Journal of Chromatography, 107 (1975) 115–123

CHROM. 8077

HIGH-PRESSURE LIQUID CHROMATOGRAPHIC DETERMINATION OF PROPOXYPHENE HYDROCHLORIDE IN TABLETS AND CAPSULES

R. K. GILPIN*, J. A. KORPI and C. A. JANICKI
McNeil Laboratories, Inc., Ft. Washington, Pa. 19034 (U.S.A.)
(First received May 2nd, 1974; revised manuscript received November 4th, 1974)

SUMMARY

The determination of propoxyphene hydrochloride by gas chromatography (GC) has been examined. Under a number of operating conditions significant on-column decomposition has occurred. The amounts of decomposition have been found to be dependent on sample size, flow-rate, column temperature, liquid phase loading, and solid support material. In view of this work a high-pressure liquid chromatographic procedure for quantitating propoxyphene hydrochloride has been developed. This method has been found to be specific for propoxyphene hydrochloride in the presence of its heat decomposition products formed with and without acetaminophen. This method does not suffer from problems associated with GC procedures.

INTRODUCTION

Much work[1–7] has been concerned with the use of gas chromatography (GC) as a means of determining propoxyphene in a variety of samples. Arising from this work has been conflicting evidence as to the stability of propoxyphene under various GC conditions. Recently, when Sparacino *et al.*[7] attempted to repeat earlier experiments of Wolem and Gruber[2], they found significant amounts of on-column decomposition. The previous authors had reported no such problems. Sparacino *et al.*[7] also found that the particular column support had a major effect on decomposition.

When the work of Sparacino was repeated in our laboratory, we also found significant on-column decomposition and a lack of quantitation under a number of GC conditions. The amounts of decomposition have been found to be dependent on a number of chromatographic variables. Among these are: column temperature, sample size, flow-rate, liquid phase film thickness, and solid support material.

In order to circumvent the problems of on-column decomposition associated with GC assay procedures, the use of high-pressure liquid chromatography (HPLC) has been examined. A number of column materials and mobile phases have been investigated. In the light of this work a HPLC assay procedure for both capsules and

* To whom correspondence should be addressed.

tablets containing propoxyphene hydrochloride in the presence of acetaminophen has been devised. This method does not suffer from the problems associated with GC procedures.

EXPERIMENTAL

Equipment

All GC work was performed on either a Perkin-Elmer Model 900 or 990 gas chromatograph equipped with dual flame ionization detectors. All gases were research grade. Dried nitrogen was used as the carrier gas. GC sample collections were obtained by the aid of a Hewlett-Packard Model 7670A automatic injector and dry-ice trap.

All HPLC work was performed on a Waters Ass. Model 202 liquid chromatograph equipped with dual 6000 p.s.i. pumps and a solvent programmer. A 254-nm UV detector was used for all work. Samples were introduced by a 25-μl Precision Sampling syringe. Columns were maintained at ambient temperature.

Columns

All GC columns were prepared in the following manner: The various packing materials were packed into either 2-mm or 4-mm-I.D. glass columns 6 ft. in length. Before packing the columns were thoroughly cleaned and dried. They were packed by a combination of gentle tapping and suction from a vacuum line. All columns were conditioned for at least 24 h prior to use. Columns used include 2-mm-I.D. and 4-mm I.D. 1.5 and 3.0% SE-30 on Gas-Chrom Q and 2-mm-I.D. 1.0 and 3.0% SE-30 on Chromosorb W HP.

All HPLC columns were prepared in the following manner except the micro-silica column: The dry packing material was packed into 2 ft. $\times$ 1/8 in. O.D. (2.4 mm I.D.) stainless-steel columns. Before packing, the tubing was cleaned with a non-polar solvent and a polar solvent, and rinsed with acetone. These columns were dried before packing. They were packed by a combination of gentle vibration and suction from a vacuum line. Columns were conditioned with solvent prior to use. Columns examined include: Corasil II, C_{18} Corasil, Phenyl Corasil, OPN Porasil (Waters Ass., Milford, Mass., U.S.A.); Acid Alumina, LiChrosorb micro-silica (10 μm) (Merck, Darmstadt, G.F.R.).

