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Note

High-performance liquid chromatographic method for the determination of dextropropoxyphene and nordextropropoxyphene in serum

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Dextropropoxyphene, a widely prescribed drug for the relief of moderate pain, frequently causes self-poisoning. The poisoning is often serious because of respiratory and circulatory insufficiency [1].

Most of the methods described for the determination of dextropropoxyphene and nordextropropoxyphene in biological specimens have utilized gas chromatographic (GC) separation with either flame-ionization [2—6], nitrogen-selective [7—9] or mass fragmentographic detection [10]. Problems associated with decomposition of dextropropoxyphene on the GC column have been reviewed by Millard et al. [11]; other analytical problems and improvements in the GC determination of dextropropoxyphene and nordextropropoxyphene have recently been described by Margot et al. [8].

High-performance liquid chromatographic (HPLC) methods appear to offer the most useful methodologies for routine clinical determination. Measurement of dextropropoxyphene and nordextropropoxyphene has no pronounced UV absorption at the wavelengths commonly used (i.e. 254 and 280 nm) and, until very recently, only HPLC methods with sensitivity adequate for the determina-

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tion of dextropropoxyphene in tablet- and capsule-dosage forms were published [12, 13]. However, the absorption of UV energy by propoxyphene in the low UV range is apparently sufficient for the determination of dextropropoxyphene and nordextropropoxyphene in biological specimens [14]. Due to an extensive clean-up there was no interference from endogenous compounds, but the selectivity with regard to other drugs and metabolites was not investigated. Compared with UV detection, electrochemical detection offers enhanced sensitivity and selectivity for these analytes when an unmodified silica column together with a non-aqueous ionic eluent are used [9]. The selectivity of the assay has been investigated by Jane et al. [15].

This paper describes an HPLC method with a relatively simple extraction procedure. The sensitivity is sufficient for measuring steady-state levels of dextropropoxyphene and its major metabolite nordextropropoxyphene after therapeutical treatment with dextropropoxyphene.

EXPERIMENTAL

Chemicals

Acetonitrile and methanol were of HPLC grade (LiChrosolv; Merck, Darmstadt, F.R.G.). All other solvents and chemicals were of analytical-reagent quality. Stock solutions of dextropropoxyphene hydrochloride, nordextropropoxyphene maleate and α-*d*-pyrroliphene hydrochloride (all from Eli Lilly, Indianapolis, IN, U.S.A.) were prepared in 0.1 *M* hydrochloric acid.

Apparatus

The chromatograph consisted of a Waters Model 6000 A pump, a Waters WISP 710 B automatic injection system and a Waters Model 450 variable-wavelength UV spectrophotometer detector operating at 215 nm (Waters Assoc., Milford, MA, U.S.A.). The column was a 5-μm Supelcosil CN 25 cm × 4.6 mm column (Supelco, Gland, Switzerland), used at room temperature.

Mobile phase

Mobile phase I, acetonitrile—methanol—10 m*M* sodium phosphate, pH 7.0 (55:25:20), was used in most cases. Mobile phase II, acetonitrile—methanol—10 m*M* sodium phosphate, pH 7.0 (40:20:35), was used in cases of interfering drugs. A flow-rate of 2 ml/min was used.

Procedure

To 1.0 ml of serum were added 0.5 ml of 1 *M* carbonate buffer (pH 10), 20 μl of internal standard solution (0.13 m*M* pyrroliphene) and 5.0 ml of *n*-butyl chloride. The sample was shaken for 5 min and, after centrifugation at 1300 *g* for 5 min, 4 ml of the organic phase were transferred into clean glass vessels with 100 μl of 33 m*M* phosphoric acid. After whirlmixing for 20 s, centrifugation and cooling in a dry-ice—acetone bath for 1 min, the organic phase was aspirated off and discarded. The acid phase (40 μl) was analysed by HPLC. Quantitation was by peak-height ratio with reference to the graphs obtained by analysing serum standards simultaneously.

RESULTS

Evaluation of the analytical procedure

Fig. 1 shows chromatograms obtained from serum analysis. Plots of the standard curves of the two drugs over the range 0.25—25 μmol/l were linear. The line of best fit for dextropropoxyphene, using 40 μl of the internal standard solution, was $y = 0.24x + 0.04$ ($n = 16$, $r = 1.00$) and for nordextropropoxyphene it was $y = 0.16x + 0.03$ ($n = 16$, $r = 1.00$), where x is the analyte concentration and y the peak-height ratio.

The lower limits of detection for dextropropoxyphene and nordextropropoxyphene were 0.1 and 0.2 μmol/l, respectively (signal-to-noise ratio = 4.0).

