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Note

Gas chromatographic assay for dextromoramide in human plasma

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Dextromoramide [(+)-1-(3-methyl-4-morpholino-2,2-diphenylbutynyl) pyrrolidine] is a non-opiate narcotic analgesic, discovered by Janssen in 1956 [1]. Its chemical structure is shown in Fig. 1. This compound is approximately five times as potent as morphine and is used in surgical theatres, intensive care units or at home. In adults, therapeutic doses are ca. 5–20 mg of dextromoramide, repeated as necessary [2]. Very little is known about the pharmacokinetics of dextromoramide, i.e. its distribution in the body and metabolic pathways. This seems to be due to the absence of a suitable method of analysis.

Spectrophotometric UV absorption techniques are time-consuming and lack specificity and sensitivity [3,4]. Thin-layer chromatography appears to be only a qualitative test [5,6]. High-performance liquid chromatographic and gas chromatographic (GC) [8] methods have included only a screening evaluation of several narcotics, including dextromoramide.

Therefore, we have developed a new, direct GC method that has permitted the analysis of more than 240 specimens without column reconditioning.

EXPERIMENTAL

Chemicals and reagents

Dextromoramide bitartrate (Delalande Laboratories, Rueil-Malmaison, France) and the internal standard (I.S.) proadifen (SKF 525 A hydrochloride;

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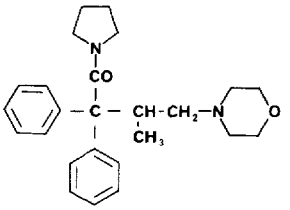

Fig. 1. Structure of dextromoramide.

Smith Kline and French Labs., Welwyn, U.K.) were generously offered by the mentioned companies. Chloroform, 2-propanol, heptane and methanol were HPLC grade (Merck, Darmstadt, F.R.G.). All other chemicals were analytical grade (Merck). Stock solutions of dextromoramide (1 mg/ml, free base) and I.S. were prepared in methanol and stored at 4°C. The dextromoramide standard concentrations, obtained by dilution with methanol, were 20, 50, 100, 200, 300 and 500 ng/ml.

Phosphate buffer was prepared with a saturated solution of dipotassium hydrogenphosphate, 40% diluted with deionized water and adjusted to pH 9.2. Buffered solutions of pH 8.4 and 10.0 were prepared in the same manner and adjusted to the desired pH.

Chromatographic conditions

GC was performed on a 1.8 m × 2 mm I.D. glass column with 3% OV-17 on 100–120 mesh Chromosorb Q (Alltech). The GC system consisted of a Perkin-Elmer (8500) chromatograph with a nitrogen–phosphorus detector and a Perkin-Elmer data collector (Sigma 15). The column, injector port and detector temperatures were 280, 290 and 300°C, respectively. The nitrogen carrier gas pressure was 2.4 bar. Quantification was done for dextromoramide by plotting peak-area ratios (drug/I.S.) against the concentration of standards to produce standard curves and by comparing the results for the case samples with the curves.

Procedure

Plasma (2 ml) was pipetted into a 15-ml pyrex centrifuge tube and followed by 50 μl of 4 *M* sodium hydroxide, 2 ml of phosphate buffer (40%, pH 9.2), 20 μl of I.S. (10 μg/ml) and 10 ml of chloroform–2-propanol–heptane (50:17:33, v/v). After agitation and centrifugation, the organic phase was purified by an additional acidic extraction (5 ml of 0.2 *M* hydrochloric acid). The aqueous layer was reextracted after addition of 2 ml of phosphate buffer (40%, pH 9.2), 0.5 ml of concentrated ammonia solution and 5 ml of chloroform. After agitation and centrifugation, the organic phase was taken off and evaporated to dryness at 45°C in a Speed Vac concentrator (Savant Instruments). The residue was dissolved in 25 μl of methanol, and 1 μl was injected into the GC column.