The micro-silica column was packed by the following procedure: Silica gel (average particle diameter 10 μm) from Merck was dried at 200° for 4 h. A slurry was prepared by placing 1.0 g of the dried gel in 10 ml of 1,2-dibromoethane which had been purified by passing through a column of silica AR from Mallinckrodt (St. Louis, Mo., U.S.A.). The columns were packed using a Waters Ass. 6000-p.s.i. high-pressure pump and a slurry reservoir at a pressure of 5500 p.s.i.

After initial packing, the column was disconnected from the slurry reservoir and enough gel removed from the up-stream side of the column to insert a quartz wool plug. The column was connected to the pump and flushed with 100 ml of methanol followed by an equivalent volume of distilled water. The column was then removed and dried at 120° for 18 h while passing dry nitrogen through the column.

HPLC assay procedure

Calibration standards were prepared as follows: 25, 32, and 38 mg of pro-

poxyphene hydrochloride were weighed into three 1-oz. screw-cap bottles. To each, 10 ml of 0.1 *M* sodium hydroxide and exactly 10 ml of chloroform were added.

For the sample preparation, tablets or capsules containing 32.5 mg propoxyphene hydrochloride with or without acetaminophen were used. The average weight of twenty tablets or capsules was determined. The sample was pulverized if needed and an equivalent of 32.5 mg propoxyphene hydrochloride was weighed into a 1-oz. screw-cap bottle. 10 ml of 0.1 *M* sodium hydroxide and exactly 10 ml of chloroform were added.

Both samples and calibration standards were shaken for 30–45 min on a mechanical shaker. The sample was centrifuged, and the aqueous layer aspirated and discarded. 10 μl of the chloroform solution (3.25 μg/μl) were injected onto the HPLC column.

Chromatography

A 5% solution of conc. ammonium hydroxide in isopropanol was prepared. 3.25 ml of this solution were added to a 500-ml volumetric flask and diluted to volume with hexane. The final solution was slightly cloudy due to undissolved ammonia. This solution (mobile phase) was warmed on a steam-bath and degassed under vacuum until clear.

The 1/8-in.-O.D. × 25-cm micro-silica column was conditioned with at least 200 ml of the mobile phase. After conditioning the flow-rate was set at 1.5 ml/min and the UV detector (254 nm) at 0.08 absorbance units full scale. After the flow had stabilized, the 10-μl sample and standard solution injections were made.

Calculations

The area of all peaks was calculated. A working curve was prepared by plotting the peak areas *vs.* the concentrations of the standard solutions. From this curve the percentage of propoxyphene in each sample was determined.

$$\% = \frac{\text{Actual amount} \times 100}{\text{Theoretical amount}}$$

Specificity of method

Decomposed samples of propoxyphene were prepared in the following manner: 32.5 mg of propoxyphene hydrochloride were placed in a glass container. To an identical container a mixture of 32.5 mg propoxyphene and acetaminophen was added. Both samples were heated for 30 min at 200°.

RESULTS AND DISCUSSION

GC decomposition

Representative chromatograms of propoxyphene hydrochloride under several sets of GC operating conditions are shown in Fig. 1. In each case on-column decomposition was evident by several additional peaks and large trailing baseline from the solvent peak. To further examine this decomposition, repetitive 3.0-μg injections of propoxyphene hydrochloride under a typical set of GC conditions as given in Fig. 1a were made and the effluent collected in a dry-ice trap. When the detector was re-

