was utilized. The distillate and a sample of pure ethchlorvynol from Abbott had the same specific gravity, 1.070 at 20°C.

\* The Government Laboratory for Forensic Chemistry.

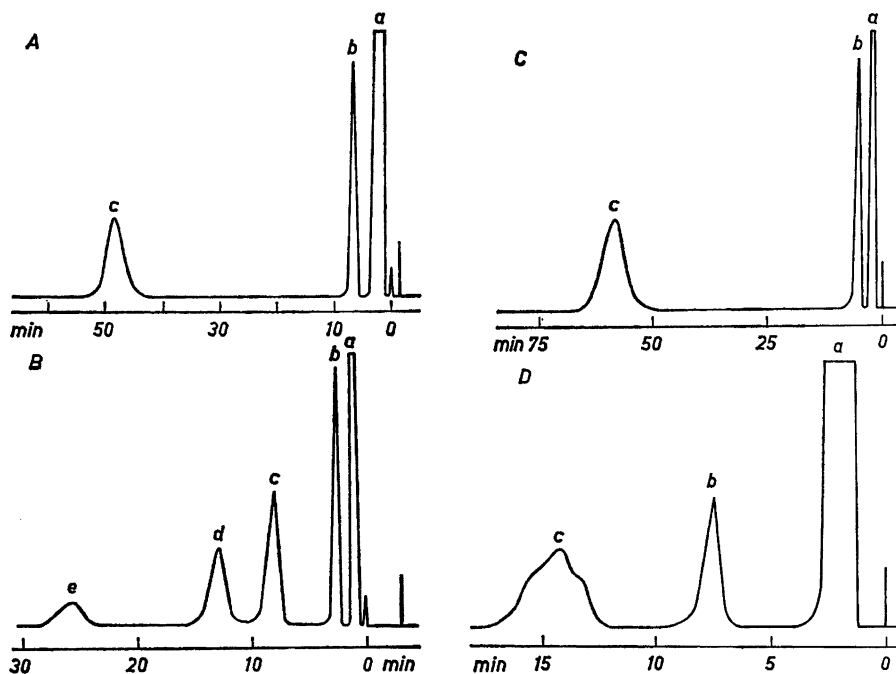


Fig 1.

- A. P-E 116 E. HWD. Col. "R". Temp. 190°. Gas pressure 1.0 kg/cm<sup>2</sup> of helium. Sample volume 5  $\mu$ l of the mixture.
- |                              |            |
|------------------------------|------------|
| a) Diethyl ether (solvent)   | Sens. 1/8. |
| b) Methylpentynol (20 mg/ml) | " 1/8.     |
| c) Ethchlorvynol (20 mg/ml)  | " 1/2.     |
- B. P-E 154 C. TBD. Col. "C". Temp. 192°. Gas pressure 1.0 kg/cm<sup>2</sup> of helium. Flow rate 3.0 l/h. Sample volume 5  $\mu$ l of the mixture.
- |                                       |            |
|---------------------------------------|------------|
| a) Acetone (solvent)                  | Sens. 1/8. |
| b) Methylpentynol (as liquid)         | " 1/8.     |
| c) Ethchlorvynol (as liquid)          | " 1/2.     |
| d) Ethinamate (100 mg/ml in acetone)  | " 1/1.     |
| e) Propinamate (100 mg/ml in acetone) | " 1/1.     |
- C. P-E 116 E. FID. Col. "W". Temp. 167°. Gas pressure 1.5 kg/cm<sup>2</sup> of nitrogen. Flow rate 4.8 l/h. Sample volume 5  $\mu$ l of the mixture.
- |                              |            |
|------------------------------|------------|
| a) Acetone (solvent)         | Sens. 1/4. |
| b) Methylpentynol (10 mg/ml) | " 1/4.     |
| c) Ethchlorvynol (10 mg/ml)  | " 1/1.     |
- D. P-E 116 E. FID. Col. "Q". Temp. 205°. Gas pressure 1.0 kg/cm<sup>2</sup> of nitrogen. Flow rate 7.0 l/h. Sample volume 5  $\mu$ l of the mixture.
- |                                   |            |
|-----------------------------------|------------|
| a) Carbon tetrachloride (solvent) | Sens. 1/4. |
| b) Ethinamate (10 mg/ml)          | " 1/4.     |
| c) Propinamate (10 mg/ml)         | " 1/4.     |
- Paper speeds varied from 120 to 1200 mm/h.

