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Note

High-performance liquid chromatographic separation of the diastereoisomers of propoxyphene

Determination of microquantities of β-*dl*-propoxyphene in commercial preparations of α-*d*-propoxyphene

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Propoxyphene, 1,2-diphenyl-2-propionoxy-3-methyl-4-dimethylaminobutane (Fig. 1), exists in two diastereoisomeric forms, α-*dl* and β-*dl*. The α-isomers are pharmacologically active; the α-*d*-isomer is an analgesic, whereas the α-*l*-isomer is a clinically useful antitussive[1]. The β-*d*- and β-*l*-isomers, however, are therapeutically almost inactive[2] and are unwanted contaminants in pharmaceutical preparations containing the α-diastereoisomers.

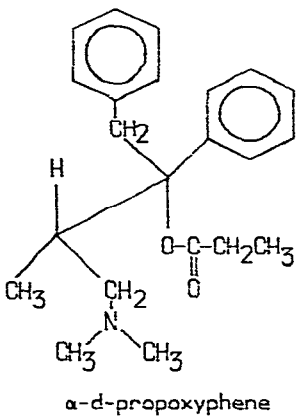

Fig. 1. Structure of propoxyphene.

Thin-layer chromatography (TLC), infrared spectroscopy and nuclear magnetic resonance spectroscopy have been used to identify the two diastereoisomeric forms of propoxyphene[3]. Although these methods are useful identification tests, they cannot be used to accurately quantify small amounts of one diastereoisomer in the presence of the other.

Soni and Van Gelder[4] have recently reported a reversed-phase high-performance liquid chromatographic (HPLC) method for the separation and characterization of the propoxyphene diastereoisomers. However, in their method the α-isomers elute

before the β-isomers, and the assay does not appear to be suitable for determination of microquantities of the β-diastereoisomers in the presence of the α-diastereoisomers.

As part of the ongoing program in our laboratory for the development of assays for the isomeric purity of therapeutic agents, we have also developed an HPLC assay for the α- and β-diastereoisomers of propoxyphene. In this assay, the β-diastereoisomers elute before the α-isomers, and as little as 0.1 % of the β-*dl*-isomer can be detected in the presence of the α-*d*-isomer. The analytical procedure is a rapid and accurate method for determining the content and isomeric purity of commercial propoxyphene preparations as well as those of standards and the bulk drug substance; it can be used for single dose analysis.

EXPERIMENTAL

Apparatus

The HPLC determinations were performed with a Spectra-Physics Model 3500 high-performance liquid chromatograph equipped with a 4000 S-P data system (Spectra-Physics, Santa Clara, CA, U.S.A.); a Spectra-Physics Model 770 UV-visible detector set at 220 nm; and a temperature-controlled column compartment. A stainless-steel DuPont-packed Zorbax Sil column, 5–6 μm particle size (25 cm × 4.6 mm I.D.) was used for all determinations. The injector (Valco Instruments, Houston, TX, U.S.A.) was equipped with a 10-μl sampling loop.

Materials

All solvents, including water, were of suitable grade for HPLC. All solutions were filtered through micropore Millipore LS filters (Millipore, Bedford, MA, U.S.A.), or equivalent and then degassed before use. α-*d*-Propoxyphene hydrochloride was a proposed United States Pharmacopeia Reference Standard, Lot H, β-*dl*-Propoxyphene hydrochloride was supplied by the Mid-Atlantic Regional Laboratory of the Drug Enforcement Administration. This sample, which was analyzed by TLC[3] and by the proposed HPLC method, was found to contain impurities. The β-*dl*-propoxyphene was isolated by preparative TLC on precoated plates of silica gel GF, 2.0 mm thick (Analtech, Newark, DE, U.S.A.). Each plate was viewed under short-wavelength UV light; the upper, isolated band, which contained the β-*dl*-propyxphene, was scraped from the plate, vigorously mixed with methanol and centrifuged. The supernatant liquid was removed, filtered through a micropore filter, and analyzed by the proposed HPLC method.

General procedures

Standard solutions of α-*d*-propoxyphene hydrochloride in the mobile phase and in methanol were prepared in concentrations ranging from 0.001 to 1 mg/ml. The standard solutions were chromatographed and a calibration curve was obtained by using the peak area measurements generated by the data system.