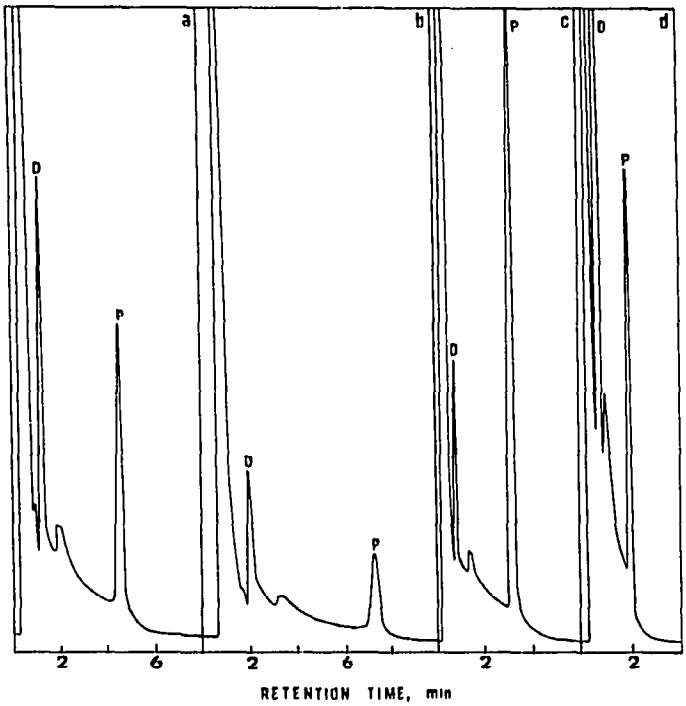

Fig. 1. Chromatograms showing on-column decomposition of propoxyphene hydrochloride as a function of various GC operating parameters: (a) Sample size, 0.78 μg; flow-rate, 41.8 ml/min; column temperature, 200°. (b) Sample size, 1.56 μg; flow-rate, 22.8 ml/min; column temperature, 200°. (c) Sample size, 1.56 μg; flow-rate, 49.5 ml/min; column temperature, 200°. (d) Sample size, 1.56 μg; flow-rate, 41.8 ml/min; column temperature, 225°. Other GC conditions: 3.0% SE-30 on Gas-Chrom Q; attenuation, ×128. P = Propoxyphene hydrochloride; D = main decomposition.

hydrochloride peak was significantly reduced in relation to other observed peaks as shown in Fig. 2a. Also the trailing baseline was almost completely eliminated due to the presence of little undecomposed material. High-pressure liquid chromatograms of the on-column decomposition collection along with a sample of undecomposed propoxyphene are shown in Figs. 2b and 2c, respectively. A peak eluting with the solvent and two additional peaks which eluted after propoxyphene were noted (Fig. 2b). The peak for propoxyphene was almost completely absent.

On-column decomposition was also studied as a function of several GC operating conditions. The various parameters examined include sample size, column temperature, and flow-rate or molecular resident time on the column. Representative chromatograms showing decomposition as a function of sample size, carrier flow-rate and column temperature appear in Fig. 1. Area ratios of the undecomposed propoxyphene peak to the main decomposition peak as a function of the various GC conditions are summarized in Figs. 3–5. These figures show general trends and not total on-column decomposition since significant amounts of decomposed material were contained in the trailing edge from the solvent and the other peaks. Also a 1.5% SE-30 on Gas-Chrom Q column has been examined. For both columns, on-column

