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Determination of Propoxyphene in Biological Materials

Propoxyphene is 4-dimethylamino-3-methyl-1, 2-diphenyl-2-butanol propionate. The dextrorotatory salt is a very popular prescription analgesic marketed as Darvon®. The levorotatory product, which is not especially popular as an antitussive, is marketed as Novrad®. Dextropropoxyphene hydrochloride is very soluble in water (2 g/ml) and is marketed in capsules by itself and together with acetylsalicylic acid, phenacetin, caffeine, and also a tranquilizer. The capsules usually contain 32 or 65 mg of dextropropoxyphene hydrochloride. Unfortunately, the ready solubility of the hydrochloride has allowed for easy abuse of this drug by injection. A capsule which contained the drug in a spherical tablet also allowed the easy separation of this drug from other ingredients. This form has been discontinued. In an effort to prevent the misuse of the drug and to increase its safety, the manufacturer has produced a water-insoluble salt (1.5 mg/ml), propoxyphene napsylate, or the 2-naphthalene sulfonate which is marketed as Darvon-N®. Because of the weight of the salt this product is supplied in 50- and 100-mg dosage forms as tablets and a suspension containing 50 mg of Darvon-N® per 5 ml. The tablets are film-coated. The insolubility of this product should restrict its abuse and might prevent deaths by allowing for a much slower absorption of the drug.

Propoxyphene is a widely used analgesic. In fact, only aspirin and codeine were prescribed more frequently in 1971. However, its efficiency and its toxicity are controversial, and it appears that the drug has been extensively abused. Propoxyphene has been reported as the most common cause of drug death in the U.S. Army in Europe [*1*] and it is believed that many deaths may have gone undiscovered because of the problems concerning the detection and quantitation of this drug.

Several methods for the detection of propoxyphene have been reported, but all have limitations. Propoxyphene absorbs ultraviolet light and has the nonspecific spectrum of a benzenoid compound [*2*]; however, its absorbance is so low that only high concentrations of the drug may be detected. A methyl orange colorimetric procedure [*3*] lacks specificity. Another colorimetric procedure, that of Rickards [*4*], has been modified for the determination of propoxyphene in urine and gastric contents. Cochin and Daly [*5*] detected the drug in urine by thin-layer chromatography. Gas chromatographic methods

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have been offered for the determination of propoxyphene in urine by Kazyak and Knoblock [*6*] and in plasma by Wolen and Gruber [*7*]; Manno, Jain, and Forney [*8*]; and Frings and Foster [*9*], and in tissue specimens by Worm [*10*]. Thompson et al [*11*] altered the ultraviolet spectrum of propoxyphene by exposing a solution of the drug to ultraviolet light. The sensitivity limit of this method appears to be 10 μg/g. Wallace et al proposed a method which involved hydrolysis of propoxyphene in concentrated hydrochloric acid followed by steam distillation and ultraviolet spectrophotometry [*12*]. Unfortunately the final product is so diluted that specimens with less than 0.25 mg of propoxyphene must be concentrated by extraction. Wallace, Ladd, and Blum pointed out the deficiency and advanced a method for improving it [*13*]. Blood or urine is made alkaline and extracted with heptane, the heptane is extracted with acid, and the aqueous solution is refluxed with concentrated hydrochloric acid. This solution is neutralized and is refluxed with heptane, whereupon the heptane solution is examined by ultraviolet spectrophotometry. The reaction product has a new spectrum. Gas chromatography greatly enhances the specificity and sensitivity of this determination, but because of these modifications the procedure is time-consuming.