A mixture containing 1 % β-*dl*-propoxyphene hydrochloride in α-*d*-propoxyphene hydrochloride was prepared both in the mobile phase and in methanol.

Single dose units (tablets or capsules) were dissolved individually in the mobile phase and in methanol in volumetric flasks with the aid of an ultrasonic bath. If

necessary, the samples were crushed with a glass rod. After dissolution, the samples were diluted to volume with either the mobile phase or methanol, and the resulting solutions were filtered through micropore filters. This procedure produced solutions with α-*d*-propoxyphene concentrations of approximately 1 mg/ml. The sample solutions were then chromatographed and the peak areas were used to determine the actual concentrations of the samples.

Chromatographic conditions

The mobile phase was isopropanol–hexane (80:20) containing 1% water. When the mobile phase was being prepared, vigorous shaking was required to ensure the miscibility of the water. A flow-rate of 0.25 ml/min and a column temperature of 25°C were maintained throughout the experiment. When the column was not in use, the mobile phase was continuously circulated over it.

RESULTS AND DISCUSSION

Under the experimental conditions, baseline separations of β-*dl*-propoxyphene and α-*d*-propoxyphene were obtained with an average resolution factor (R_s) of 5.60 (Fig. 2). The retention time of the β-isomer ranged from 20.9 to 21.7 min; the retention time of the α-isomer ranged from 27.8 to 28.7 min. The separation between the two peaks and their characteristics permitted determination of as little as 0.1% of β-*dl*-propoxyphene in the presence of α-*d*-propoxyphene (Fig. 2).

Methanol was used as the diluent in initial work; however, spurious peaks were observed in the chromatograms. These peaks were shown to be associated with the

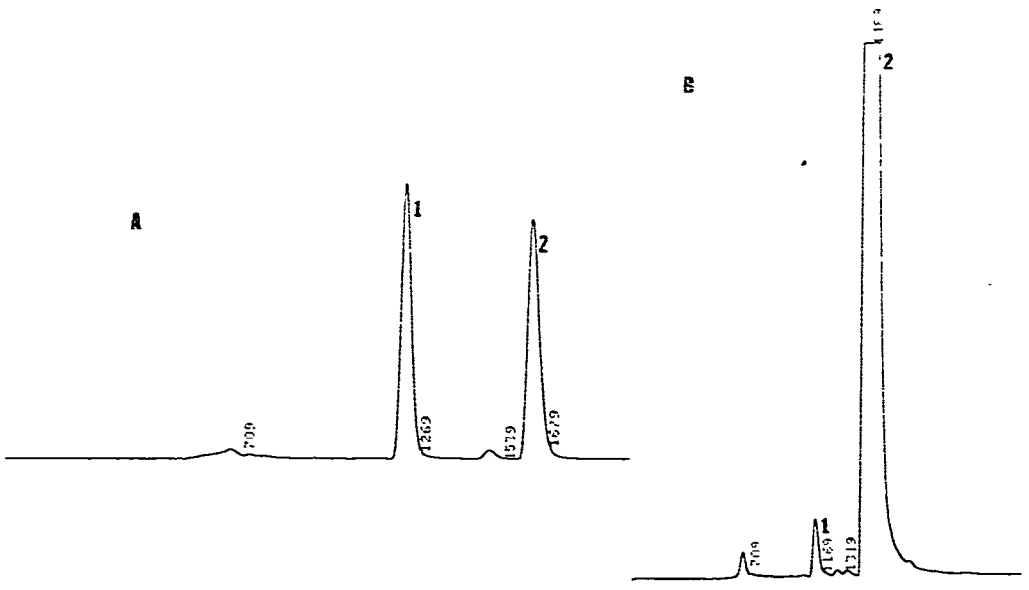

Fig. 2. Chromatograms of mixtures of β-*dl*-propoxyphene (1) and α-*d*-propoxyphene (2): (A) Equimolar solutions of β-*dl*-propoxyphene and α-*d*-propoxyphene, [1] = [2] = 100 μg/ml; (B) 0.5% β-*dl*-propoxyphene in α-*d*-propoxyphene, [1] = 10 μg/ml. [2] = 2000 μg/ml.

methanol. The problem was avoided by preparing the drug standards and dosage forms in the mobile phase. The relationship of the area under the UV response curve to the concentration of α-*d*-propoxyphene was linear over a 1000-fold range (0.001 to 1 mg/ml) (Fig. 3). The response curves with the mobile phase and with methanol as diluents were identical.