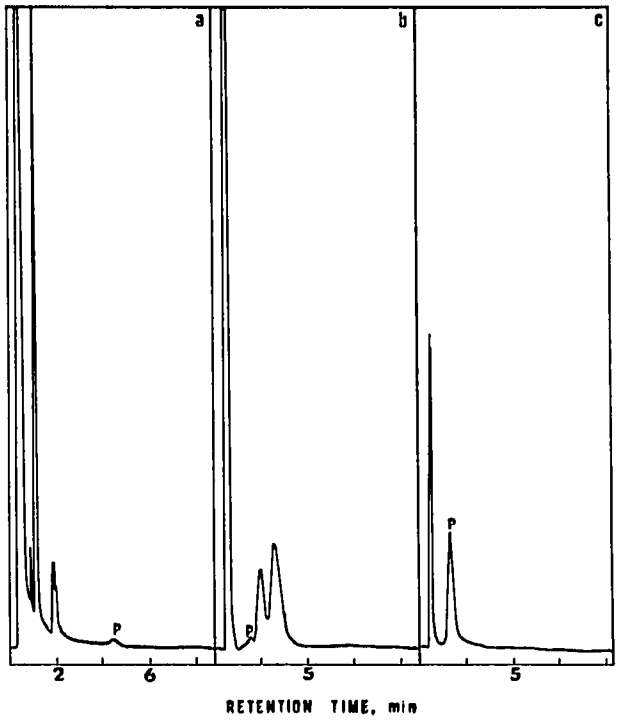

Fig. 2. (a) Gas chromatogram of GC column effluent. GC conditions same as Fig. 1a. (b) Liquid chromatogram of mixture shown in a. (c) Liquid chromatogram of undecomposed propoxyphene hydrochloride. LC conditions: 2 ft. × 1/8 in. C_{18} Corasil column; mobile phase, acetonitrile–0.01 *M* ammonium carbonate (7:3); flow-rate, 2.0 ml/min; range, 0.08 AUFS.

decomposition of propoxyphene hydrochloride has been found to be dependent on sample size, flow-rate and temperature.

1.5 and 3.0% SE-30 on Chromosorb W HP columns were also studied. Similar decomposition behavior to that of the Gas-Chrom Q columns was noted. However, the baseline before and after the propoxyphene peak was noticeably improved. The columns were further examined by temperature programming. 3.0% SE-30 on both Gas-Chrom Q and on Chromosorb W HP were investigated. Typical chromatograms obtained are shown in Fig. 6. The Chromosorb W HP columns were found to be preferable to Gas-Chrom Q columns. These data are in good agreement with data reported by Sparacino *et al.*[7]. However, in all cases, some on-column decomposition of propoxyphene hydrochloride was observed.

HPLC assay

Propoxyphene hydrochloride has been satisfactorily eluted with the following column/mobile phase combinations: Corasil II/isopropanol–hexane (1:99); OPN Porasil/isopropanol–chloroform (7:93); Acid Alumina/methanol–dioxane (1:9); C_{18} Corasil and Phenyl Corasil/acetonitrile–0.01 *M* ammonium carbonate (7:3); Li-Chrosorb SI 60 (10 μm silica/0.65% basic isopropanol (ammonium hydroxide–isopropanol, 5:95) in hexane.

