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Short Communication

Determination of dextropropoxyphene and norpropoxyphene in plasma by high-performance liquid chromatography

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ABSTRACT

A simple and sensitive procedure for the routine assay of the analgesic drug dextropropoxyphene and its main metabolite, norpropoxyphene, in plasma is described. After liquid–liquid extraction from alkalinized plasma and back-extraction into a small volume of an acidic aqueous phase, the aqueous phase was injected into a column packed with 3-μm octadecylsilica particles. Ultraviolet absorbance detection at 210 nm was used. Concentrations down to 2 n*M* could be determined for both compounds; at this level, the intra-assay coefficient of variation was 5%.

INTRODUCTION

Dextropropoxyphene is a widely prescribed drug for the relief of moderate pain. In humans, N-demethylation to form norpropoxyphene is the major route of metabolism [1,2].

A number of chromatographic methods for the determination of dextropropoxyphene and norpropoxyphene in biological samples have been published. In gas chromatography (GC), nitrogen-selective [3–5] or mass-selective detection [6] were used. In liquid chromatography, reversed-phase columns combined with UV detection [7–9] or unmodified silica columns and electrochemical detection have been used [3,10]. The limit of quantification for most of the published liquid chromatographic methods is in the range 100–200 n*M*, which is insufficient for the calculation of pharmacokinetic parameters. A GC method with nitrogen-selective detection offers sufficient sensitivity, but the sample pretreatment is very laborious [4].

This paper describes a sensitive and simple method based on reversed-phase high-performance liquid chromatography (HPLC). The low quantification limit (2 n*M*) was accomplished by a combination of a selective extraction procedure and efficient chromatography on a column packed with small (3 μm) particles. The simple sample preparation also makes the method appropriate for analysis of the large number of samples obtained in pharmacokinetic studies.

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EXPERIMENTAL

Materials and reagents

Sodium decyl sulphate (DS) was purchased from Research Plus (Bayonne, NJ, USA) and N,N-dimethyloctylamine (DMOA) from ICN Biomedicals (Plainview, NY, USA). Other chemicals were of HPLC or analytical grade. Norpropoxyphene maleate was obtained from Sigma (St. Louis, MO, USA) and dextropropoxyphene hydrochloride from Eli Lilly (Indianapolis, IN, USA). The internal standard, 1-benzyl-3-methylethylamino-2-methyl-1-phenylpropyl propionate picrate, was synthesized at Astra Pharmaceutical Productions (Södertälje, Sweden). Standard stock solutions of norpropoxyphene, dextropropoxyphene and the internal standard were prepared in water once a month and stored in a refrigerator at 4°C.

Liquid chromatographic system

The solvent-delivery system was an LKB 2150 pump. Samples were injected with a Perkin-Elmer ISS-100 autosampler. The analytical column (100 mm × 4.6 mm I.D.) was a factory-packed YMC S-3 120A ODS (YMC, Kyoto, Japan). The UV detector was a Spectra 100 (Spectra-Physics, San Jose, CA, USA) operated at 210 nm. The chromatograms were recorded and analysed with a Spectra-Physics SP4270 integrator using peak-height measurements.

The mobile phase, degassed with helium before use, was acetonitrile–phosphate buffer (0.05 *M* sodium dihydrogenphosphate adjusted to pH 2.0 with orthophosphoric acid) (39.5:60.5, v/v). To the mobile phase were added 0.2 m*M* DMOA and 1.0 m*M* DS. The flow-rate was 1.3 ml/min.

Sample preparation

Plasma samples were stored in polypropylene tubes (Nunc, Roskilde, Denmark) at −20°C until analysis.

For the extraction, 0.50 ml of plasma was transferred to a glass tube. To each tube, 20 μl of internal standard solution (20 μ*M*), 0.15 ml of 0.1 *M* sodium carbonate buffer (pH 10.0) and 3 ml of an organic solvent mixture, *n*-hexane–diethyl ether–*n*-butanol (70:25:5, v/v), were added. After gentle agitation in a rotating mixer for 10 min and centrifugation for 10 min at 3200 *g* and 2°C, the organic layer was separated from the aqueous phase and transferred to a smaller tube. Phosphate buffer (pH 2.0) (250 μl) was added, and the tube was agitated for 15 s on a vortex mixer. After centrifugation the aqueous phase was transferred to an autosampler vial, and 175 μl were injected into the liquid chromatographic system.

To improve the limit of quantification, the extraction can be performed from 1 ml of plasma. In such cases, 0.3 ml of carbonate buffer and 4 ml of organic phase are added.

Quantification and validation

Standard and control samples were prepared by adding a stock solution (not more than 2% of the total sample volume), containing both norpropoxyphene and dextropropoxyphene, to blank plasma. The linearity of the method was initially established by running a number of multipoint calibration graphs. Quantifications were thereafter made from a two-point calibration graph, the first point being the origin (confirmed each day of analysis) and the second point the mean ratio (analyte peak height *versus* internal standard peak height) of six standard samples with the same concentration.