Crystalline samples of ethinamate (Schering) and of propinamate (Orion) were acquired through the Department of Forensic Medicine (Chemical Division) of the University of Helsinki, Finland. "Mekos" of Hälsingborg were kind enough to supply samples of propinamate crystals and tablets.

Two Perkin-Elmer gas chromatographs (P-E 154 C and P-E 116 E) were used: Model 154 C with a thermistor bead detector (TBD), and Model 116 E with both a flame ionization detector (FID) and a hot wire detector (HWD). A Siemens 2.5 mV and a Philips 1.0 mV recorder were employed. They were either connected both to the same detector or each to a different one when two detectors were used simultaneously.

The columns were packed by vibration into 1/4" aluminium tubing of 2 m length, using the following standard column material from the Perkin-Elmer Corporation:

"C" Silicone oil DC-200, dimethylsiloxane polymer, on chromosorb.

"Q" Apiezon "L" grease on GC-22 firebrick.

"R" Ucon oil LB-550-X, 20 % by weight on chromosorb.

"W" Carbowax 1500, polyethylene glycol, 10 % by weight on Teflon.

In order to stabilize the columns, a continuous flow of nitrogen was passed through them for two to three days in a thermostat.

Infrared spectra were recorded in a Hilger H 800 spectrophotometer, equipped with a microscope.

The drugs were injected as such in the liquid state (methylpentynol, ethchlorvynol), or as solutions in acetone, diethyl ether, carbon tetrachloride etc. Analytical grade solvents were used, or the solvents were distilled before use. 1 to 10 microliter samples were injected by the aid of Hamilton syringes.

When methylpentynol and ethchlorvynol were injected as such into the instrument, very low sensitivities had to be used, *viz* 2/32 with the flame ionization detector. The retention times stated in this paper are those measured from the air peak (corrected retention times) obtained when thermistor bead or hot wire detectors were used. With the flame ionization detector, a "signal giver" indicated the injection time electronically. Examples of the gas chromatograms obtained are seen in Fig. 1.

The compounds were assayed quantitatively by direct measurements of the peaks. Sample and standard were always injected consecutively under the same conditions. Since the peak height is very sensitive to variations in operating conditions, particularly the temperature, and the peak area is sensitive mainly to changes in the gas flow rate, both parameters were used in parallel for quantitative interpolation. The peak areas

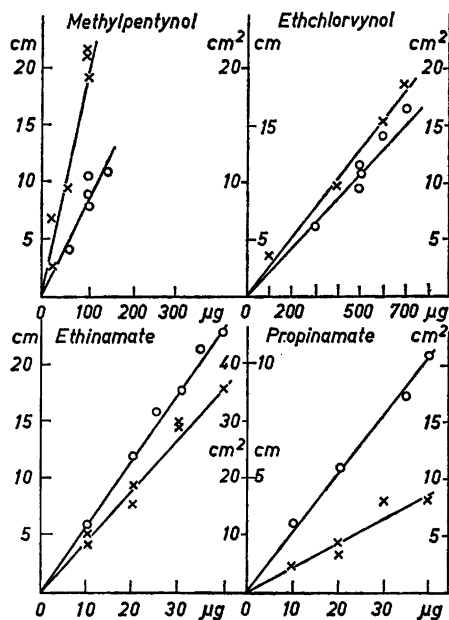


Fig. 2. Some calibration curves.  $\times$  Height of the peak in cm.  $\circ$  Area of the peak in  $\text{cm}^2$ . Operating conditions:

- A. Methylpentynol and ethchlorvynol in heptane. P-E 154 C. TBD. Column "C". Temp.  $145^\circ$ . Gas pressure 1.0 kg/ $\text{cm}^2$  of helium. Flow rate 3.0 l/h. Sens. 1/4. Paper speed 300 mm/h.
- B. Ethinamate and propinamate in acetone. P-E 116 E. FID. Column "Q". Temp.  $210^\circ$ . Gas pressure 1.0 kg/ $\text{cm}^2$  of nitrogen. Flow rate 8.0 l/h. Sens. 1/4. Paper speed 120 mm/h.

were measured by multiplication of the peak height by the width at half height. Some series of the peak height and area measurements are plotted in Fig. 2.