In our experience, all of the above methods leave something to be desired. None dealt with metabolized propoxyphene which represents most of the product in the urine [*14*] and, in our experience, in the liver. In an attempt to increase the yield of propoxyphene, liver was hydrolyzed with concentrated hydrochloric acid. The solution was made alkaline and extracted with ether and back-extracted with a dilute solution of hydrochloric acid. The acid solution had an ultraviolet spectrum with a broad peak at 254 nm but did not resemble the spectrum of propoxyphene. Ultraviolet irradiation of this solution increased the peak absorption and had a unique spectrum. The hydrolysis increased the absorption to about 30 times that of propoxyphene. Propoxyphene was recovered up to about 10 times more by strong acid hydrolysis of urine and liver. The irradiation increased the absorption to about 80 times that of propoxyphene. The procedure utilizes an ultraviolet spectrophotometric procedure which is specific and sensitive. Thin-layer chromatography, gas-liquid chromatography, and infrared spectrophotometry allow alternate methods of detection and quantitation of the acid-hydrolyzed product and of the irradiated product.

Propoxyphene is a poor absorber of ultraviolet light. The nonspecific benzenoid spectrum has an absorbance of a 1% solution in a 1 cm cell ($E_{1\ \mathrm{cm}}^{1\%}$) of 12 at 263 nm. This spectrum is obliterated by the spectrum of 2-naphthalene sulfonic acid when propoxyphene napsylate is examined. This has an $E_{1\ \mathrm{cm}}^{1\%}$) of 65 at 277 nm. The presence of the 2-naphthalene sulfonic acid may interfere with the ultraviolet spectrophotometric identification of the drug from dosage forms, but should not interfere with the determination of the drug in biological specimens. Propoxyphene metabolizes by demethylation and by conjugation to norpropoxyphene. In strongly alkaline solution norpropoxyphene rearranges to norpropoxyphene amide. When specimens are extracted at high pH, the amide will form and will not be extracted from the organic solvent by an acid solution. The formation of the amide may be prevented by extracting the specimen at about pH 9. Propoxyphene will not form this amide. The high solubility of propoxyphene hydrochloride in chloroform further complicates the extraction by allowing the salt to remain in the organic phase rather than going into the aqueous acid phase. Some of the difficulties of analyzing specimens for this drug are produced by the different properties of propoxyphene and of the products of metabolism and chemical reaction.

Most of the drug in the urine is probably present as conjugated norpropoxyphene and in the blood as propoxyphene. In poisonings, the drug probably is present as free and

conjugated norpropoxyphene and propoxyphene in high concentrations in the liver. The method of analysis presented, which hydrolyzes with concentrated hydrochloric acid, breaks the conjugation and allows all of the drug to be determined first as the hydrolyzed product and then as the ultraviolet irradiated hydrolysis product.

Experimental

Instrumentation

Ultraviolet measurements were performed using a Perkin-Elmer 402 double-beam spectrophotometer with linear presentation of wavelength. A Perkin-Elmer 257 spectrophotometer was used for infrared absorption measurements. A Varian Aerograph 2100 gas chromatograph with a U-shaped, silanized, 10-ft-long, 1.8-mm inside diameter glass column, with 2.5 percent SE-30 on Chromosorb G AW-DMCS 100–120 mesh, was used for gas chromatographic analysis. Spectrofluorometric measurements were made using a Farrand Mark I Spectrofluorometer with excitation monochromator slits at 10 mm and emission monochromator slits at 5 mm.

Procedure

Ten ml of blood and 15 ml of concentrated hydrochloric acid are placed in a 60-ml test tube. The mixture is heated in a boiling water bath for 20 min with occasional stirring. Ten-gram amounts of homogenized tissue, 10 ml of water, and 15 ml of concentrated hydrochloric acid are treated in the same way. The test tube is cooled and the contents are transferred to a 500-ml, glass-stoppered erlenmeyer flask. Fifteen millilitres of 50 percent sodium hydroxide solution are added. After cooling the flask, 150 ml of ethyl ether are added and the mixture is shaken vigorously. The ether layer is removed and filtered through dry Whatman No. 1 filter paper. The filtrate is shaken vigorously with 10 ml of water, the water is removed, and the ether layer is filtered through dry Whatman No. 1 filter paper. The volume of the filtrate is recorded. Five milliliters of 0.25 *N* hydrochloric acid solution are added to the filtrate and the mixture is shaken vigorously. The aqueous layer is removed and filtered through water-washed Whatman No. 5 filter paper. The absorbance from 220 to 310 nm is determined using 0.25 *N* hydrochloric acid as the reference solution. A broad peak at 255 nm indicates the probable presence of propoxyphene. If the absorbance of the peak at 255 nm exceeds 0.3, dilute the solution with 0.25 *N* hydrochloric acid solution to give an absorbance of about 0.3. Irradiate the solution for 5 min in a quartz cuvet placed a few millimetres from a 257.3-nm ultraviolet light. Determine the absorbance of the irradiated solution from 220 to 310 nm. Ultraviolet irradiated, acid-hydrolyzed propoxyphene has a sharp maximum at 255 nm with a shoulder at 247 nm and minor peaks at 277, 287, and 298 nm (Fig. 1). A reference solution containing propoxyphene should be determined and analyzed simultaneously with each batch of test samples.