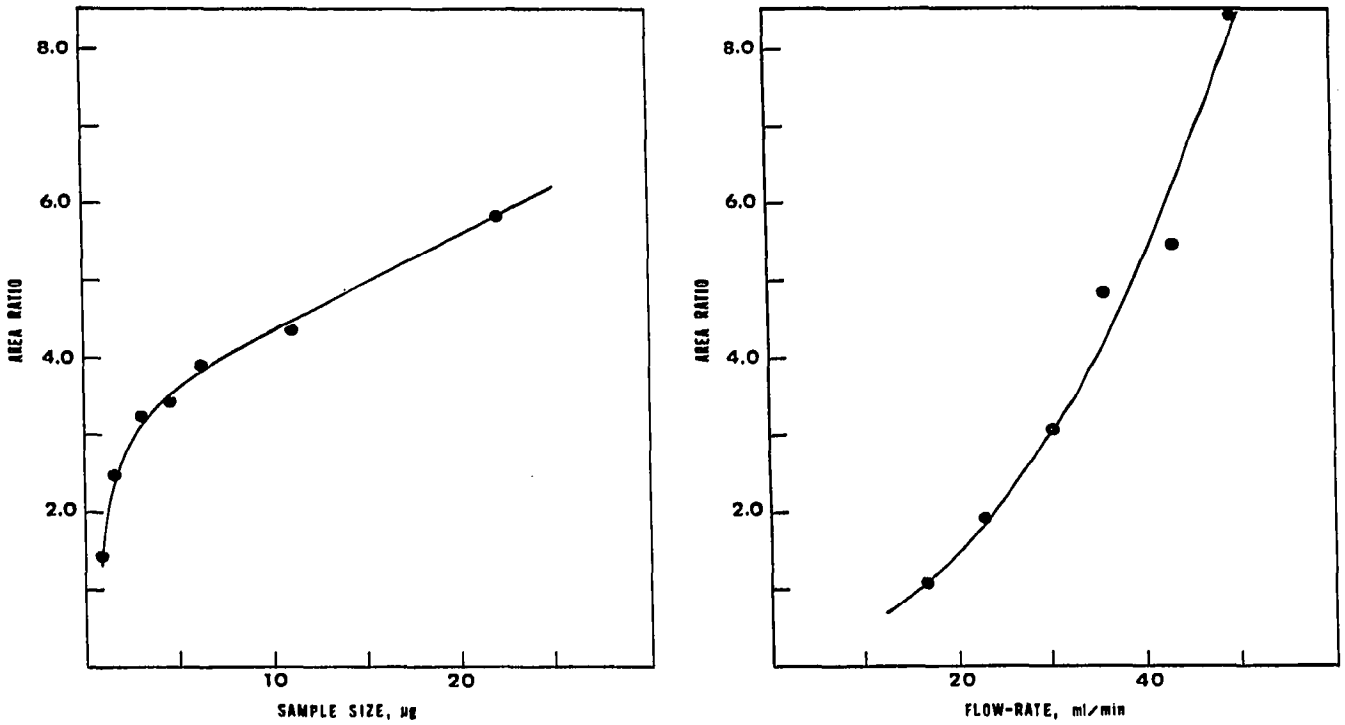

Fig. 3. Peak area ratio as a function of varying amounts of propoxyphene hydrochloride (area ratio = area propoxyphene peak/area main decomposition peak). GC conditions: column, 3.0% SE-30 on Gas-Chrom Q; column temperature 200°; flow-rate, 41.8 ml/min; attenuation, ×128.

Fig. 4. Peak area ratio as a function of carrier flow-rate (area ratio, see Fig. 3). GC conditions: column, 3.0% SE-30 on Gas-Chrom Q; column temperature, 200°; sample size, 1.56 µg; attenuation, ×128.

Corasil II, OPN Porasil, and Acid Alumina all failed to separate propoxyphene from its degradation products. C_{18} Corasil and Phenyl Corasil separated propoxyphene only from heat decomposition products formed in the absence of acetaminophen. Only with the micro-silica column could propoxyphene be completely resolved from both heat degradation products and decomposition products formed in the presence of acetaminophen.

A mobile phase containing only isopropanol and hexane and a micro-silica column were found to separate propoxyphene from both its heat and acetaminophen degradation products but significant peak tailing was evident. Addition of small amounts of ammonium hydroxide resulted in improved peak shapes. This system was found to be extremely sensitive to changes in solvent composition.

The mobile phase which has been found to give the best combination of resolution and minimum analysis time was 0.65% basic isopropanol (ammonium hydroxide–isopropanol, 5:95) in hexane. When the mobile phase is initially prepared it is slightly cloudy due to undissolved ammonia. To insure proper chromatographic performance, this solution must be warmed slightly on a steam-bath and degassed under vacuum until clear.