In order to check the yield of ethinamate and propinamate, added to biological material, 20 mg of either drug were added to 50 g of minced tissue. After the addition of water and solid sodium chloride, the mixture was steam distilled. An aliquot (100 ml) of the distillate was extracted with 3 portions of 100 ml diethyl ether. The extracts were combined, dehydrated over sodium sulfate, filtered, and evaporated to dryness without heating. The residue was weighed and dissolved in 1 ml of acetone. Aliquots were injected into the gas chromatograph.

### RESULTS

At the high temperatures necessary for the analysis of ethinamate and propinamate, methylpentynol appears so close to the solvents used that an analysis is impossible. Furthermore, under these conditions ethchlorvynol lies close to ethinamate. For separation of the peaks in columns "C" and "Q" at high temperatures, reduction of the gas flow rate proved helpful. The effect of an increase in temperature on the retention times of the compounds studied is seen in Fig. 3.

Ethinamate, and in particular propinamate, showed multiple peaks. The number of peaks for propinamate decreased with increasing temperature; at 190° there were 5 distinguishable peaks but at 245° only 2. However, the appearance of the peaks was the same for standard and tissue samples. Figs. 4 A and B illustrate chromatograms of propinamate standard and of a distillate from liver from a propinamate poisoning case; the corresponding chromatograms of ethinamate can be seen in Figs. 4 D and E.

In order to investigate possible chemical changes in the propinamate molecule during the gas chromatographic procedure, infrared spectra were recorded. No significant differences were observable in the propinamate spectrum after steam distillation and extraction with diethyl ether, after sublima-

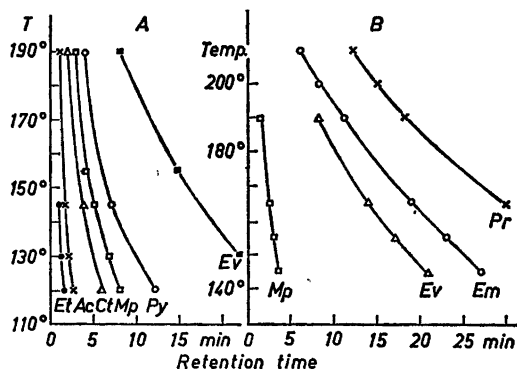
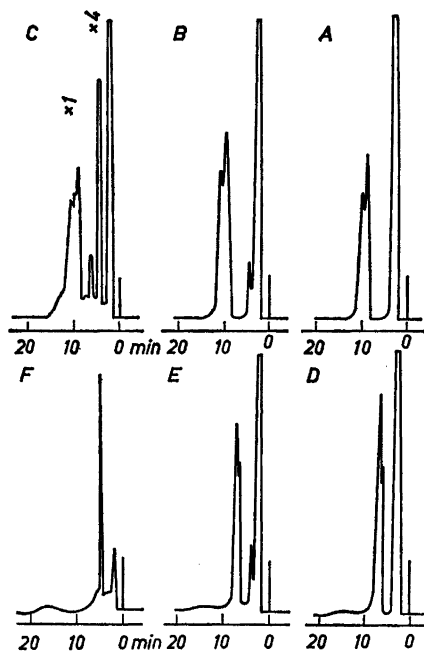


Fig. 3. Operating conditions:

- A. P-E 154 C. TBD. Column "C". Gas pressure 1.0 kg/cm<sup>2</sup> of helium. Flow rate 3.0 l/h. Sens. 2/32.
- B. P-E 116 E. FID. Column "Q". Gas pressure 1.0 kg/cm<sup>2</sup> of nitrogen. Flow rate 8.0 l/h. Sens. 2/32. Et=ether; Ac=acetone; Ct=carbon tetrachloride; Py=pyridine; Mp=methylpentynol; Ev=ethchlorvynol; Em=ethinamate; Pr=propinamate.