Preparation of Reference Solutions

Five ml of a 50 μg/ml solution of propoxyphene and 2.5 ml of concentrated hydrochloric acid are treated along with the sample. After cooling the solution it is made strongly alkaline by adding 50 percent sodium hydroxide solution (about 2–2.5 ml). The resulting solution is acidified with a few drops of concentrated hydrochloric acid and diluted to 25 ml with water. This solution contains hydrolyzed propoxyphene equivalent to 10 μg/ml of propoxyphene. After the absorbance spectrum is recorded, this solution is irradiated along with the sample and the absorbance spectrum is rerecorded. A difference of absorbance

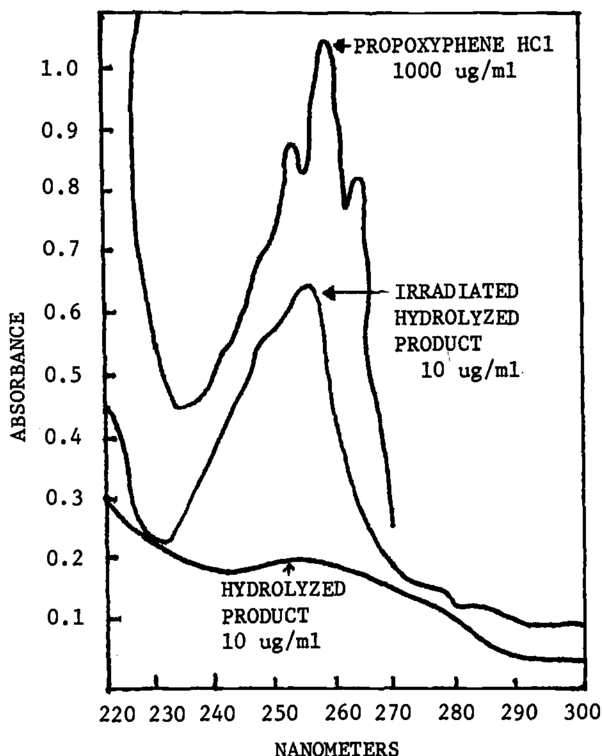

FIG. 1—*Ultraviolet absorption spectra of propoxyphene, the hydrolyzed product, and the irradiated hydrolyzed product.*

between the hydrolyzed product and the ultraviolet irradiated hydrolyzed product should be about 0.5 at 255 nm.

Gas Chromatography

Propoxyphene, hydrolyzed propoxyphene, and ultraviolet irradiated hydrolyzed propoxyphene were each examined using a gas chromatographic method after extraction from basic solutions into methylene chloride. The methylene chloride fractions were pooled and evaporated to appropriate volume for gas chromatographic analysis. The oven temperature was 190°C, the detector 220°C, and the injector 220°C.

Propoxyphene has a major peak whose retention time is 2.5 min. When hydrolyzed this peak disappears at 2.5 min and three other peaks occur at 4.3, 4.7, and 6.9 min. The ultraviolet irradiated product has the same gas chromatographic pattern as the hydrolyzed product. The same gas chromatographic pattern was observed after extracts of blood, liver, kidney, urine, and stomach content of individuals who allegedly ingested propoxyphene were hydrolyzed.