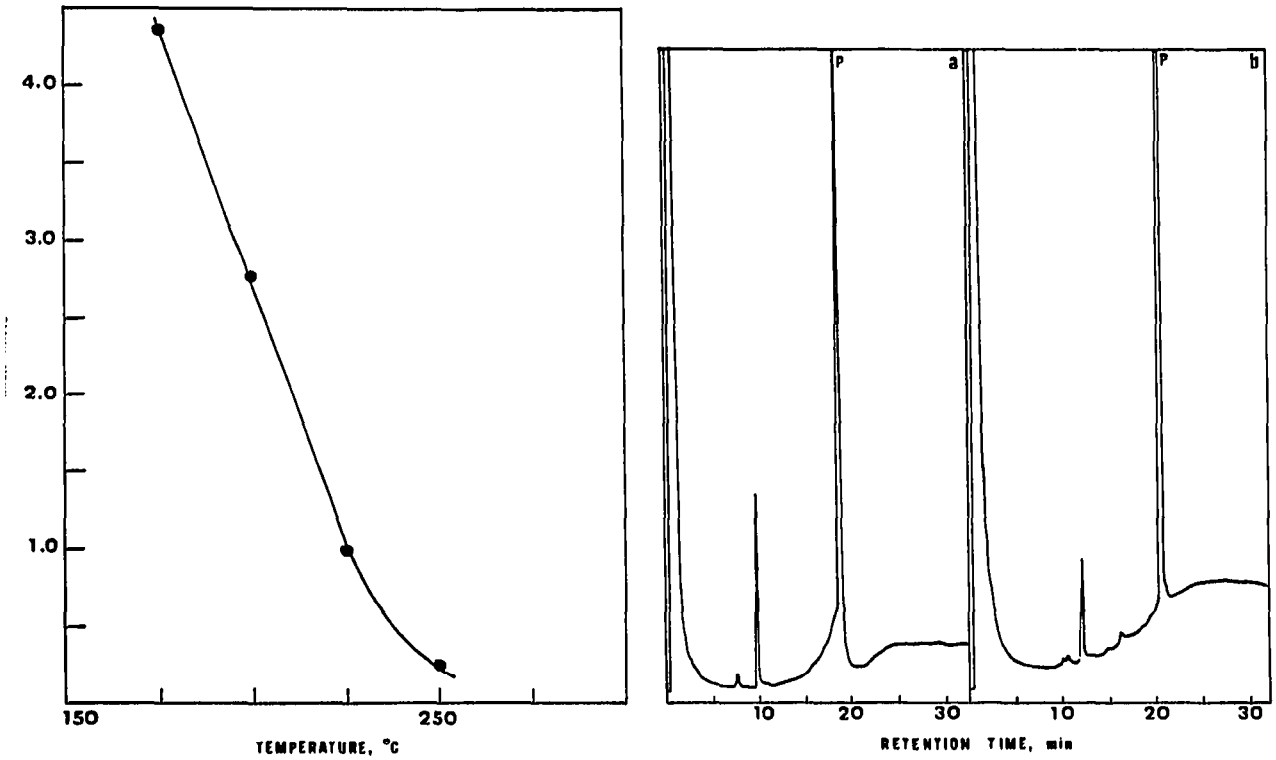

Fig. 5. Peak area ratio as a function of column temperature (area ratio, see Fig. 3). GC conditions: column, 3.0% SE-30 on Gas-Chrom Q; flow-rate, 41.8 ml/min; sample size, 1.56 μg; attenuation, ×128.

Fig. 6. Typical chromatograms for temperature-programming study. GC conditions: flow-rate, 41.8 ml/min; sample size, 3.0 μg; column temperature, 70 to 200° at 6°/min; attenuation, × 128. Column: (a) 3.0% SE-30 on Gas-Chrom Q; (b) 3.0% SE-30 on Chromosorb W HP.

A representative chromatogram is shown in Fig. 7. A calibration curve was run and found to be linear and reproducible in the range needed to assay single tablets or capsules of acetaminophen with 32.5 mg propoxyphene hydrochloride as shown in Fig. 8.

The specificity of the method was examined by placing 32.5 mg propoxyphene hydrochloride and an excess of acetaminophen in a glass container and heating for 30 min at 200°. The mixture was treated in a similar manner to capsule samples. When the solution was chromatographed, no peak for propoxyphene was observed. This was also confirmed by GC.

The precision of the method was tested on a series of tablet and capsule sample composites. The results obtained for both are summarized in Table I. Mean values of 96.9 and 99.9 were found for the seven tablet and capsule sample composites, respectively. The standard deviations of these were ± 1.56 and ± 1.96. In the light of these data and the specificity data the HPLC method has been found to provide a convenient and precise assay procedure free from the worry of possible on-column decomposition associated with GC procedures.