*Fig. 4.* Gas chromatograms of ethinamate, propinamate and glucuronic acid. P-E 116 E. FID. Colum "Q". Temp. 210°. Gas pressure 1.0 kg/cm<sup>2</sup> of nitrogen. Flow rate 9.5 l/h. Paper speed 120 mm/h. Sens. 1/2. Sample volume 2  $\mu$ l for A, B, D, E, and F; 5  $\mu$ l for C.

- A. Propinamate standard (10 mg/ml in carbon tetrachloride).  
 B. Propinamate, steam distilled from liver (calculated concentration 20 mg/ml).  
 C. Propinamate in urine, dose 0.7 g/day.  
 D. Ethinamate standard (10 mg/ml in carbon tetrachloride).  
 E. Ethinamate steam distilled from liver (calculated concentration 20 mg/ml).  
 F. Glucuronic acid standard (100 mg/ml in water).



tion in vacuo (1 mm Hg; 100°C), or after heating the dry crystals to 190° for 10 min in an oil bath (the brown liquid obtained recrystallized when cooling). Thus the appearance of multiple peaks in the gas chromatograms cannot be explained.

Table 1.

|                | Column | Temperature, °C. | Flow rate l/h | Carrier gas    | Detector | Sample size, $\mu$ g |
|----------------|--------|------------------|---------------|----------------|----------|----------------------|
| Methylpentynol | "C"    |                  |               | N <sub>2</sub> | FID      | 2-5                  |
|                | "Q"    | 130-             | 2.0-5.0       | He             | HWD      | 20-50                |
|                | "R"    | 150              |               | He             | TBD      | 20-50                |
|                | "W"    |                  |               |                |          |                      |
| Ethchlorvynol  | "C"    |                  |               | N <sub>2</sub> | FID      | 5-10                 |
|                | "Q"    | 150-             | 3.0-7.0       | He             | HWD      | 100-300              |
|                | "R"    | 200              |               | He             | TBD      | 100-300              |
|                | "W"    |                  |               |                |          |                      |
| Ethinamate     | "C"    | 160-200          | 4.0-8.0       | N <sub>2</sub> | FID      | 20-50                |
|                | "Q"    | 160-250          |               | He             | HWD      | 500-800              |
|                |        |                  |               | He             | TBD      | 500-800              |
| Propinamate    | "C"    | 160-200          | 4.0-8.0       | N <sub>2</sub> | FID      | 20-50                |
|                | "Q"    | 160-250          |               | He             | HWD      | 500-800              |
|                |        |                  |               | He             | TBD      | 500-800              |

The yield of ethinamate after steam distillation from liver was only 20 %, of propinamate 25 %. Since these solid compounds easily evaporate together with the solvent in the same way as methylpentynol and ethchlorvynol, distillation into the solvent might be preferable to extraction.<sup>4</sup> Studies of the isolation and quantitative estimation methods are still in progress, and will be published later in conjunction with the findings from some cases of intoxication with these drugs.

Since the flame ionization detector is sensitive to 2–5  $\mu\text{g}$  of methylpentynol, and 5–10  $\mu\text{g}$  of ethchlorvynol and does not react for water and ammonia, it can be used for the direct analysis of urine and of steam distillates from biological material. Columns "R" and "W" are particularly suitable for the analysis of aqueous solutions; however, the low maximum temperatures and the resultant long retention times limit their use. Propinamate was detected in urine after the administration of therapeutic doses (0.7 g/day) only after chemical isolation of the drug (Fig. 4 C). One of the peaks observed in the analysis of urine (Fig. 4 C) was due to glucuronic acid (Fig. 4 F). Glucuronic acid is evidently detectable in urine samples in this way.

Suitable conditions for the analysis of the four compounds are listed in Table 1. It is apparent from this table, that columns "C" and "Q" are the most suitable for the analysis of unknown samples. After a preliminary run at 150–160°C, the temperature can be increased or decreased depending on the presence of low boiling or high boiling compounds. This can be done without major manipulation of the instrument or the column. In our laboratory, columns "C" and "Q", in conjunction with a flame ionization detector and nitrogen as carrier gas, have been the most successful in the estimation of the four compounds in biological samples.

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Received October 28, 1963.