Spectrofluorometry

Propoxyphene, hydrolyzed propoxyphene, and ultraviolet irradiated hydrolyzed propoxyphene each were observed fluorometrically after extraction from a basic medium with methylene chloride. For the emission spectra, the excitation was set at 295 nm and

emission was scanned between 250 to 450 nm. For the excitation spectra, the emission was set at 370 nm and excitation was scanned between 200 to 450 nm.

No significant fluorometric spectrum was found for propoxyphene (100 μg/ml). However, the hydrolyzed product and the ultraviolet irradiated hydrolyzed product have characteristic spectra which are as follows: Emission peaks are observed at 355 and 370 nm with a shoulder at 387 nm. Excitation peaks are observed at 255 and 295 nm.

The hydrolyzed propoxyphene and the ultraviolet irradiated hydrolyzed propoxyphene have identical fluorometric spectra. The only observed difference is that the ultraviolet irradiated hydrolyzed product had an increased peak intensity. The characteristic fluorometric emission and excitation spectra of the hydrolyzed product were observed after extraction of blood, liver, kidney, and urine of individuals who allegedly had ingested propoxyphene. Drug-free specimens of the above-mentioned tissues were examined using the above procedure. Nothing was found to interfere with the fluorometric spectra obtained for the hydrolyzed propoxyphene. Propoxyphene, which is irradiated but not hydrolyzed, also had the characteristic hydrolyzed propoxyphene spectrum; however, the sensitivity for detecting the irradiated propoxyphene is greatly decreased.

Infrared Spectrophotometry

Infrared spectra were prepared both before and after hydrolysis of propoxyphene and after ultraviolet irradiation of the hydrolyzed product. The infrared spectrum of the hydrolyzed propoxyphene was determined in chloroform solution in a sodium chloride cell. The ultraviolet irradiated, hydrolyzed propoxyphene was prepared for infrared spectroscopy by the standard potassium bromide pellet technique.

The infrared spectrum of hydrolyzed propoxyphene was identical to the infrared spectrum that Wallace [*13*] observed for the refluxed propoxyphene product. The infrared spectrum of ultraviolet irradiated, hydrolyzed propoxyphene as compared to that of hydrolyzed propoxyphene had slight changes in intensity in the aromatic (1200 to 650 cm^{-1} absorption bands) and double-bond areas.

It is believed that the increase in ultraviolet absorbance following ultraviolet irradiation of hydrolyzed propoxyphene is due to changes in the aromatic and double-bond areas.

Thin-Layer Chromatography

Ascending thin-layer chromatography was conducted on 5 by 20 cm glass plates coated with a 0.25-mm layer of silica gel G. After the solvent, ammonium hydroxide:methanol (1.5:100) ascended 10 cm the plates were air-dried and sprayed with acidified iodoplatinate solution. The R_F values obtained were propoxyphene 0.89, hydrolyzed propoxyphene 0.84, norpropoxyphene 0.60, and hydrolyzed norpropoxyphene 0.60.

Liver from a propoxyphene overdose case was hydrolyzed and extracted for thin-layer chromatography. Thin-layer spots from the liver extract had the same R_F values and reacted similarly to standards of hydrolyzed propoxyphene and hydrolyzed norpropoxyphene when applied to the same plate. Unhydrolyzed liver extracts prepared for thin-layer chromatography had R_F values matching unhydrolyzed standards of propoxyphene and norpropoxyphene. An extract of hydrolyzed liver irradiated with ultraviolet light prior to thin-layer chromatography gave an R_F value of 0.65, as did irradiated samples of propoxyphene and norpropoxyphene.