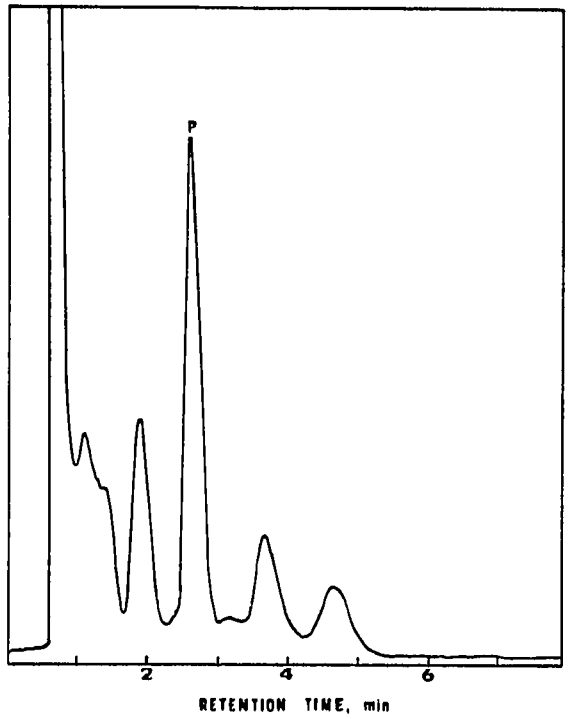

Fig. 7. Chromatogram showing partial heat decomposition of propoxyphene hydrochloride in the presence of acetaminophen (single capsule, 32.5 mg propoxyphene hydrochloride). HPLC conditions: column, microsilica; mobile phase, 0.65% basic isopropanol in hexane; flow-rate, 1.5 ml/min; range, 0.08 AUFS.

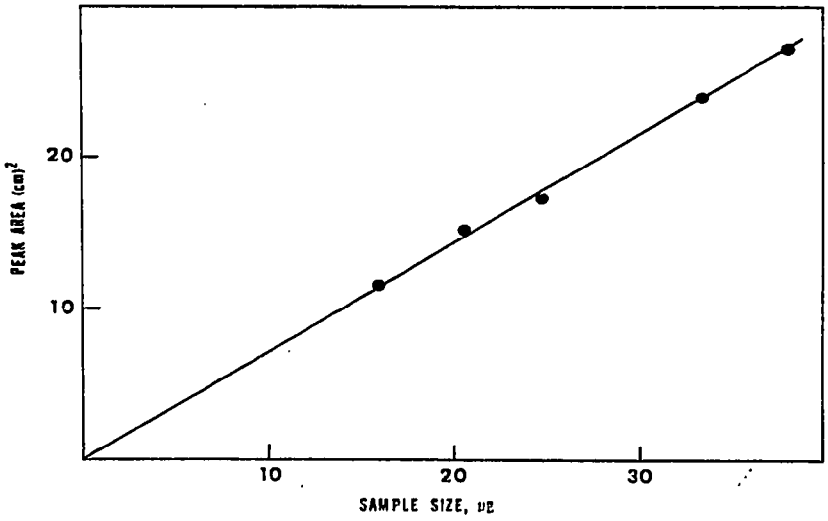

Fig. 8. Peak area as a function of varying amounts of propoxyphene hydrochloride.

TABLE I

PRECISION OF HPLC ASSAY PROCEDURE FOR PROPOXYPHENE HYDROCHLORIDE IN TABLET AND CAPSULE SAMPLES

Tablets		*Capsules*	
Sample No.	*% Propoxyphene*	*Sample No.*	*% Propoxyphene*
1	96.6	1	97.5
2	95.2	2	99.1
3	94.9	3	99.2
4	98.7	4	99.6
5	96.3	5	101.3
6	98.7	6	103.6
7	98.0	7	99.2
	$\bar{x} = 96.9$		$\bar{x} = 99.9$
	$\sigma = \pm 1.56$		$\sigma = \pm 1.96$

REFERENCES

1 L. Kazyak and E. C. Knoblock, *Anal. Chem.*, 35 (1963) 1448.
2 R. L. Wolem and C. M. Gruber, Jr., *Anal. Chem.*, 40 (1968) 1243.
3 H. L. Thompson and W. J. Decker, *Amer. J. Clin. Pathol.*, 49 (1968) 103.
4 C. S. Frings and L. B. Foster, *Amer. J. Clin. Pathol.*, 53 (1970) 944.
5 J. M. Manno, N. Jain and R. Forney, *J. Forensic Sci.*, 15 (1970) 403.
6 K. Verebely and C. E. Inturrisi, *J. Chromatogr.*, 75 (1973) 195.
7 C. M. Sparacino, E. D. Pellizzari, C. E. Cook and M. W. Wall, *J. Chromatogr.*, 77 (1973) 413.