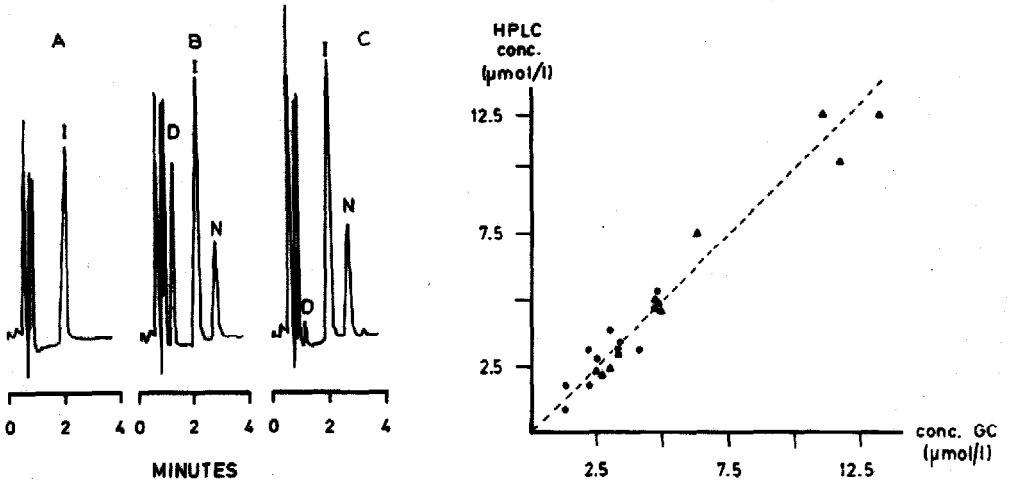

Fig. 1. Chromatograms of human serum extracts, analysed as described in the text (mobile phase I). (A) Blank serum; (B) serum to which dextropropoxyphene and nordextropropoxyphene were added (1.06 and 0.95 μmol/l, respectively); (C) serum from a patient therapeutically treated with 300 mg of dextropropoxyphene napsylate per day. (Dextropropoxyphene and nordextropropoxyphene were determined to be 0.15 and 0.99 μmol/l, respectively.) Peaks: D = dextropropoxyphene; N = nordextropropoxyphene; I = internal standard.

Fig. 2. Comparison of results from analysis of samples from intoxicated patients by our earlier GC method [7] and our HPLC method. (•) Dextropropoxyphene; line of best fit: $y = 0.89x + 0.30$ ($n = 11$, $r = 0.89$). (▲) Nordextropropoxyphene; line of best fit: $y = 0.96x + 0.08$ ($n = 11$, $r = 0.98$).

The reproducibility was determined using spiked serum samples analysed at random on different days. These spiked samples were stored at −20°C from one day to eight months. The reasonable reproducibility shown in Table I demonstrates that the two drugs are stable for several months under the conditions mentioned above.

Fig. 2 compares results from analyses of serum from intoxicated patients by our HPLC method and our earlier GC method [7].

TABLE I

REPRODUCIBILITY OF REPLICATE ANALYSIS OF DEXTROPROPOXYPHENE AND NORDEXTROPROPOXYPHENE ADDED TO HUMAN SERUM

Compound	Serum concentration* (μmol/l)	Coefficient of variation (%)
Dextropropoxyphene	1.1	4.7
	5.0	2.4
	9.8	2.9
Nordextropropoxyphene	1.1	5.6
	4.8	3.5
	9.9	3.1

*Mean values from six duplicate samples of each concentration.

Selectivity

Serum blanks from twenty drug-free patient samples did not show any interference from endogenous compounds.

The following drugs were investigated for possible interfering peaks in the same region as dextropropoxyphene, nordextropoxyphene and pyrroliphene: amitriptyline, aprobarbitone, barbitone, carbamazepine, chlordiazepoxide, chlorpromazine, chlorprothixene, clomipramine, clopenthixol, codeine, desimipramine, diazepam, disopyramide, flupenthixol, imipramine, ketobemidone, levomepromazine, lidocaine, methadone, morphine, nitrazepam, nortriptyline, oxazepam, paracetamol, pentobarbitone, pethidine, phenobarbitone, phenytoin, theophylline and thioridiazine. Table II shows the relative retention times of those drugs that did interfere when using mobile phase I. By using mobile phase II instead, the interference from these drugs could be eliminated, but the time of the HPLC analysis increased to 10 min.

TABLE II

RELATIVE RETENTION TIMES (RRT) OF INTERFERING DRUGS

Compound	RRT	
	Mobile phase I	Mobile phase II
Dextropropoxyphene	0.62	0.65
Nordextropropoxyphene	1.36	1.04
Pyrroliphene	1.00	1.05
Codeine	0.61	0.37
Ketobemidone	0.65	0.46
Methadone	1.38	1.15
Pethidine	0.59	0.48
Clomipramine	0.99	1.21
Clopenthixol	0.56	0.50
Levomepromazine	0.70	0.76

DISCUSSION

The advantage of using a chemically bonded stationary phase for HPLC is that it allows the injection of the acid phase directly into the column. Dextropropoxyphene and nordextropoxyphene appeared to be rather stable in the acid phase. No loss of the two compounds was observed after storage at 2–8°C for five days.

The cyano-bonded phase provides good selectivity for separating many basic compounds and their metabolites. It has been used for the determination of tricyclic antidepressants [16–19], antiarrhythmics [20] and phenothiazines [21, 22]. With the cyano material from Supelco, satisfactory peak shape was obtained without the addition of amine modifiers, which simplified the development of this application.

Mobile phase I was chosen in order to obtain a short HPLC analysis time. In cases where the patients have taken interfering drugs (Table II), mobile phase II should be used instead. The intake of drugs can easily be checked by a qualitative urine thin-layer chromatographic screen [23] and/or by an abnormal ratio between the dextropropoxyphene and nordextropropoxyphene concentrations in serum [24].

The HPLC method described has replaced the earlier GC method [7] in our laboratory because it is much easier and less time-consuming to perform, especially for just a few samples at a time, and has a better sensitivity in spite of the use of a smaller volume of serum.

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