Specificity

In order to eliminate drugs which might interfere with this ultraviolet method of propoxyphene determination, the compounds discussed hereafter were subjected to the hy-

drolysis and ultraviolet irradiation procedure. Ultraviolet spectra were determined for each of the compounds in acid solution before hydrolysis, after hydrolysis, and after ultraviolet irradiation of the hydrolyzed solution. Compounds showing no significant ultraviolet spectrum changes included ephedrine, imipramine, meperidine, methaqualone, pentazocine, and phenylephrine. The following drugs showed spectral changes which should not interfere with the quantitation of propoxyphene.

Amitriptyline—Hydrolysis caused no spectral change. Ultraviolet irradiation of the hydrolyzed solution causes a slight decrease in absorbance at 245 nm and a slight increase at 275 nm.

Chlordiazepoxide—Hydrolysis reduces the maxima at 250 and 312 nm. Ultraviolet irradiation does not cause any further change.

Diazepam—Hydrolysis reduces the maxima at 245 and 288 nm to a shallow curve from 250 to 320 nm. Ultraviolet irradiation does not cause any further change.

Methadone—Hydrolysis does not change the normal absorbance spectrum; thus, the hydrolyzed spectra of propoxyphene and methadone are readily distinguishable. Ultraviolet irradiation of hydrolyzed methadone yields an increase in absorbance at 255 nm similar to that of propoxyphene. These spectra are also readily differentiated because methadone does not have a minimum before the peak at 255 nm, nor shoulders at 278, 287, and 298 nm. Instead methadone has a broad peak extending from 280 through 300 nm.

Discussion

Overdose of the drug was first reported in 1960 [*15*]. The first report of a fatality was in 1964. It involved a 15-year-old girl who allegedly ingested about 1.3 g of propoxyphene [*16*]. One of the authors [*17*] reported a death in 1966 in which tissue concentrations were given as 5.7 mg/100 ml of blood and 10 mg/100 g of liver. A 65-year-old patient died 9 days after taking 2.3 g of the drug [*18*]. A 17-year-old girl who died 2½ months after allegedly ingesting about 1.6 g of propoxyphene, had a concentration of 0.3 mg of drug in 100 ml of blood [*19*]. In 17 cases where death was attributed to propoxyphene, the concentration of the drug in the liver ranged from 3.1–12.6 mg/100 g with a median of 6.7 mg/100 g [*11*].

Three cases of intoxication were reported as follows [*20*]: a 19 year-old pregnant housewife recovered after ingesting 1.6 g of the drug; a 23-year-old woman was found dead following the ingestion of 1.8 g; and an 18-year-old boy who died following the ingestion of 3.25 g of the drug had concentrations of propoxyphene of 4 mg/100 g in the brain, 1.1 mg/100 ml in the blood, and 9 mg/100 g in the liver.

In nine deaths attributed to propoxyphene [*21*], the livers of six contained 2.7 to 27.3 mg/100 g and the bloods of two contained 1.3 and 13.2 mg/100 ml. An 18-year-old male ingested 0.7 g of the drug and a 24-year-old female ingested 2 g of the drug.

Nine cases of fatal poisoning, where propoxyphene was the sole or contributory cause of death, have been reported [*10*]. Concentrations of the drug are given in the following specimens: gastric contents, brain, blood, liver, bile, small intestinal contents, kidneys, and urine. The blood samples contained 0.04 to 2.3 mg/100 ml and the liver samples contained 0.02 to 2.2 mg/100 grams.

Sturner and Garriott [*22*] reported on 41 deaths involving propoxyphene dihydrochloride. The blood concentrations of propoxyphene in patients who overdosed were from 0.05–1.1 mg/100 ml, with the majority between 0.2 and 0.9 mg/100 ml. The liver and lungs had 10 to 20 times the levels found in the blood.