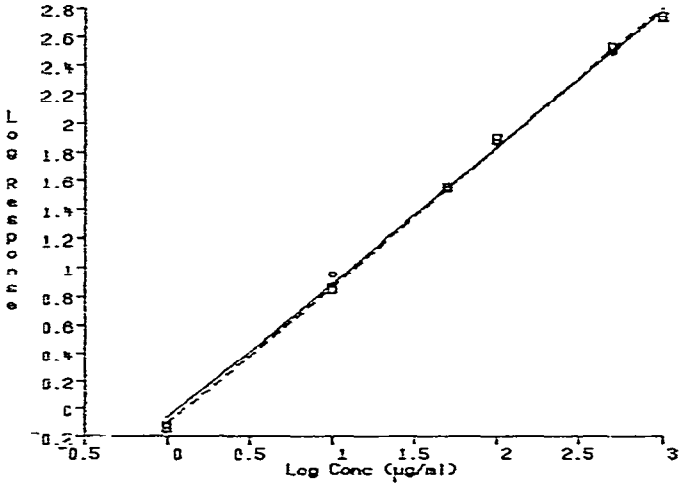

Fig. 3. UV response curve for α-*d*-propoxyphene concentrations of 0.001 to 1 mg/ml. O——O, methanol; □—□, isopropanol–hexane (80:20).

Six dosage forms of commercially available α-*d*-propoxyphene hydrochloride were analyzed for β-*dl*-propoxyphene and for content (Table I). Propoxyphene is often formulated in combination with such drugs as aspirin, acetaminophen and naloxone. Aspirin, acetaminophen and naloxone hydrochloride elute very slowly from the column and do not interfere with the analysis. However, after a series of samples, the column must be flushed with mobile phase to prevent interference with the assay.

Solubility problems were encountered with two of the preparations —sample 4 (propoxyphene HCl, sustained action capsule) and sample 5 (propoxyphene N/acetaminophen, tablet). These problems were overcome by placing each sample in a 100-ml volumetric flask, adding 20 ml of isopropanol and 1 ml of water, and sonicating until dissolution. After the solutions cooled to room temperature, hexane was added to volume. The samples were then treated as described.

Single tablet assays for α-*d*-propoxyphene content varied from 97.8 to 113.7% of label claim (Table I). After the determination of α-*d*-propoxyphene content, the samples were rerun at maximum detector sensitivity to assay for the β-isomer. Of six samples analyzed, four samples contained the β-isomer in concentrations ranging from 0.16 to 0.45%; samples 2 and 3 contained no β-isomer (Table I).

This method is a rapid and sensitive assay for the content and isomeric purity of α-*d*-propoxyphene preparations and can be used for commercial samples as well as for the bulk drug.

TABLE I

ANALYSES OF INDIVIDUAL PROPOXYPHENE TABLETS AND CAPSULES FROM COMMERCIAL SOURCES FOR *β-dl*-PROPOXYPHENE AND CONTENT

Sample	*Dosage form*	*Label claim*	*α-Propoxyphene found (mg/tablet or mg/capsule)*	*Percent of label claim*	*Percent of β-isomer*
1	Capsule	Propoxyphene HCl, 65 mg/capsule	73.3	112.7	0.35
2	Tablet	Propoxyphene napsylate (N), 100 mg/tablet	97.8	97.8	0.00
3	Tablet	Propoxyphene N, 100 mg/tablet Aspirin, 325 mg/tablet	113.7	113.7	0.00
4	Sustained-action capsule	Propoxyphene HCl, 130 mg/capsule	127.9	98.4	0.24
5	Tablet	Propoxyphene N, 50 mg/tablet Acetaminophen, 325 mg/tablet	53.4	106.7	0.16
6	Capsule	Propoxyphene HCl, 65 mg/capsule Naloxone HCl, 0.5 mg/capsule	72.5	111.5	0.45

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