The control samples were made in batches, in number and concentration appropriate for the study to be analysed, and stored frozen. The control samples were made at two concentrations, one low and one high, and duplicates of each were analysed on every occasion when unknown samples were assayed. The results from these determinations from a single batch of control samples were used to calculate the inter-assay precision.

The intra-assay precision data were determined from ten spiked plasma samples at each level and determined at the same occasion.

The absolute recovery was obtained by comparing the mean peak heights (separate for norpropoxyphene, dextropropoxyphene and the internal standard) from eight spiked plasma samples (500 n*M*), processed as described in Experi-

mental but using exactly known volumes, and the peak heights for direct injections of water solutions with the same nominal concentrations. The recovery for each substance was calculated as the quotient between the mean peak heights of the plasma samples and the aqueous references.

RESULTS AND DISCUSSION

The absolute recoveries found for the extractions from 0.5 ml of plasma were 87% (coefficient of variation, C.V. 4%), 97% (C.V. 3%) and 99% (C.V. 3%) for norpropoxyphene, dextropropoxyphene and the internal standard, respectively. When 1.0 ml of plasma was used, the recovery for norpropoxyphene decreased to 82% while no significant decrease was observed for the two other compounds, results that are in agreement with the change in phase volume ratio.

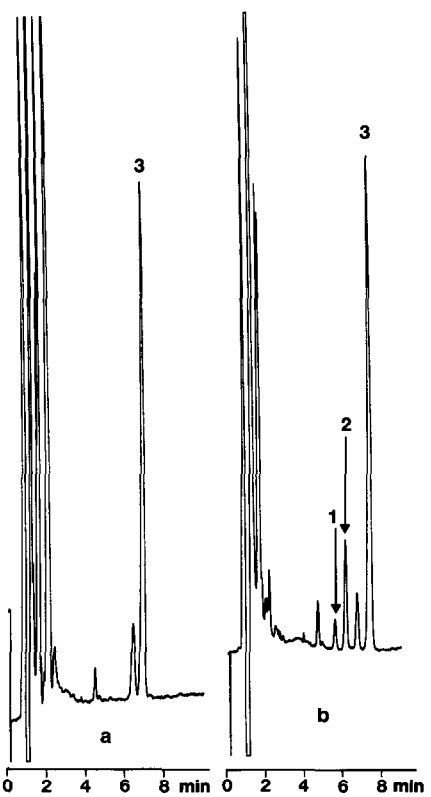

Fig. 1. Chromatograms obtained from (a) blank plasma and (b) a plasma sample from a patient 1 h after a 35-mg of dose of dextropropoxyphene; the found concentrations were 17 n*M* for norpropoxyphene (1) and 61 n*M* for dextropropoxyphene (2). The internal standard (3) concentration in both samples was 800 n*M*. The sample volume was 0.5 ml. Detector setting: 0.008 a.u.f.s.

TABLE I

INTRA-ASSAY ACCURACY AND PRECISION

Added concentration (n*M*)	Accuracy (found) (%)	Precision (C.V.) (%)
Norpropoxyphene		
250	100	2.1
25.0	101	4.4
5.0	102	6.4
2.0[a]	85	5.2
Dextropropoxyphene		
250	100	1.2
25.0	100	1.6
5.0	104	5.0
2.0[a]	100	5.7

[a] Extraction from 1 ml of plasma; $n = 10$.

The chromatographic efficiency, calculated from the peak width at half the peak height, was *ca.* 120 000 theoretical plates per metre for all three compounds. Chromatograms from a plasma blank and a patient sample are presented in Fig. 1. In all chromatograms two extra peaks of unknown origin were seen, one peak eluting before the analytes and one peak eluting between

TABLE II

INTER-ASSAY ACCURACY AND PRECISION

Added concentration (n*M*)	Accuracy (found) (%)	Precision (C.V.)[a] (%)
Norpropoxyphene		
250	103	4.6
25	104	3.7
Dextropropoxyphene		
250	101	1.3
25	100	4.1

[a] Two samples at each concentration and on eight occasions: $n = 16$.

dextropropoxyphene and the internal standard. By adjustment of the mobile phase, the selectivity between the extra peaks and the analytes was easily regulated. An increase in DMOA or acetonitrile content decreased the retention of the analytes more than the retention of the extra peaks.

The linearity of the method was tested up to 1 μM for norpropoxyphene and 0.6 μM for dextropropoxyphene. No deviations from linearity were observed. The intra-assay and inter-assay precision and accuracy were both good (Tables I and II).

For both dextropropoxyphene and norpropoxyphene, the instrumental limit of detection and the limit of quantification, defined as described previously [11], were 0.9 pmol and 5 nM, respectively. When the plasma volume was increased from 0.5 to 1.0 ml, the limit of quantification was 2 nM for both compounds.

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