Interpretation of Tissue Concentrations

Plasma concentrations reached a maximum of 0.02 mg/100 ml in 1 h following a 195-mg oral dose, and 0.03 mg/100 ml in 30 min after a 50-mg intravenous dose [*23*]. Plasma concentration reached a maximum of 0.03 mg percent following the administration of 13 oral doses of 65 mg of the propoxyphene hydrochloride or 100 mg of the newer propoxyphene napsylate [*24*]. In fatal poisonings, concentrations of at least 0.2 mg/100 ml of blood and 3 mg/100 g of liver are attained. In Table 1 where death was attributed to propoxyphene, the blood levels had a range of 0.2 to 2.0 mg/100 ml, with the majority from 0.5 to 0.7 mg/100 ml. Liver concentrations ranged from 1.5 to 30 mg/100 g, with a majority from 5 to 13 mg/100 g. In the few cases where death was attributed to other causes (see Table 2), three blood samples contained less than 0.1 mg/100 ml, four liver concentrations were less than 1 mg/100 g, and the other two were in the same range as those in Table 1. It appears that in order to establish that death might be due to propoxyphene, the blood concentration must be 0.2 mg/100 ml or more. A high concentration in the liver might be indicative of chronic use of the drug.

TABLE 1—*Cases where death was attributed to propoxyphene.*

			Concentration of Propoxyphene		Blood	
Case No.	Sex	Age	Blood, mg/100 ml	Liver, mg/100 g	Ethanol, mg/100 ml	Salicylate, mg/100 ml
1	F	37	...	13	50	N.D.[a]
2	M	30	...	8	0	N.D.
3	F	31	1.5	30	0	N.D.
4	F	43	0.4	5.5	0	N.D.
5	M	24	0.7	12	0	N.D.
6	F	48	0.8	14	0	13[b]
7	M	48	0.3	12	0	0
8	F	29	0.4	9.5	0	14[c]
9	M	9	0.8	4	0	5
10	M	52	0.6	5	150	5
11	F	29	0.2	3	0	N.D.
12	F	44	0.7	22	50	12
13	M	50	0.5	7.5	160	N.D.
14	M	41	0.5	20	0	6
15	F	20	2.0	...	280	...
16	M	40	0.5	...	230	N.D.
17	F	50	0.6	17	0	6
18	F	37	0.2	1.5	220	0

[a] N.D. = none detected, screening method.
[b] Blood ethchlorvynol—3 mg/100 ml.
[c] Blood theophylline—0.8 mg/100 ml.

Summary

A rapid method for the quantitative determination of propoxyphene in biological materials is presented. The drug is extracted from acid-hydrolyzed tissue with ether and after extraction is taken up in dilute acid, and the ultraviolet spectrum is determined. An aliquot of the acid solution is exposed to ultraviolet radiation and the ultraviolet spectrum is again recorded using the unexposed solution as a reference. The absorbance difference at 255 nm between the irradiated and unirradiated solution is proportional to the propoxyphene concentration. Most of the propoxyphene in the liver and urine is conjugated. Acid hydrolysis not only liberates the conjugated propoxyphene but also

TABLE 2—*Cases where death was attributed to causes other than propoxyphene.*

Case No.	Sex	Age	Propoxyphene Blood, mg/100 ml	Propoxyphene Liver, mg/100 g	Blood Ethanol, mg/100 ml	Cause of Death
1	F	46	0.01	7	0	Cardiac tamponade
2	F	43	...	3	140	Ventricular fibrillation during injection[a]
3	F	31	...	0.3	0	CO, blood 75% saturated, auto exhaust
4	M	48	...	0.2	0	Blood glutethimide—2 mg/100 ml Blood phenobarbital—1.4 mg/100 ml
5	F	19	0.09	0.9	0	Viral pneumonia[b]
6	M	55	0.07	0.4	220	CO, blood 20% saturated, auto crash fire

[a] Blood acetone—30 mg/100 ml, blood isopropanol—20 mg/100 ml.
[b] Blood salicylate—16 mg/100 ml.

causes the formation of a new compound which has an absorbance about 30 times greater than that of propoxyphene. Ultraviolet irradiation further increases the absorbance to about 80 times that of propoxyphene. The various ultraviolet spectra and changes provide specificity to the method, and gas-liquid and thin-layer chromatographic techniques provide increased confidence in the determination. Case studies containing tissue concentrations are presented.

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