RESULTS AND DISCUSSION

Retention times were 2.15 and 12.98 min for I.S. and dextromoramide, respectively. Proadifen was chosen as internal standard because the molecule has a

narcotic-like structure close to that of methadone. The compound is not marketed and has no reported history of abuse, so will not interfere in the assay monitoring since drug abusers often prepare their own drug mixtures.

Dextromoramide has a very long retention time relative to I.S., and thus the measurement of both compounds would require temperature programming. This procedure was not adopted because it would lack reproducibility, especially with post-mortem materials. No interferences from plasma components were observed. A typical chromatogram obtained after extraction of a patient's plasma is shown in Fig. 2. Results were linear over the range 20–500 ng/ml for dextromoramide (correlation coefficient of 0.9934).

The accuracy of the method was assessed by carrying six replicate samples of six concentrations of dextromoramide (25, 50, 100, 200, 300 and 400 ng/ml of plasma) through the entire procedure in one analysis day. The results (expressed as relative error to the least-squares equation) are reported in Table I.

The day-to-day precision was studied by adding dextromoramide to blank plasma at therapeutic and toxic concentrations. Analyses were performed every day over a period of three weeks. The precision was found to be ca. 7% (Table II). The within-run studies are also summarized in Table II. The extraction re-

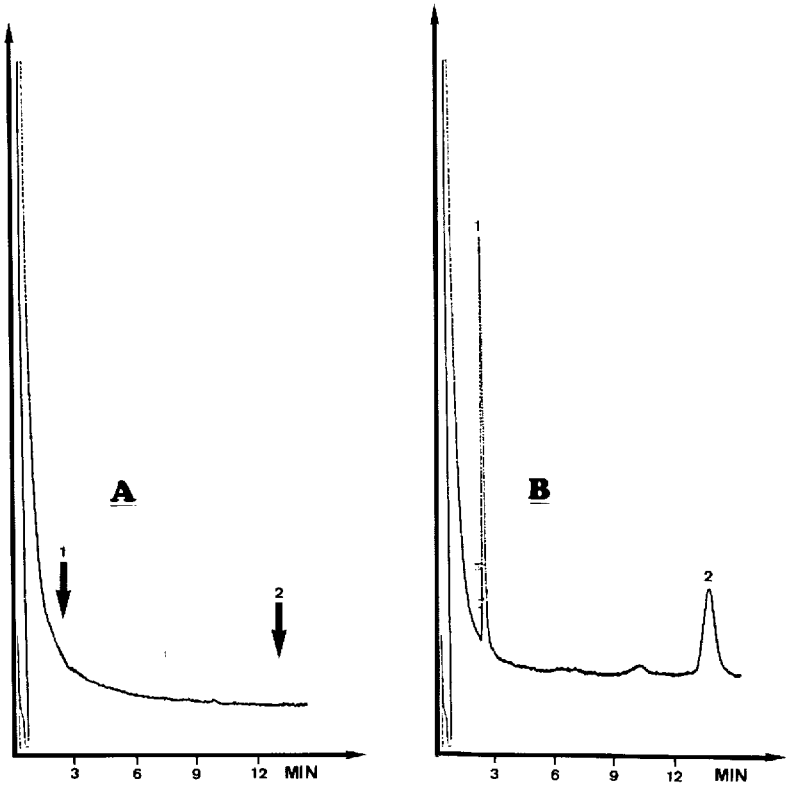

Fig. 2. (A) Gas chromatogram of a control plasma. (B) Gas chromatogram of an extract of test plasma sample obtained from a subject under dextromoramide treatment (concentration, 24 ng/ml). Peaks: 1 = I.S.; 2 = dextromoramide.

TABLE I

ACCURACY FOR THE DETERMINATION OF DEXTROMORAMIDE IN PLASMA

Concentration given (ng/ml)	Concentration found after extraction (mean ± S.D.) (ng/ml)	R.S.D. (%)	Error (%)
25	24.03 ± 1.42	6.9	−3.9
50	51.73 ± 2.79	4.6	3.5
100	106.41 ± 5.37	3.2	6.4
200	197.09 ± 10.11	3.9	−1.5
300	308.4 ± 13.07	4.1	2.8
400	391.9 ± 18.40	4.4	−2.0

TABLE II

REPRODUCIBILITY IN PLASMA FOR DEXTROMORAMIDE

Concentration (ng/ml)	Within-run precision ($n=9$) (%)	Day-to-day precision over three weeks ($n=6$) (%)
50	4.8	6.9
200	4.2	6.6

TABLE III

COMPARISON OF ORGANIC SOLVENTS FOR THE EXTRACTION OF DEXTROMORAMIDE FROM PLASMA

Results are the means ± S.D. of four separate experiments. Each plasma was spiked to contain 100 ng/ml dextromoramide.

Solvent	Dextromoramide recovery (%)		
	pH 8.4	pH 9.2	pH 10.0
Chloroform	82.1 ± 3.4	76.9 ± 5.2	74.3 ± 4.9
n-Hexane	13.1 ± 8.2	17.0 ± 9.1	19.5 ± 6.3
Ethyl acetate	27.5 ± 9.9	34.7 ± 11.3	28.7 ± 10.7
Chloroform–2-propanol–*n*-heptane (50:17:33, v/v)	79.0 ± 4.1	86.0 ± 4.0	86.4 ± 4.0

covery was determined for dextromoramide by comparing the representative peak areas of extracted plasma with the peak areas of methanolic standards at the same concentration (external standard quantification).

Various common extraction solvents were investigated for recovering dextromoramide from plasma. The results are summarized in Table III. Chloroform–2-propanol–*n*-heptane was selected because it produced emulsion-free extracts with an acceptable extraction efficiency. These conditions were also found to be adequate for the extraction of opiates.

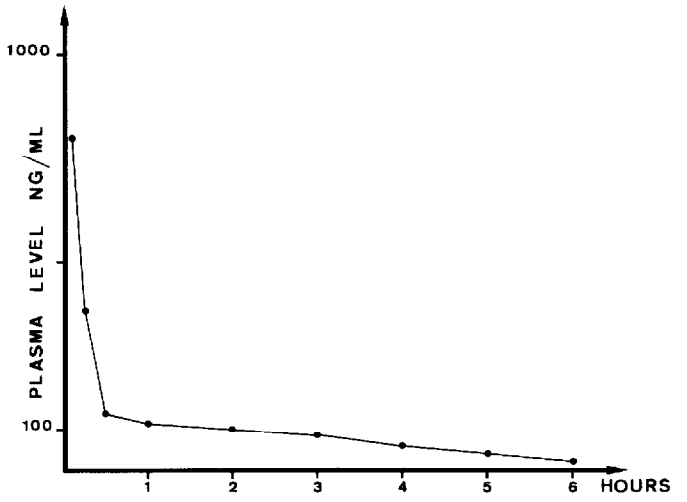

Fig. 3. Plasma levels of dextromoramide in a patient after an intravenous dose of 5 mg.

The limit of detection was determined by spiking plasma with decreasing concentrations of dextromoramide until a response equivalent to three times the background noise was observed. The lower limit of detectability was found to be 2 ng/ml in plasma. This detection limit is adequate for forensic and clinical analyses.

The detection and measurement not only of the parent drug but also of its major metabolites would be an indication of chronic abuse. However, dextromoramide metabolism is still hypothetical: several pathways have been proposed, including N-oxidation, 2-hydroxylation and amide hydrolysis [2,4]. Since these metabolites have not yet been isolated, their chromatographic identification remained unreliable.

The suitability of the method for dextromoramide was demonstrated by the analysis of plasma samples from a patient after an intravenous dose of 5.0 mg of dextromoramide base. Fig. 3 shows the time course of the plasma concentration of dextromoramide.

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